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An Efficient Method to Construct the A,B-Rings Component toward Total Syntheses of Phycocyanobilin and Its Derivative as a Photoprobe

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Total syntheses of phycocyanobilin and its derivative bearing a photoreactive group at the D-ring were accomplished by developing a new and efficient method for the construction of A,B-rings component, which consists of the Wittig-type new coupling reaction of 4-(1-methoxyethyl or 1-tosylethyl)-3-methyl-5-tosyl-1,5-dihydro-2*H*-pyrrol-2-one and a 2-formyl pyrrole derivative in the presence of $^{n}Bu_3P$ and a base, followed by reduction with aluminum amalgam and a subsequent acid or base treatment.

Phycocyanin and phytochrome belonging to biliproteins, which contain bile pigments as chromophoric units, exist in plants. Their chromophores, namely phycocyanobilin (**1**) and phytochromobilin (**2**), are linear tetrapyrrole derivatives, and are covalently bonded to their apoproteins at the A-ring. Even though such bile pigments, phycobilins, could be isolated from natural sources, knowledge of the relationship between the structure of synthetic pigments and the biochemical properties of the biliproteins prepared by combining them with an apoprotein is quite interesting and important to reveal the precise function of the phycobilins. The recent developments in gene technology have made it possible to assemble such chromophores as **1** and **2** with an apoprotein obtained by the over-expression of the corresponding cDNA in bacteria and yeast. Compound **1** has often been used as a substitute for the natural chromophore **2** in phytochrome reconstitution experiments. Moreover, the photophysical and photochemical properties of wild type phytochrome are quite similar to those of the reconstituted chromoprotein containing phycocyanobilin (**1**).[1]

For the structure/function analysis of phytochrome, we have been studying the syntheses of phycobilin derivatives.[2] In the previous paper,[2g] we reported a preliminary results of a new method for the construction of A,B-rings component utilizing 4-(1-methoxyethyl)-3-methyl-5-tosyl-1,5-dihydro-2*H*-pyrrol-2-one (**6a**) and its application to the total syntheses of phycocyanobilin (**1**) and its derivative (**3**) having a photoreactive group at the D-ring. Detailed results of the study are presented in this paper together with new results utilizing 3-methyl-5-tosyl-4-(1-tosylethyl)-1,5-dihydro-2*H*-pyrrol-2-one (**6b**).

Results and Discussion

Though there have been reports on the syntheses of phycobilin ester derivatives,[3—5] most of the published studies in this area for the synthesis of the A,B-rings component have been carried out by utilizing either the Eschenmoser's sulfide contraction,[3] the thio-Wittig coupling,[4,5] or the photochemical rearrangement of *N*-pyrroloenamide.[6] Although these methods provide viable routes to the required A,B-rings component, the former two usual methods require removing a carboxylic acid ester group at the meso-position at the later stage toward the total syntheses of phycobilins. Therefore, an alternative route has been developed for the synthesis of the A,B-rings component, as illustrated in Fig. 1, as retrosynthetic analyses of the A,B- and C,D-rings components (**4** and **5**), both of which apply our original Wittig-type new coupling reaction between 5-tosyl pyrrolinones (**6** and **8**) and a common formyl pyrrole (**7**).[2b—2d]

The 3,4-disubstituted 5-tosylpyrrolinones (**8**) have so far been synthesized by the acid hydrolysis of the corresponding 2-bromo-5-tosylpyrroles according to our method.[2a] This time we established an alternative method, starting from the 2-pyrrolecarboxylic acid ester derivatives (**9**), which are easily prepared by the reaction of β-acetoxy nitroalkanes and *t*-butoxycarbonylmethyl isocyanide in the presence of a base,[7] as shown in Scheme 1. Iodination at the 5-position of **9** with *N*-iodosuccinimide (NIS) afforded the corresponding iodinated products[8] (**10**) in quantitative yields, which were oxidized by $Pb(OAc)_4$ without any further purification to give the pyrrolinone derivatives **11** in excellent yields. When these derivatives were treated with *p*-toluenesulfinic acid in the presence of diethyl ether–boron trifluoride (1/1), the desired 5-tosylpyrrolinones (**8**) were obtained in high yields.

Similarly, compounds **11** underwent the substitution reaction with triethyl phosphite to give the corresponding diethyl 3,4-disubstituted 1,5-dihydro-5-oxo-2*H*-pyrrol-2-ylphosphonates (**12**),[2d,9] which are also useful synthons to be coupled with formyl pyrroles, probably through an elimination/addition mechanism accompanied by a decarboxylation reaction (Scheme 2).

The preparation of A-ring **6a** from the 5-tosylpyrrolinone

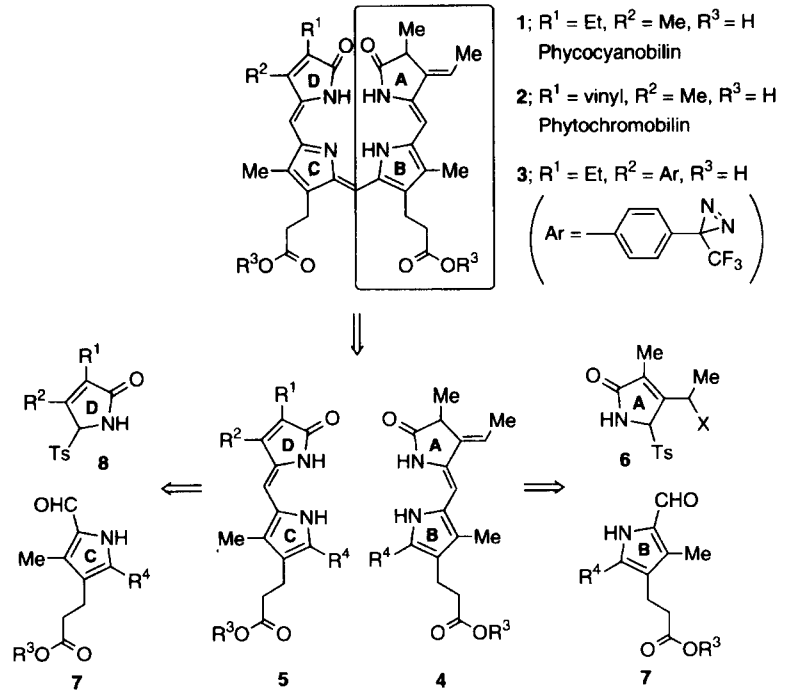
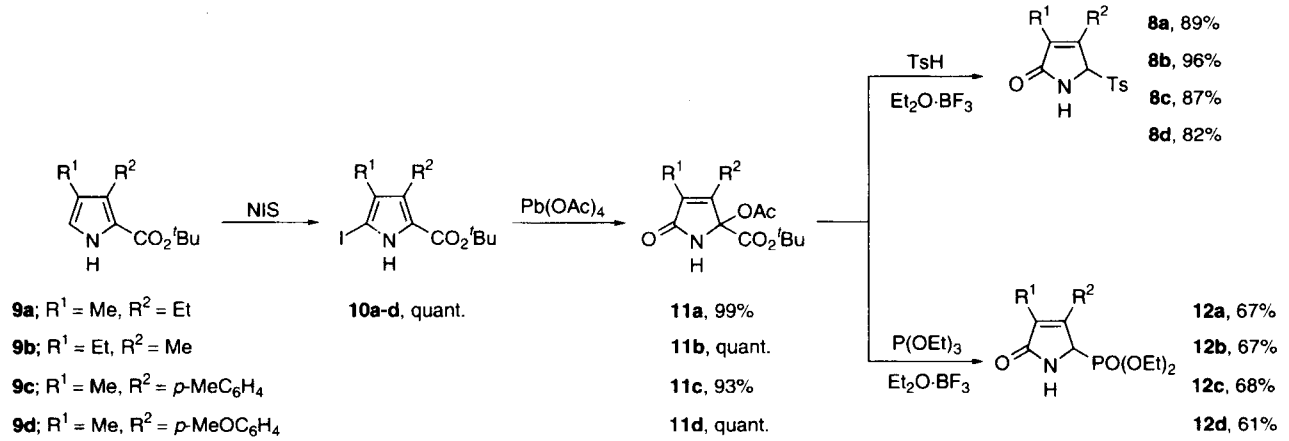
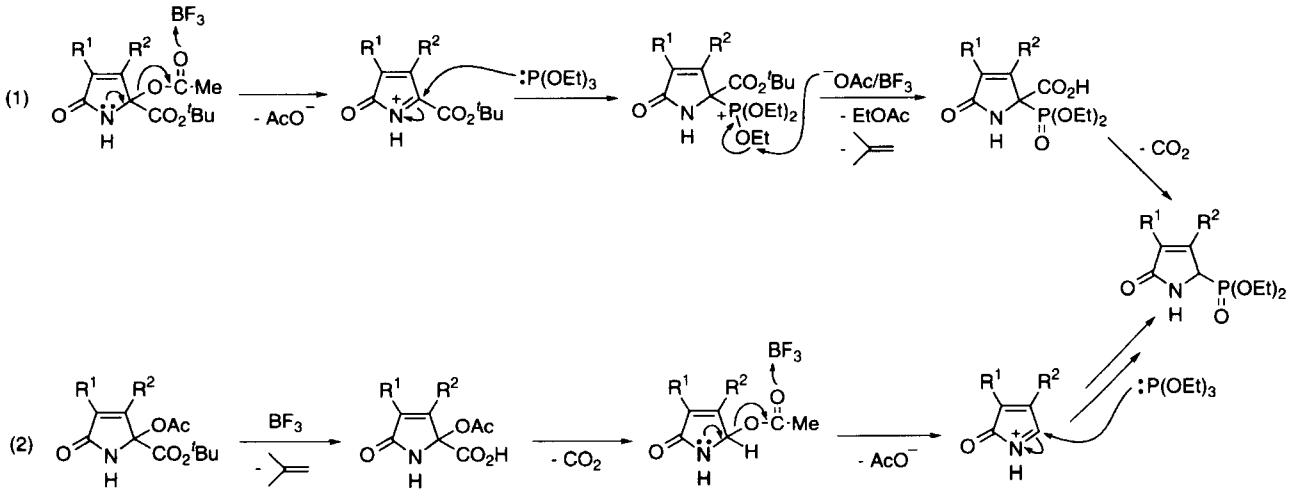


Fig. 1.



Scheme 1.



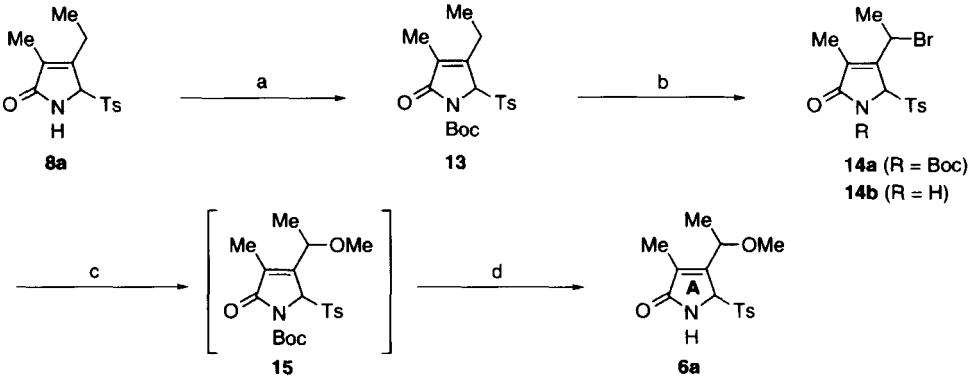
Scheme 2.

8a was achieved through the *N*-protected derivative (**13**), as illustrated in Scheme 3. At first, direct bromination of compound **8a** with *N*-bromosuccinimide (NBS) was examined to afford **14b** under various conditions. However, in all cases, the corresponding maleimide derivative was produced as the main product, and the desired product **14b** could be obtained only in a trace amount. Therefore, the nitrogen atom of **8a** was protected using di-*t*-butyl dicarbonate in the presence of 4-(dimethylamino)pyridine (DMAP), and the resulting compound **13** was brominated with NBS to afford **14a** regioselectively in good yield. Then, the treatment of **14a** with NaOAc in MeOH, followed by deprotection of the Boc group with trifluoroacetic acid, afforded the desired compound **6a** as a mixture of two diastereomers in good yield.

The formation of a maleimide derivative by direct bromination seemed to be due to the existence of a methine proton at the 5-position of **8a**. Therefore, the direct bromination of the intermediary pyrrolinone **11a** was next examined (Scheme 4). Bromination of the pyrrolinone derivative **11a** with one molar amount of NBS was found to give the corresponding brominated compound **16** in quantitative yield. Then, the treatment of **16** with NaOMe in MeOH, followed by introducing a tosyl group, afforded the desired tosyl pyrrolinone **6a** in reasonable yield. In a similar manner, the tosyl group could be introduced instead of the methoxy group by a treatment of **16** with sodium *p*-toluenesulfinate in refluxing THF, followed by decarboxylation with trifluoroacetic acid to afford **6b** in high yield.

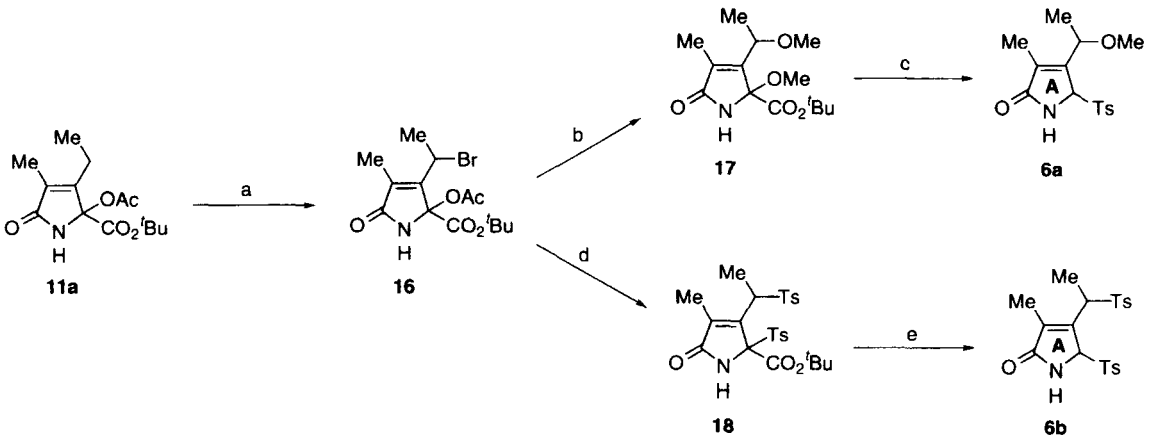
The compounds **6a** and **6b** thus obtained were coupled with a formyl pyrrole **7a** as a B-ring by the Wittig-type coupling reaction in the presence of $^{n}Bu_3P$ and $^{t}BuOK$ in CH_2Cl_2 to afford **19a,b** as mixtures of *E*- and *Z*-isomers in good yields, respectively. The resulting compounds **19a,b** were reduced with aluminum amalgam to give **20a,b**.[10] These intermediates **20a,b** were treated with pyridinium *p*-toluenesulfonate (PPTS, for **20a**) or with 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU, for **20b**) without further purification to give the desired A,B-rings component **4a** as a single *Z*-isomer (confirmed by NOE measurement) via the elimination of methanol or *p*-toluenesulfinic acid in good yields, as shown in Scheme 5.

The C,D-rings components **5a,b** were prepared from **7a** and **8b,e** by the Wittig-type coupling reaction as described



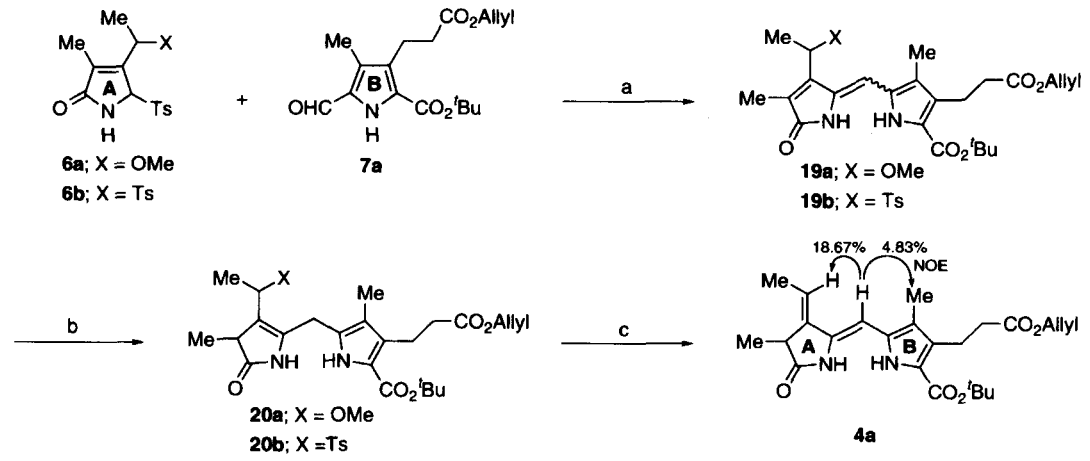
a) $(Boc)_2O$ (1.5 eq.), DMAP (0.1 eq.) in MeCN at -40 - 0 °C, 1 h. **13** 91%. b) NBS (1.2 eq.) in benzene at rt, 2 d. **14a** 71%. c) NaOAc (5 eq.) in refluxing MeOH, 1 h. **15** (crude). d) TFA in CH_2Cl_2 at 0 °C - rt, 10 min. **6a** 80% (from **14a**).

Scheme 3.



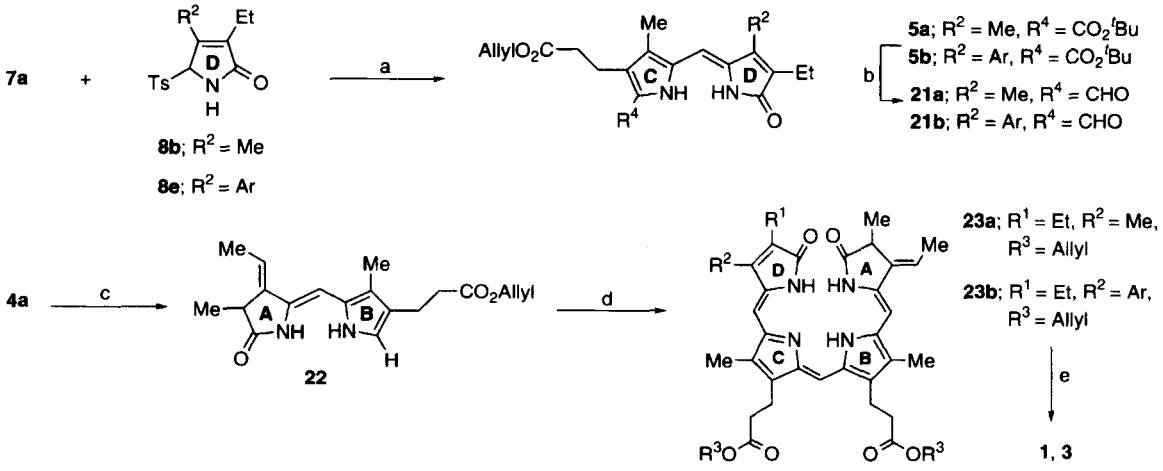
a) NBS (1.2 eq.) in benzene at rt, overnight. **16** quant. b) NaOMe (3.1 eq.) in MeOH at 0 °C - rt, 2 h. **17** 53%. c) TsH (4 eq.), $Et_2O{\cdot}BF_3$ (3 eq.) in toluene at rt, 2 d. **6a** 60%. d) TsNa (5 eq.) in refluxing THF, 1h. **18** 94%. e) TFA in CH_2Cl_2 at 0 °C - rt, 10 min. **6b** 80%

Scheme 4.



a) A CH_2Cl_2 solution of **6a** or **6b** (1.4 eq.) was added dropwise over the period of 30 min to the mixed solution of **7a** (1.0 eq.), $^nBu_3P$ (2.4 eq.) and ^{t}BuOK (1.2 eq.) in CH_2Cl_2 at -70 °C - rt, overnight at rt. **19a** 84% (*E*/*Z* = 37/63), **19b** 73% (*E*/*Z* = 40/60). b) Al(Hg), in THF/H_2O (10/1, v/v) at rt, 2 h. **20a,b** (not isolated in general). c) PPTS (0.1 eq.) in CH_2Cl_2 at 0 °C - rt, 30 min. **4a** 72% (from **19a**); DBU (2 eq.) in CH_2Cl_2 at 0 °C - rt, 30 min. **4a** 68% (from **19b**).

Scheme 5.



a) $^nBu_3P$ (2 eq.), **7a** (1.1 eq.), **8b** (1 eq.) and DBU (1.2 eq.) in CH_2Cl_2 at 0 °C - rt, 4 h. **5a** 84%; ^{t}BuOK (1.2 eq.), **7a** (1.1 eq.), $^nBu_3P$ (2.4 eq.) and **8e** (1 eq.), in CH_2Cl_2 at -78 °C - rt, overnight at rt. **5b** 73%. b) TFA at rt, 40 min, $HC(OMe)_3$ at rt, 1 h. **21a** 81%; **21b** 66%. c) TFA at rt, 1 h. **22** (not isolated). d) **21a** (0.9 eq.) in EtOH, cat. conc. H_2SO_4 at rt, 2 h. **23a** 36% (based on **21a**); **21b** (0.9 eq.) in MeOH/CH_2Cl_2 (6/1, v/v), cat. $MeSO_3H$ at rt, 2 h. **23b** 32% (based on **21b**). e) $[Pd(PPh_3)_4]$ (0.2 eq.), morpholine (10 eq.) in THF at rt, 1 h. **1** 96%, **3** 80%.

Scheme 6.

previously.[2e] Subsequant decarboxylation and formylation afforded the aldehydes **21a,b** (Scheme 6). Then, these components **21a,b** were reacted with the A,B-rings component **22** obtained by an acid treatment of **4a** to construct the corresponding tetrapyrrole derivatives **23a,b**. The allyl ester group were deprotected by a treatment with morpholine in the presence of a catalytic amount of Pd(0)[2f] to obtain the acid forms of phycocyanobilin (**1**) and its derivative **3** bearing a photoreactive group at the D-ring for a photoaffinity study.[2f,11]

As described above, the Wittig-type coupling reaction proved to be useful not only for the preparation of the C, D-rings component, but also for the construction of A,B-rings component by introducing an eliminating group, like methoxy or tosyl group, into the precursor of the A-ring. An investigation of the reconstituted chromoproteins using the synthesized phycobilins (**1** and **3**) is in progress for the structure/function analysis of phytochrome.

Experimental

All of the melting points were determined with a micro melting apparatus (Yanagimoto Seisakusho) and were uncorrected. The ^{1}H NMR, IR, and MS spectra were recorded on JEOL JNM-LA 300FT (300 MHz) and LA 400 FT (400 MHz) NMR spectrometers, a JASCO FT/IR-230 infrared spectrometer, and a JEOL SX-102A mass spectrometer, respectively. The chemical shifts of NMR are reported in the δ-scale relative to TMS as an internal standard. All of the solvents were distilled and stored over a drying agent. Thin-layer chromatography (TLC) and flash column chromatography were performed by using Merck's silica gel 60 PF_{254} (Art. 7749) and Cica-Merck's silica gel 60 (No. 9385-5B), respectively.

Preparation of *t*-Butyl 3,4-Disubstituted 5-Iodo-2-pyrrolecarboxylate (10a—d). A solution of **9a**[7] (209 mg, 1 mmol)

and NIS (228 mg, 1 mmol) in 6 ml of acetone was stirred for 1 h at room temperature. After evaporation of the solvent, the residue was taken up in ethyl acetate. The organic layer was successively washed with a saturated aqueous solution of $NaHSO_3$, a saturated aqueous solution of $NaHCO_3$, and brine, and dried over Na_2SO_4. Evaporation of the solvent afforded the desired product[8] **10a** in quantitative yield (335 mg). It was used for the next reaction without further purification. A part of the product was recrystallized for elemental analysis. In a similar manner, **10b—d** were prepared in quantitative yields, respectively. Their physical and spectral data are given in the following:

***t*-Butyl 3-Ethyl-5-iodo-4-methyl-2-pyrrolecarboxylate (10a).** Mp 110—111 °C (decomp) (from hexane); IR (KBr) 3286, 3008, 2971, 2928, 2872, 1668, 1557, 1456, 1397, 1365, 1240, 1175, 1142, 1104, 861, 776, 721 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.09 (t, J = 7.47 Hz, 3H), 1.56 (s, 9H), 1.97 (s, 3H), 2.73 (q, J = 7.47 Hz, 2H), 8.84 (brs, 1H). Found: C, 42.93; H, 5.43; N, 4.06%. Calcd for $C_{12}H_{18}INO_2$: C, 43.00; H, 5.41; N, 4.18%.

***t*-Butyl 4-Ethyl-5-iodo-3-methyl-2-pyrrolecarboxylate (10b).** Mp 120—121 °C (decomp) (from hexane); IR (KBr) 3302, 2963, 2925, 2867, 1668, 1556, 1458, 1405, 1364, 1318, 1257, 1239, 1173, 1144, 1091, 862, 773, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.04 (t, J = 7.56 Hz, 3H), 1.56 (s, 9H), 2.29 (s, 3H), 2.37 (q, J = 7.56 Hz, 2H), 8.72 (brs, 1H). Found: C, 43.14; H, 5.45; N, 4.04%. Calcd for $C_{12}H_{18}INO_2$: C, 43.00; H, 5.41; N, 4.18%.

***t*-Butyl 5-Iodo-4-methyl-3-(*p*-tolyl)-2-pyrrolecarboxylate (10c).** Mp 164—165 °C (decomp) (from EtOAc); IR (KBr) 3271, 2978, 2920, 2860, 1658, 1551, 1519, 1473, 1455, 1390, 1266, 1231, 1160, 1038, 999, 848, 822, 778 739 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.35 (s, 9H), 1.91 (s, 3H), 2.37 (s, 3H), 7.15 (d, J = 8.42 Hz, 2H), 7.17 (d, J = 8.42 Hz, 2H), 8.87 (brs, 1H). Found: C, 51.43; H, 5.07; N, 3.43%. Calcd for $C_{17}H_{20}INO_2$: C, 51.40; H, 5.07; N, 3.53%.

***t*-Butyl 5-Iodo-3-(*p*-methoxyphenyl)-4-methyl-2-pyrrolecarboxylate (10d).** Mp 159—161 °C (decomp) (from EtOAc); IR (KBr) 3284, 2979, 2963, 2934, 2832, 1654, 1610, 1548, 1518, 1456, 1391, 1367, 1287, 1248, 1229, 1177, 1157, 1042, 994, 834, 778, 736 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.36 (s, 9H), 1.91 (s, 3H), 3.83 (s, 3H), 6.91 (d, J = 8.78 Hz, 2H), 7.19 (d, J = 8.78 Hz, 2H), 9.04 (brs, 1H). Found: C, 49.60; H, 4.89; N, 3.27%. Calcd for $C_{17}H_{20}INO_3$: C, 49.41; H, 4.88; N, 3.39%.

Preparation of 3,4-Disubstituted 5-Acetoxy-5-(*t*-butoxycarbonyl)-1,5-dihydro-2*H*-pyrrol-2-one (11a—d). A solution of **10a** (1.005 g, 3 mmol) in 10 ml of toluene was added to a suspension of $Pb(OAc)_4$ (1.994 g, 4.5 mmol) in 10 ml of toluene at room temperature under a nitrogen atmosphere; the mixture was allowed to stand for 2 d at room temperature. The mixture was filtered through celite and the filtrate was successively washed with a saturated aqueous solution of $NaHSO_3$, a saturated aqueous solution of $NaHCO_3$, and brine, and dried over Na_2SO_4. After evaporation of the solvent, the resulting residue was recrystallized from EtOAc/hexane to afford **11a** in 99% yield (841 mg). In a similar manner, **11b—d** were prepared in quant., 93%, and quantitative yields, respectively. Their physical and spectral data are given in the following:

5-Acetoxy-5-(*t*-butoxycarbonyl)-4-ethyl-1,5-dihydro-3-methyl-2*H*-pyrrol-2-one (11a). Mp 102—103 °C (from EtOAc/hexane); IR (KBr) 3231, 3102, 2982, 2942, 2881, 1760, 1739, 1714, 1456, 1436, 1409, 1391, 1372, 1364, 1291, 1260, 1230, 1156, 1144, 1116, 1054, 1040, 1022, 901, 821, 730 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.14 (t, J = 7.72 Hz, 3H), 1.45 (s, 9H), 1.86 (s, 3H), 2.13 (s, 3H), 2.41 (m, 2H), 6.66 (brs, 1H). Found: C, 59.29; H, 7.57; N, 4.86%. Calcd for $C_{14}H_{21}NO_5$: C, 59.35; H, 7.47; N, 4.94%.

5-Acetoxy-5-(*t*-butoxycarbonyl)-3-ethyl-1,5-dihydro-4-methyl-2*H*-pyrrol-2-one (11b). Mp 87—88 °C (from hexane); IR (KBr) 3254, 3104, 2980, 2942, 2882, 1759, 1739, 1715, 1457, 1366, 1287, 1260, 1231, 1156, 1142, 1040, 1024, 899, 820, 769, 709 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.09 (t, J = 7.52 Hz, 3H), 1.45 (s, 9H), 1.95 (s, 3H), 2.14 (s, 3H), 2.29 (d, J = 7.52 Hz, 2H), 6.64 (brs, 1H). Found: C, 59.24; H, 7.45; N, 4.94%. Calcd for $C_{14}H_{21}NO_5$: C, 59.35; H, 7.47; N, 4.94%.

5-Acetoxy-5-(*t*-butoxycarbonyl)-1,5-dihydro-3-methyl-4-(*p*-tolyl)-2*H*-pyrrol-2-one (11c). Mp 157—159 °C (from benzene/hexane); IR (KBr) 3218, 3100, 2974, 2929, 1747, 1719, 1510, 1431, 1382, 1354, 1277, 1258, 1235, 1169, 1151, 1119, 1048, 1033, 1009, 848, 815, 762 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.23 (s, 9H), 1.97 (s, 3H), 2.20 (s, 3H), 2.39 (s, 3H), 6.91 (brs, 1H), 7.22 (d, J = 8.25 Hz, 2H), 7.36 (d, J = 8.25 Hz, 2H). Found: C, 66.07; H, 6.73; N, 3.90%. Calcd for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06%.

5-Acetoxy-5-(*t*-butoxycarbonyl)-1,5-dihydro-4-(*p*-methoxyphenyl)-3-methyl-2*H*-pyrrol-2-one (11d). Mp 128.5—129.5 °C (from benzene/hexane); IR (KBr) 3221, 3099, 2999, 2976, 2936, 2842, 1745, 1721, 1607, 1511, 1442, 1371, 1354, 1290, 1276, 1252, 1234, 1178, 1149, 1119, 1031, 1014, 1006, 896, 847, 836, 817, 791, 761 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.23 (s, 9H), 1.99 (s, 3H), 2.21 (s, 3H), 3.85 (s, 3H), 6.91 (brs, 1H), 6.94 (d, J = 8.99 Hz, 2H), 7.45 (d, J = 8.99 Hz, 2H). Found: C, 63.11; H, 6.50; N, 3.72%. Calcd for $C_{19}H_{23}NO_6$: C, 63.14; H, 6.42; N, 3.88%.

Preparation of 3,4-Disubstituted 1,5-Dihydro-5-tosyl-2*H*-pyrrol-2-ones (8a—d). To a mixed suspention of **11a** (849 mg, 3 mmol) and *p*-toluenesulfinic acid (1.872 g, 12 mmol) in 15 ml of toluene was added dropwise $Et_2O{\cdot}BF_3$ (1.278 g, 9 mmol) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 2 d at room temperature. The mixture was poured into 50 ml of ice water, and partitioned between ethyl acetate and 10% $NaHCO_3$. The organic layer was washed with brine, and dried over Na_2SO_4. Evaporation of the solvent and recrystallization of the resulting residue from EtOAc/hexane gave the expected product **8a** as a colorless solid (746 mg) in 89% yield.

4-Ethyl-1,5-dihydro-3-methyl-5-tosyl-2*H*-pyrrol-2-one (8a). Mp 155—156 °C (from EtOAc/hexane); IR (KBr) 3184, 3081, 2964, 2943, 2877, 1707, 1598, 1459, 1387, 1355, 1315, 1303, 1290, 1217, 1166, 1148, 1085, 1016, 962, 893, 831, 809, 790, 748, 703, 669 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.21 (t, J = 7.61 Hz, 3H), 1.58 (s, 3H), 2.43 (s, 3H), 2.57 (m, 1H), 2.70 (m, 1H), 5.15 (s, 1H), 6.41 (brs, 1H), 7.30 (d, J = 8.16 Hz, 2H), 7.64 (d, J = 8.16 Hz, 2H). Found: C, 60.24; H, 6.14; N, 4.79%. Calcd for $C_{14}H_{17}NO_3S$: C, 60.19; H, 6.13; N, 5.01%.

In a similar manner, **8b—d** were prepared in 96, 87, and 82% yields, respectively. Their physical and spectral data were consistent with those given in Ref. 8.

Preparation of Diethyl 3,4-Disubstituted 1,5-Dihydro-5-oxo-2*H*-pyrrol-2-ylphosphonates (12a—d). To a solution of **11a** (57 mg, 0.2 mmol) and triethyl phosphite (100 mg, 0.6 mmol) in 2.5 ml of dry CH_2Cl_2 was added $Et_2O{\cdot}BF_3$ (85 mg, 0.6 mmol) at 0 °C under a nitrogen atmosphere. The mixed solution was stirred for 2 d at room temperature. The solvent was removed in vacuo, and the resulting residue was partitioned between ethyl acetate and 10% $NaHCO_3$. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent and separation of the residue with a preparative TLC (SiO_2, EtOAc/EtOH = 20/1, v/v) gave 35 mg (67%) of **12a** as a colorless oil. In a similar manner, **12b—d** were prepared in 67, 68, and 61% yields, respectively. Their physical and spectral data were consistent with those given in Ref. 9.

1-*t*-Butoxycarbonyl-4-ethyl-1,5-dihydro-3-methyl-5-tosyl-2*H*-pyrrol-2-one (13). A solution of DMAP (31 mg, 0.25 mmol) in 3 ml of dry MeCN was added dropwise to a mixed solution of pyrrolinone **8a** (698 mg, 2.5 mmol) and di-*t*-butyl dicarbonate (818 mg, 3.75 mmol) in 12 ml of dry MeCN at −40 °C under a nitrogen atmosphere and the mixture was stirred for 1 h allowing to reach to 0 °C. After removal of the solvent, the residue was partitioned between ethyl acetate and water. The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ and brine, and dried over Na_2SO_4. The filtrate was concentrated in vacuo and the solid residue was recrystallized from EtOAc/hexane to give the desired compound **13** as colorless crystals in 91% yield (860 mg). Mp 130—132 °C (from EtOAc/hexane); IR (KBr) 3068, 3060, 2979, 2888, 1759, 1721, 1665, 1595, 1455, 1372, 1302, 1257, 1146, 1104, 1081, 984, 848, 818, 804, 778, 715, 666 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.24 (t, J = 7.61 Hz, 3H), 1.45 (s, 9H), 1.67 (s, 3H), 2.42 (s, 3H), 2.56—2.82 (m, 2H), 5.74 (s, 1H), 7.31 (d, J = 8.25 Hz, 2H), 7.67 (d, J = 8.25 Hz, 2H). Found: C, 60.18; H, 6.69; N, 3.51%. Calcd for $C_{19}H_{25}NO_4S$: C, 60.14; H, 6.64; N, 3.69%.

4-(1-Bromoethyl)-1-*t*-butoxycarbonyl-1,5-dihydro-3-methyl-5-tosyl-2*H*-pyrrol-2-one (14a). A solution of **13** (95 mg, 0.25 mmol) and NBS (57 mg, 0.3 mmol) in 5 ml of benzene was kept at room temperature for 2 d with stirring under a nitrogen atmosphere. The solvent was removed in vacuo, and the residue was taken up in ethyl acetate. The organic layer was successively washed with water, a saturated aqueous solution of $NaHSO_3$, a saturated aqueous solution of $NaHCO_3$, and brine, and dried over Na_2SO_4. Evaporation of the solvent and separation of the residue with a preparative TLC (SiO_2, hexane/EtOAc = 3/1, v/v) gave **14a** (81 mg, 71%) as an oily mixture of diastereomers (Major/Minor = ca. 2/1). IR (neat) 3032, 2981, 2934, 1784, 1752, 1728, 1660, 1596, 1456, 1370, 1331, 1307, 1290, 1257, 1145, 1082, 989, 849, 817, 803, 757, 689 cm^{-1}; ^1H NMR ($CDCl_3$) Major δ = 1.39 (s, 9H), 1.98 (d, J = 1.65 Hz, 3H), 2.12 (d, J = 7.15 Hz, 3H), 2.43 (s, 3H), 5.41 (q, J = 7.15 Hz, 1H), 5.85 (d, J = 1.47 Hz, 1H), 7.34 (d, J = 8.16 Hz, 2H), 7.70 (d, J = 8.16 Hz, 2H). Minor δ = 1.46 (s, 9H), 1.90 (d, J = 1.65 Hz, 3H), 1.97 (d, J = 7.15 Hz, 3H), 2.42 (s, 3H), 5.28 (q, J = 7.15 Hz, 1H), 5.73 (d, J = 1.65 Hz, 1H), 7.31 (d, J = 8.16 Hz, 2H), 7.78 (d, J = 8.16 Hz, 2H). HRMS (FAB) (M^++1), Found: m/z 458.0631. Calcd for $C_{19}H_{25}{}^{79}BrNO_5S$: M, 458.0637. Found: m/z 460.0622. Calcd for $C_{19}H_{25}{}^{81}BrNO_5S$: M, 460.0616.

1-*t*-Butoxycarbonyl-1,5-dihydro-4-(1-methoxyethyl)-3-methyl-5-tosyl-2*H*-pyrrol-2-one (15). A solution of **14a** (50 mg, 0.11 mmol) and sodium acetate (44 mg, 0.55 mmol) in 5 ml of methanol was refluxed for 1 h under a nitrogen atmosphere. After cooling to room temperature, the solvent was removed in vacuo, and the residue was taken up in ethyl acetate. The organic layer was washed with water and brine, and dried over Na_2SO_4. After evaporation of the solvent, the resulting crude product **15** (45 mg) was used for the next reaction without further purification. A solid mixture of diastereomers (Major/Minor = ca. 2/1). IR (KBr) 2979, 2946, 2828, 1745, 1714, 1663, 1596, 1458, 1370, 1326, 1292, 1256, 1159, 1129, 1114, 1083, 979, 915, 849, 814, 813, 746, 734, 688 cm^{-1}; ^1H NMR ($CDCl_3$) Major δ = 1.37 (s, 9H), 1.45 (s, 3H), 1.95 (d, J = 6.51 Hz, 3H), 2.43 (s, 3H), 3.44 (s, 3H), 4.77 (q, J = 6.48 Hz, 1H), 5.64 (d, J = 1.47 Hz, 1H), 7.33 (d, J = 8.25 Hz, 2H), 7.74 (d, J = 8.25 Hz, 2H). Minor δ = 1.36 (s, 9H), 1.47 (s, 3H), 1.60 (d, J = 6.48 Hz, 3H), 2.42 (s, 3H), 3.36 (s, 3H), 4.49 (q, J = 6.48 Hz, 1H), 5.84 (d, J = 1.47 Hz, 1H), 7.31 (d, J = 8.25 Hz, 2H), 7.66 (d, J = 8.25 Hz, 2H). MS m/z 409 (M^+; 0.75%), 353 (1.90), 323 (0.68), 292 (1.26), 254 (50.08), 224 (12.30), 199 (4.88), 155 (12.88), 154 (100), 124 (98.06), 91 (27.40), 57 (67.97).

1,5-Dihydro-4-(1-methoxyethyl)-3-methyl-5-tosyl-2*H*-pyrrol-2-one (6a). To a solution of **15** (45 mg, 0.11 mmol) in 1 ml of CH_2Cl_2 was added dropwise 0.5 ml of TFA at 0 °C under a nitrogen atmosphere and kept at room temperature for 10 min. The solvent was then removed in vacuo and the residue was partitioned between ethyl acetate and water. The organic layer was washed with water, a saturated aqueous solution of $NaHCO_3$, and brine, and dried over Na_2SO_4. Evaporation of the solvent and separation of the residue with a preparative TLC (SiO_2, hexane/AcOEt = 2/1, v/v) gave 27 mg (80% from **14a**) of **6a** as a colorless solid, a part of which was recrystallized for elemental analysis. **6a**: A mixture of two diastereomers (Major/Minor = ca. 2/1); mp 161—162 °C (from EtOAc/hexane); IR (KBr) 3170, 3068, 2979, 2925, 2824, 1704, 1654, 1597, 1437, 1378, 1339, 1314, 1288, 1212, 1170, 1147, 1114, 1082, 985, 904, 806, 786, 746, 704, 674 cm^{-1}; ^1H NMR ($CDCl_3$) Major δ = 1.44 (d, J = 6.71 Hz, 3H), 1.83 (s, 3H), 2.45 (s, 3H), 3.37 (s, 3H), 4.68 (q, J = 6.71 Hz, 1H), 5.01 (s, 1H), 6.24 (brs, 1H), 7.34 (d, J = 8.42 Hz, 2H), 7.74 (d, J = 8.42 Hz, 2H). Minor δ = 1.57 (d, J = 6.34 Hz, 3H), 1.87 (s, 3H), 2.45 (s, 3H), 3.31 (s, 3H), 4.45 (q, J = 6.34 Hz, 1H), 5.23 (s, 1H), 6.19 (brs, 1H), 7.34 (d, J = 8.42 Hz, 2H), 7.74 (d, J = 8.42 Hz, 2H). Found: C, 58.08; H, 6.17; N, 4.32%. Calcd for $C_{15}H_{19}NO_4S$: C, 58.23; H, 6.19; N, 4.53%.

5-Acetoxy-4-(1-bromoethyl)-5-(*t*-butoxycarbonyl)-1,5-dihydro-3-methyl-2*H*-pyrrol-2-one (16). A solution of **11a** (57 mg, 0.2 mmol) in 3 ml of dry benzene was added to a solution of NBS (43 mg, 0.24 mmol) in 3 ml of dry benzene at room temperature under a nitrogen atmosphere and allowed to stand overnight. After removal of the solvent, the residue was partitioned between ethyl acetate and water. The organic layer was successively washed with a saturated aqueous solution of $NaHSO_3$, $NaHCO_3$, and brine, and dried over Na_2SO_4, and concentrated in vacuo to afford **16** quantitatively as a mixture of diastereomers (ca. 1 : 1). A part of the product was recrystallized for elemental analysis. Mp 110—111 °C (from hexane); IR (KBr) 3355, 2992, 2932, 1741, 1442, 1370, 1291, 1257, 1243, 1156, 1120, 1048, 1032, 978, 840, 757 cm^{-1}; ^1H NMR ($CDCl_3$) Isomer-1 δ = 1.48 (s, 9H), 1.92 (d, J = 7.15 Hz, 3H), 2.02 (s, 3H), 2.13 (s, 3H), 5.04 (q, J = 7.15 Hz, 1H), 6.70 (brs, 1H). Isomer-2 δ = 1.49 (s, 9H), 1.96 (d, J = 7.15 Hz, 3H), 2.07 (s, 3H), 2.16 (s, 3H), 4.93 (q, J = 7.15 Hz, 1H), 6.80 (brs, 1H). Found: C, 46.14; H, 5.40; N, 4.06%. Calcd for $C_{14}H_{20}BrNO_5$: C, 46.42; H, 5.57; N, 3.87%.

5-*t*-Butoxycarbonyl-1,5-dihydro-5-methoxy-4-(1-methoxyethyl)-3-methyl-2*H*-pyrrol-2-one (17). To a solution of **16** (250 mg, 0.69 mmol) in 10 ml of MeOH was added sodium methoxide (3.1 equiv) at 0 °C under a nitrogen atmosphere and allowed to stand for 2 h at room temperature. After neutralization with 1 M (= mol dm^{-3}) HCl, the solvent was removed in vacuo. The resulting residue was taken up in ethyl acetate. The resulting organic layer was washed with brine and dried over Na_2SO_4. The filtrate was concentrated, and the residue was separated by a preparative TLC (SiO_2, hexane/EtOAc = 3/1, v/v) to give **17** in 53% yield (104 mg) as a colorless oily mixture of diastereomers (ca. 1 : 1). IR (neat) 3246, 2980, 2936, 2830, 1714, 1458, 1394, 1370, 1279, 1254, 1117, 999, 841, 821, 760 cm^{-1}; ^1H NMR ($CDCl_3$) Isomer-1 δ = 1.39 (d, J = 6.52 Hz, 3H), 1.48 (s, 9H), 1.97 (s, 3H), 3.25 (s, 3H), 3.29 (s, 3H), 4.24 (q, J = 6.42 Hz, 1H), 7.27 (brs, 1H). Isomer-2 δ = 1.41 (d, J = 6.52 Hz, 3H), 1.48 (s, 9H), 1.98 (s, 3H), 3.25 (s, 3H), 3.29 (s, 3H), 4.26 (q, J = 6.42 Hz, 1H), 7.39 (brs, 1H). MS m/z 285 (M^+; 0.19%), 254 (1.15), 227 (8.36), 198 (7.50), 184 (100), 168 (14.41), 152 (98.99), 124 (15.60), 120 (22.93), 92 (13.45), 83 (10.45), 67 (15.69), 59 (30.36), 57 (67.49).

Conversion of **17** to **6a** was carried out in a similar manner described above for the preparation of **8a**.

5-*t*-Butoxycarbonyl-1,5-dihydro-3-methyl-5-tosyl-4-(1-tosylethyl)-2*H*-pyrrol-2-one (18). A mixed suspension of **16** (36 mg, 0.1 mmol) and anhydrous sodium *p*-toluenesulfinate (89 mg, 0.5 mmol) in 5 ml of THF was refluxed for 1 h under nitrogen. After removal of the solvent, the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to afford **18** as a colorless solid in 94% yield (50 mg, single isomer). A part of the product was recrystallized for elemental analysis. Mp 135—136 °C (from EtOAc/hexane); IR (KBr) 3188, 3093, 2981, 1713, 1596, 1457, 1397, 1372, 1325, 1290, 1150, 1082, 1038, 817, 724, 707, 656 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.53 (s, 9H), 1.70 (d, J = 7.15 Hz, 3H), 2.07 (s, 3H), 2.45 (s, 3H), 2.48 (s, 3H), 5.31 (q, J = 7.15 Hz, 1H), 6.56 (brs, 1H), 7.33 (d, J = 8.25 Hz, 2H), 7.40 (d, J = 8.44 Hz, 2H), 7.67 (d, J = 8.44 Hz, 2H), 7.87 (d, J = 8.25 Hz, 2H). Found: C, 58.22; H, 6.15; N, 2.44%. Calcd for $C_{26}H_{31}NO_7S_2$: C, 58.52; H, 5.86; N, 2.62%.

1,5-Dihydro-3-methyl-5-tosyl-4-(1-tosylethyl)-2*H*-pyrrol-2-one (6b). To a solution of **18** (107 mg, 0.2 mmol) in 1 ml of CH_2Cl_2 was added dropwise 0.5 ml of TFA at 0 °C under a nitrogen atmosphere and kept at room temperature for 10 min. The solvent was then removed in vacuo and the residue was partitioned between ethyl acetate and water. The organic layer was washed with water, a saturated aqueous solution of $NaHCO_3$, and brine, and dried over Na_2SO_4. Evaporation of the solvent and separation of the residue with a preparative TLC (SiO_2, hexane/AcOEt = 2/1, v/v) gave 69 mg (80%) of **6b** as a colorless solid (single isomer). A part of the product was recrystallized for elemental analysis. Mp 170.5—172 °C (from EtOAc/hexane); IR (KBr) 3217, 3088, 2991, 2927, 2906, 1708, 1596, 1452, 1402, 1383, 1362, 1318, 1302, 1241, 1199, 1170, 1138, 1085, 1039, 820, 736, 707 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.64 (d, J = 7.20 Hz, 3H), 1.69 (s, 3H), 2.44 (s, 3H), 2.48 (s, 3H), 4.79 (q, J = 7.20 Hz, 1H), 5.12 (s, 1H), 6.36 (brs, 1H), 7.33 (d, J = 8.54 Hz, 2H), 7.39 (d, J = 8.54 Hz, 2H), 7.63 (d, J = 8.30 Hz, 2H), 7.73 (d, J = 8.30 Hz, 2H). Found: C, 58.17; H, 5.39; N, 3.00%. Calcd for $C_{21}H_{23}NO_5S_2$: C, 58.18; H, 5.35; N, 3.23%.

5-[4-(2-Allyloxycarbonylethyl)-5-*t*-butoxycarbonyl-3-methyl-2-pyrrolylmethylene]-1,5-dihydro-4-(1-methoxyethyl)-3-methyl-2*H*-pyrrol-2-one (19a). A solution of tributylphosphine (121 mg, 0.6 mmol) in 12 ml of dry CH_2Cl_2 was added to the suspention of ^{t}BuOK (34 mg, 0.3 mmol) and **7a** (80 mg, 0.25 mmol) in 3 ml of dry CH_2Cl_2 at −70 °C under a nitrogen atmosphere. Then, a solution of **6a** (108 mg, 0.35 mmol) in 3 ml of dry CH_2Cl_2 was added dropwise over the period of 30 min and the reaction mixture was kept at room temperature overnight. The product was extracted with ethyl acetate from the residue obtained by the removal of the solvent. The extract was successively washed with saturated aqueous solutions of $NaHCO_3$, $NaHSO_3$, and brine, and dried over Na_2SO_4. Evaporation of the solvent and separation of the residue with a preparative TLC (SiO_2, hexane/EtOAc = 2/1, v/v) afforded (*Z*)- and (*E*)-isomers of **19a** as yellow solids in 53% (58 mg) and 31% (34 mg) yields, respectively. The structure of (*Z*)-isomer of **19a** was confirmed by NOE measurement irradiating the proton at *meso*-position (MeC<u>H</u>(OMe) 6.67%; C<u>H</u>$_3$-py 6.01%) and also by elemental analysis after recrystallization.

19a (*Z*)-isomer: A yellow solid; mp 193—194 °C (from EtOAc/hexane); IR (KBr) 3408, 3130, 2978, 2932, 2820, 1735, 1677, 1449, 1367, 1276, 1162, 1115, 1052, 985, 849, 772 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.50 (d, J = 6.69 Hz, 3H), 1.56 (s, 9H), 2.03 (s, 3H), 2.09 (s, 3H), 2.55 (t, J = 8.11 Hz, 2H), 3.02 (t, J = 8.11 Hz, 2H), 3.30 (s, 3H), 4.53 (q, J = 6.69 Hz, 1H), 4.59 (dt, J = 1.28, 5.69 Hz, 2H), 5.22 (dq, J = 1.28, 10.45 Hz, 1H), 5.30 (dq, J = 1.28, 17.24 Hz, 1H), 5.90 (ddt, J = 5.69, 10.45, 17.24 Hz, 1H), 6.48 (s, 1H), 9.61 (brs, 1H), 9.80 (brs, 1H). Found: C, 65.25; H, 7.51; N, 6.04%. Calcd for $C_{25}H_{34}N_2O_6$: C, 65.50; H, 7.42; N, 6.11%.

(*E*)-isomer: A yellow solid (contaminated with a small amount of (*Z*)-isomer); ^1H NMR ($CDCl_3$) δ = 1.44 (d, J = 6.79 Hz, 3H), 1.58 (s, 9H), 2.06 (s, 3H), 2.10 (s, 3H), 2.57 (t, J = 7.15 Hz, 2H), 3.02 (t, J = 7.15 Hz, 2H), 3.42 (s, 3H), 4.58 (dt, J = 1.28, 5.69 Hz, 2H), 4.69 (q, J = 6.76 Hz, 1H), 5.21 (dq, J = 1.28, 10.45 Hz, 1H), 5.29 (dq, J = 1.28, 17.24 Hz, 1H), 5.90 (ddt, J = 5.69, 10.45, 17.24 Hz, 1H), 6.39 (s, 1H), 9.31 (brs, 1H), 12.12 (brs, 1H).

In a similar manner, **19b** was prepared.

5-[4-(2-Allyloxycarbonylethyl)-5-*t*-butoxycarbonyl-3-methyl-2-pyrrolylmethylene]-1,5-dihydro-3-methyl-4-(1-tosylethyl)-2*H*-pyrrol-2-one (19b). (*Z*)-isomer: A yellow solid; mp 158.5—160.0 °C (from EtOAc/hexane); IR (KBr) 3322, 2977, 2930, 1734, 1697, 1671, 1598, 1453, 1368, 1316, 1276, 1163, 1147, 1082, 1037, 1018, 991, 848, 815, 731, 708, 671 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.56 (s, 9H), 1.77 (br, 3H), 1.88 (d, J = 7.34 Hz, 3H), 2.05 (brs, 3H), 2.33 (brs, 3H), 2.53 (t, J = 7.89 Hz, 2H), 3.00 (t, J = 7.89 Hz, 2H), 4.36 (br, 1H), 4.60 (dt, J = 1.28, 5.87 Hz, 2H), 5.24 (dq, J = 1.28, 10.27 Hz, 1H), 5.32 (dq, J = 1.28, 17.24 Hz, 1H), 5.55 (br, 1H), 5.93 (ddt, J = 5.87, 10.27, 17.24 Hz, 1H), 7.24 (d, J = 8.25 Hz, 2H), 7.65 (d, J = 8.25 Hz, 2H), 10.17 (br, 2H). Found: C, 63.88; H, 6.59; N, 4.55%. Calcd for $C_{31}H_{38}N_2O_7S$: C, 63.90; H, 6.57; N, 4.81%.

(*E*)-isomer: A yellow solid (contaminated with a small amount of (*Z*)-isomer); ^1H NMR ($CDCl_3$) δ = 1.52 (d, J = 6.97 Hz, 3H), 1.59 (s, 9H), 1.84 (s, 3H), 2.19 (s, 3H), 2.39 (s, 3H), 2.31—2.50 (m, 2H), 2.90 (m, 2H), 4.40—4.55 (m, 3H), 5.20 (dq, J = 1.28, 10.45 Hz, 1H), 5.26 (dq, J = 1.28, 17.24 Hz, 1H), 5.93 (ddt, J = 5.69, 10.45, 17.24 Hz, 1H), 6.04 (s, 1H), 7.23 (d, J = 8.25 Hz, 2H), 7.42 (d, J = 8.25 Hz, 2H), 9.15 (brs, 1H), 10.25 (brs, 1H).

(4*E*,5*Z*)-5-[4-(2-Allyloxycarbonylethyl)-5-*t*-butoxycarbonyl-3-methyl-2-pyrrolylmethylene]-4-ethylidene-3-methylpyrrolidin-2-one (4a). Method 1: A solution of **19a** (52 mg, 0.12 mmol) in 3 ml of THF/H_2O (10/1, v/v) was treated with aluminum amalgam (10 mg, 0.36 mmol), and the resulting suspention was stirred for 2 h at room temperature. The reaction mixture was filtered through celite and the filtrate was concentrated. The resulting residue was partitioned between ethyl acetate and water, and the organic layer was washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the resulting crude product **20a**[12] (52 mg, 0.12 mmol) was dissolved in 2 ml of dry CH_2Cl_2, and a solution of pyridinium *p*-toluenesulfonate (3 mg, 0.012 mmol) in 2 ml of dry CH_2Cl_2 was added at 0 °C under a nitrogen atmosphere. After stirring for 30 min at room temperature, the solvent was removed in vacuo and the resulting residue was partitioned between ethyl acetate and water. The organic layer was washed with brine and dried over Na_2SO_4. After concentration, the residue was separated by a preparative TLC (SiO_2, hexane/EtOAc = 2/1, v/v) to afford the desired product **4a** (37 mg) in 72% yield.

Method 2: A solution of **19b** (63 mg, 0.12 mmol) in 3 ml of THF/H_2O (10/1, v/v) was treated with aluminum amalgam (10 mg, 0.36 mmol) and the resulting suspention was stirred for 2 h at room temperature. The reaction mixture was filtered through celite and the filtrate was concentrated. The resulting residue was partitioned between ethyl acetate and water and the organic layer was washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the resulting crude product **20b**[12] (63 mg, 0.12 mmol) was dissolved in 2 ml of dry CH_2Cl_2, and a solution of DBU (36 mg, 0.24 mmol)

in 2 ml of dry CH_2Cl_2 was added at 0 °C under nitrogen. After stirring for 30 min at room temperature, the solvent was removed in vacuo and the resulting residue was partitioned between ethyl acetate and water. The organic layer was washed with brine and dried over Na_2SO_4. After concentration, the residue was separated by a preparative TLC (SiO_2, hexane/EtOAc = 2/1, v/v) to afford the desired product **4a** (33 mg) in 68% yield. **4a**: (*Z*)-form; mp 112.0—113.5 °C (from cyclohexane/hexane); IR (KBr) 3369, 3169, 2979, 2928, 2884, 1732, 1699, 1681, 1640, 1442, 1364, 1318, 1278, 1250, 1156, 1127, 1053, 989, 934, 772, 713 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.39 (d, *J* = 7.43 Hz, 3H), 1.55 (s, 9H), 1.85 (d, *J* = 7.15 Hz, 3H), 1.98 (s, 3H), 2.54 (t, *J* = 8.07 Hz, 2H), 3.00 (t, *J* = 8.07 Hz, 2H), 3.23 (q, *J* = 7.43 Hz, 1H), 4.59 (dt, *J* = 1.23, 5.69 Hz, 2H), 5.23 (dq, *J* = 1.23, 10.27 Hz, 1H), 5.30 (dq, *J* = 1.23, 17.06 Hz, 1H), 5.68 (s, 1H), 5.92 (ddt, *J* = 5.69, 10.27, 17.06 Hz, 1H), 6.18 (dq, *J* = 2.30, 7.15 Hz, 1H), 8.20 (brs, 1H), 8.85 (brs, 1H). The structure of (4*E*,5*Z*)-form was confirmed by measurement of NOE as shown in Scheme 5. 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%.

Diallyl (3*E*,4*Z*,10*Z*,15*Z*)-18-Ethyl-3-ethylidene-1,2,3,19,22,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo-21*H*-biline-8,12-dipropanoate (Phycocyanobilin Diallyl Ester) (23a). Compound **4a** (50 mg, 0.116 mmol) was dissolved in 0.5 ml of trifluoroacetic acid at room temperature under a nitrogen atmosphere and was kept for 1 h. The solvent was removed in vacuo and 2 ml of ethanol was added to the resulting crude **22**. Then a solution of **21a** (38 mg, 0.105 mmol) in 2 ml of ethanol was added, followed by the addition of 1 drop of concd H_2SO_4. The resulting mixture was kept at room temperature for 2 h. The mixture was treated with a few drops of a saturated aqueous solution of $NaHCO_3$, and the solvent was removed in vacuo. The residue was taken up in ethyl acetate and the organic layer was successively washed with water, a saturated aqueous $NaHCO_3$, and brine, and dried over Na_2SO_4. Evaporation of the solvent and separation of the residue by a preparative TLC (SiO_2, hexane/EtOAc/EtOH = 20/5/1, v/v/v) afforded the desired product **23a** (25 mg, 36%) as a greenish blue color solid. **23a**: Mp above 230 °C (from methanol/hexane); IR (KBr) 3200, 2960, 2928, 2876, 2852, 1732, 1697, 1617, 1590, 1455, 1374, 1273, 1242, 1223, 1165, 987, 962, 932, 804, 740 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.10 (t, *J* = 7.52 Hz, 3H), 1.33 (d, *J* = 7.52 Hz, 3H), 1.85 (d, *J* = 6.79 Hz, 3H), 2.03 (s, 3H), 2.12 (s, 3H), 2.13 (s, 3H), 2.33 (q, *J* = 7.52 Hz, 2H), 2.57 (m, 4H), 2.90 (t, *J* = 7.70 Hz, 2H), 2.95 (t, *J* = 7.52 Hz, 2H), 3.13 (q, *J* = 7.52 Hz, 1H), 4.56—4.58 (m, 4H), 5.19—5.32 (m, 4H), 5.78 (s, 1H), 5.83—5.97 (m, 2H), 5.99 (s, 1H), 6.34 (dq, *J* = 2.20, 7.52 Hz, 1H), 6.64 (s, 1H), 9.67 (brs, 1H), 10.72 (brs, 1H). Another NH was not observed clearly. Stereochemistry was confirmed by NOESY measurement. HRMS (FAB) (M^++1), Found: *m/z* 667.3501. Calcd for $C_{39}H_{47}N_4O_6$: M, 667.3496.

Diallyl (3*E*,4*Z*,10*Z*,15*Z*)-18-Ethyl-3-ethylidene-17-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}-1,2,3,19,21,24-hexahydro-2,7,13-trimethyl-1,19-dioxo-21*H*-biline-8,12-dipropanoate (23b). Prepared in a similar manner described above for **23a** using CH_3SO_3H as an acid catalyst instead of concd H_2SO_4 from **22** and **21b** in MeOH/CH_2Cl_2 (6/1, v/v) for 2 h at room temperature. Mp above 230 °C (from methanol/hexane); IR (KBr) ca. 3200, 2968, 2932, 2876, 2856, 1736, 1676, 1616, 1588, 1454, 1412, 1377, 1343, 1275, 1230, 1183, 1156, 1099, 1051, 938, 829, 688 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.08 (t, *J* = 7.34 Hz, 3H), 1.31 (d, *J* = 7.34 Hz, 3H), 1.82 (d, *J* = 7.34 Hz, 3H), 1.97 (s, 3H), 2.02 (s, 3H), 2.29 (m, 2H), 2.26 (m, 4H), 2.53—2.60 (m, 4H), 2.91 (m, 4H), 3.11 (q, *J* = 6.97 Hz, 1H), 4.55—4.59 (m, 4H), 5.70 (s, 1H), 5.18—5.33 (m, 4H), 5.79 (s, 1H), 5.81—5.98 (m, 4H), 6.35 (q, *J* = 7.34 Hz, 1H), 6.64 (s, 1H), 7.30 (d, *J* = 8.44 Hz, 2H), 7.48 (d, *J* = 8.44 Hz, 2H), 10.35 (brs, 1H), 11.35 (brs, 1H). Stereochemistry was confirmed by NOESY measurement. HRMS (FAB) (M^++1), Found: *m/z* 837.3577. Calcd for $C_{46}H_{48}F_3N_6O_6$: M, 837.3541.

(3*E*, 4*Z*, 10*Z*, 15*Z*)- 18- Ethyl- 3- ethylidene- 1, 2, 3, 19, 22, 24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo-21*H*-biline-8,12-dipropanoic Acid (Phycocyanobilin) (1). To a mixed solution of **23a** (20 mg, 0.03 mmol) and $[Pd(PPh_3)_4]$ (7 mg, 0.006 mmol) in 5 ml of dry THF, a solution of morpholine (26 mg, 0.3 mmol) in 1 ml of THF was added at room temperature under a nitrogen atmosphere. The resulting mixture was stirred for 1 h at room temperature. Evaporation of the solvent and separation of the residue by successive column chromatography [$CHCl_3$/MeOH/AcOH = 200/15/1 (v/v/v), then with EtOAc/MeOH/AcOH/TFA = 200/15/0.38/0.27 (v/v/v/v)] afforded the desired product **1** (17 mg, 96%) as a greenish blue solid. Mp above 300 °C (from EtOAc/hexane); IR (KBr) 3417, 3260, 2969, 2932, 2873, 1694, 1597, 1539, 1455, 1396, 1279, 1236, 1210, 1159, 1110, 1066, 1040, 964, 896, 744, 688 cm^{-1}; ^1H NMR (pyridine-d_5) δ = 1.26 (t, *J* = 7.56 Hz, 3H), 1.50 (d, *J* = 7.56 Hz, 3H), 1.72 (d, *J* = 7.32 Hz, 3H), 2.03 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 2.44—2.58 (m, 2H), 2.86 (t, *J* = 7.32 Hz, 2H), 2.88 (t, *J* = 6.95 Hz, 2H), 3.12 (t, *J* = 7.32 Hz, 2H), 3.21 (t, *J* = 6.95 Hz, 2H), 3.37 (brq, *J* = 7.32 Hz, 1H), 5.87 (s, 1H), 6.09 (s, 1H), 6.34 (dq, *J* = 2.68, 7.32 Hz, 1H), 7.29 (s, 1H). Stereochemistry was confirmed by NOESY measurement. UV/vis (MeOH) λ_{max} 364 (ε = 48000), 621 (ε = 16000) nm. HRMS (FAB) (M^++1), Found: *m/z* 587.2873. Calcd for $C_{33}H_{39}N_4O_6$: M, 587.2870.

In a similar manner **3** was prepared.

(3*E*,4*Z*,10*Z*,15*Z*)-18-Ethyl-3-ethylidene-17-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}-1,2,3,19,21,24-hexahydro-2,7,13-trimethyl-1,19-dioxo-21*H*-biline-8,12-dipropanoic Acid (3). Mp (decomp) above 230 °C (from $CHCl_3$/hexane); IR (KBr) 3400, 3208, 2920, 2851, 1702, 1613, 1589, 1456, 1441, 1408, 1385, 1343, 1312, 1231, 1186, 1156, 1050, 938, 829, 746, 693 cm^{-1}; ^1H NMR (pyridine-d_5) δ = 1.27 (t, *J* = 7.44 Hz, 3H), 1.50 (d, *J* = 7.32 Hz, 3H), 1.73 (d, *J* = 7.32 Hz, 3H), 1.91 (s, 3H), 1.96 (s, 3H), 2.46—2.59 (m, 2H), 2.78 (brt, 2H), 2.80 (brt, 2H), 3.07 (brt, 2H), 3.14 (brt, 2H), 3.36 (q, *J* = 7.07 Hz, 1H), 5.84 (s, 1H), 5.87 (s, 1H), 6.34 (dq, *J* = 1.95, 7.32 Hz, 1H), 7.38 (d, *J* = 8.05 Hz, 2H), 7.24 (s, 1H), 7.74 (d, *J* = 8.05 Hz, 2H). Stereochemistry was confirmed by NOESY measurement. UV/vis (MeOH) λ_{max} 369 (ε = 32000), 628 (ε = 13000) nm. HRMS (FAB) (M^++1), Found: *m/z* 757.2969. Calcd for $C_{40}H_{40}F_3N_6O_6$: M, 757.2961.

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12 Compound **20a** was isolable by TLC as a mixture of diastereomers, but not **20b**. They were used without purification for the next reaction. Their spectral data are given in the following. **20a** (An oily mixture of two diastereomers. Major/Minor = ca. 2/1): IR (neat) 3287, 3090, 2976, 2931, 1694, 1448, 1368, 1279, 1169, 1138, 1116, 1053, 990, 848, 777, 756 cm^{-1}; ^1H NMR ($CDCl_3$) Major δ = 1.36 (d, J = 7.65 Hz, 3H), 1.37 (d, J = 6.42 Hz, 3H), 1.53 (s, 9H), 1.98 (s, 3H), 2.53 (t, J = 8.02 Hz, 2H), 2.99 (t, J = 8.02 Hz, 2H), 3.10 (q, J = 7.65 Hz, 1H), 3.29 (s, 3H), 3.52—3.69 (m, 2H), 4.21 (q, J = 6.42 Hz, 1H), 4.58 (dt, J = 1.28, 5.69 Hz, 2H), 5.22 (dq, J = 1.28, 10.27 Hz, 1H), 5.29 (dq, J = 1.28, 17.24 Hz, 1H), 5.90 (ddt, J = 5.69, 10.27, 17.24 Hz, 1H), 7.96 (brs, 1H), 9.55 (brs, 1H). Minor δ = 1.35 (d, J = 7.65 Hz, 3H), 1.37 (d, J = 6.42 Hz, 3H), 1.53 (s, 9H), 1.99 (s, 3H), 2.53 (t, J = 8.02 Hz, 2H), 2.99 (t, J = 8.02 Hz, 2H), 3.04 (q, J = 7.65 Hz, 1H), 3.34 (s, 3H), 3.52—3.69 (m, 2H), 4.04 (q, J = 6.42 Hz, 1H), 4.58 (dt, J = 1.28, 5.69 Hz, 2H), 5.22 (dq, J = 1.28, 10.27 Hz, 1H), 5.29 (dq, J = 1.28, 17.24 Hz, 1H), 5.90 (ddt, J = 5.69, 10.27, 17.24 Hz, 1H), 7.81 (brs, 1H), 9.48 (brs, 1H). HRMS (EI) (M^+), Found: *m/z* 460.2578. Calcd for $C_{25}H_{36}N_2O_6$: M, 460.2573.

20b [An oily mixture of two diastereomers. Major/Minor = ca. 1/1 (estimated from ^1H NMR spectrum of the crude product)]: IR (crude, neat) 3341, 2978, 2931, 1711, 1686, 1449, 1368, 1288, 1144, 1050, 756 cm^{-1}; ^1H NMR spectrum was not clear enough for full assignment. HRMS (FAB) (M^++1), Found: *m/z* 585.2636. Calcd for $C_{31}H_{41}N_2O_7S$: M, 585.2637.