Regular Article

Chemical Synthetic Platform for Chlorpromazine Oligomers That Were Reported as Photo-degradation Products of Chlorpromazine

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A synthetic platform for chlorpromazine (CPZ) oligomers, which could be generated *via* photo-reaction of CPZ, is essential to promote their biological and structural studies. In this paper, the first synthetic platform for CPZ oligomers is described. A photo-irradiation experiment of CPZ to confirm whether the structure of the CPZ dimer generated by the photo-irradiation was identical to that prepared by our synthetic method is also reported.

Key words chlorpromazine (CPZ); chlorpromazine dimer; chlorpromazine trimer; chlorpromazine oligomer; photo-reaction; photosensitization

Chlorpromazine (CPZ) **1** (n=1) is the first synthetic antipsychotic drug employed for treatment of mental disorders[1,2] (Fig. 1). It was first developed by Charpentier *et al.* as an antihistaminic agent,[3] and its potency in psychiatric treatment was then reported by Laborit *et al.* in 1952.[4] Schizophrenia is one of the major diseases treatable by CPZ, and its mechanism of action is thought to be antagonistic effect on the dopamine D2 receptor.[5] As CPZ was widely used, its photo-toxic and photo-allergic adverse effects were also reported[6]; therefore, its photo-degradation reaction has been intensely studied.[7–11] In the previous studies, the main focus was on structures and bioactivities of monomeric derivatives that were generated *via* photo-irradiation of CPZ. However, bioactivities of several photo-generated CPZ oligomers have also been shown so far. The oligomers were first reported as potential causative agents of unfavorable effects such as hemolysis and inflammation.[12–14] A beneficial bioactivity, which could be an alternative action of mechanism, was recently clarified by Fukui and colleagues.[15] They have been studying on human D-amino acid oxidase (hDAO) that is a potential risk factor for schizophrenia and is involved in glutamate-mediated neurotransmission. Because antipsychotic drugs could affect not only dopaminergic but also glutamatergic neurotransmission,[16] they focused on hDAO-inhibitory activity of CPZ and its photo-generated oligomers. In their paper, photo-generation of CPZ dimer, trimer and tetramer was confirmed and the high activity of the trimer was unveiled. The trimer was therefore suggested as a potential active substance contributing to the therapeutic effect of CPZ. A proposed structure of the oligomers is shown in Fig. 1.[8,15] Huang and Sands carried out several experiments to determine the structure of the oligomer, but a position of C–C bond formation was proposed not based on experimental results, but based on speculation of a reaction mechanism.[8] Fukui's group attempted to clarify the structure using NMR, but the effort was hampered by broadening of the peaks.[15] Therefore, there is no experimental evidence that supports the position of the C–C bond formation. To promote biological and structural studies on the oligomers, we considered that constant supply of the structurally defined oligomers is essential. In this paper, development of a synthetic platform for the CPZ oligomers **1**, the structure had been proposed,[8,15] is first reported. Whether dimer **1** (n=2) could be generated by UV-induced photo-degradation of CPZ monomer **1** (n=1) is also described.

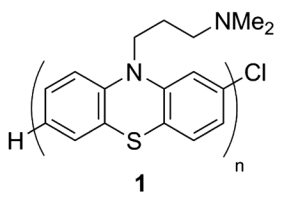

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Fig. 1. Structure of CPZ (n=1) and Its Oligomers (n>1)

Results and Discussion

A synthetic strategy for the preparation of CPZ oligomer **1** is shown in Chart 1. In this study, not dimethylamino derivatives, but *tert*-butoxycarbonyl (Boc)-protected building blocks **2** and **3** were employed to facilitate purification of synthetic intermediates by standard normal phase column chromatography. The synthetic platform is as follows: Step 1) replacement of chlorine with boron; Step 2) coupling of the obtained boron derivative with aryl bromide **3**; Step 3) removal of Boc groups; Step 4) methylation of the generated secondary amines. This platform should enable synthesis of CPZ oligomer **1** by repetition of Steps 1 and 2.

We first attempted to synthesize building blocks **2** (Chart 2). Starting from chlorophenothiazine **4**, a chloropropyl unit was introduced in accordance with a report, with slight modification.[17] Treatment of chloride **5** with NaI followed by substitution by methylamine and subsequent Boc protection generated building block **2**. Preparation of brominated building block **3** was next attempted (Chart 3). Regioselective bromination of **4** with *N*-bromosuccinimide (NBS)[18] afforded a brominated phenothiazine, and it was converted to **3** similarly as conversion of **4** to **2** in Chart 2. Dimer **1** (n=2) was then synthesized as follows (Chart 4): Chlorine of **2** was replaced by a pinacol borane in the presence of palladium catalyst and 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) to generate **7**.[19] Suzuki–Miyaura coupling[20,21] of boronate **7**

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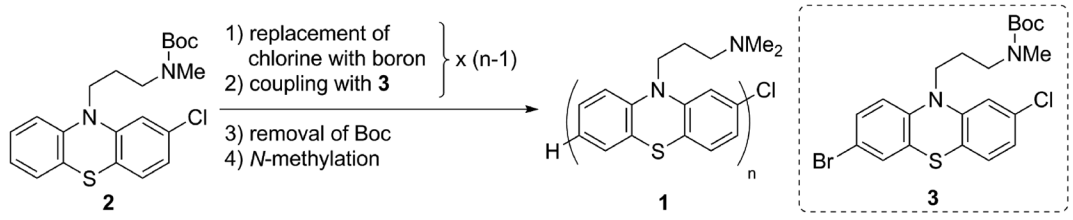
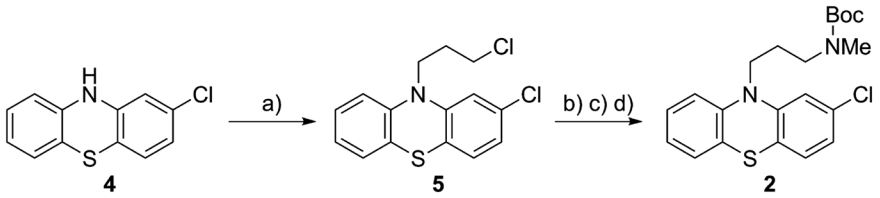
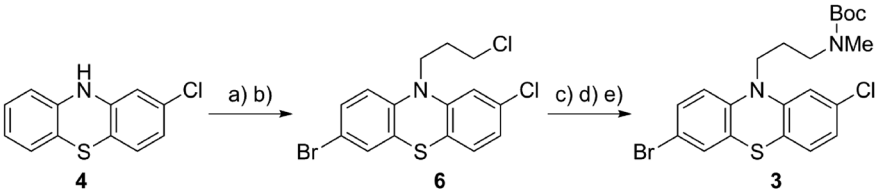


Chart 1. Synthetic Strategy for CPZ Oligomers



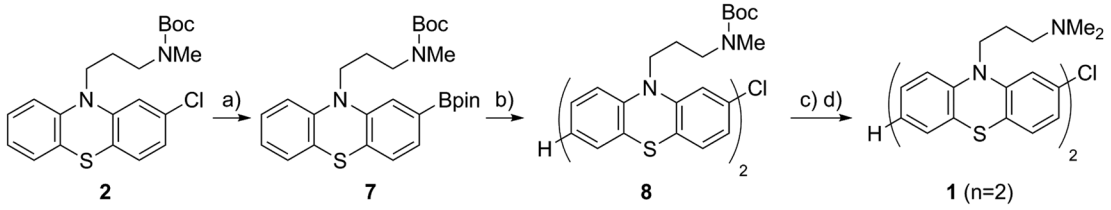
Reagents and conditions: a) NaH, $Br(CH_2)_3Cl$, DMF, 93%; b) NaI, acetone, reflux; c) H_2NMe/MeOH, THF; d) Boc_2O, Et_3N, CH_2Cl_2, 89% (3 steps).

Chart 2. Synthesis of Intermediate **2**



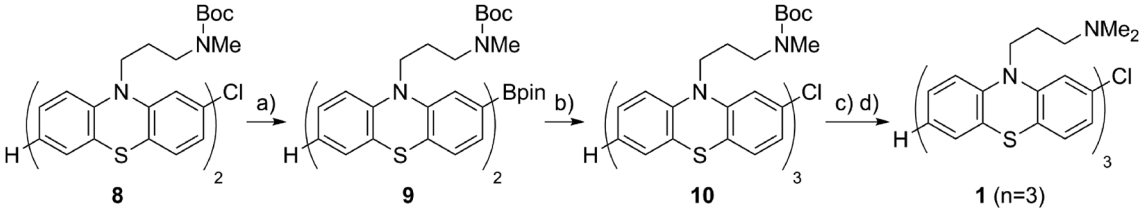
Reagents and conditions: a) NBS, THF; b) NaH, $Br(CH_2)_3Cl$, DMF, 59% (2 steps); c) NaI, acetone, reflux; d) H_2NMe/MeOH, THF; e) Boc_2O, Et_3N, CH_2Cl_2, 77% (3 steps).

Chart 3. Synthesis of Intermediate **3**



Reagents and conditions: a) bis(pinacol)diboron, $Pd(OAc)_2$, XPhos, KOAc, 1,4-dioxane, reflux, 90%; b) **3**, $Pd(PPh_3)_4$, K_2CO_3, 1,2-dimethoxyethane (DME), H_2O, reflux, 88%; c) HCl/1,4-dioxane; d) formalin, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 88% (2 steps).

Chart 4. Synthesis of CPZ Dimer **1** (n=2)



Reagents and conditions: a) bis(pinacol)diboron, $Pd(OAc)_2$, XPhos, KOAc, 1,4-dioxane, reflux, 87%; b) **3**, $Pd(PPh_3)_4$, $NaHCO_3$, DME, H_2O, reflux, 82%; c) HCl/1,4-dioxane; d) formalin, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 87% (2 steps).

Chart 5. Synthesis of CPZ Trimer **1** (n=3)

and bromide **3**[22] followed by removal of the Boc groups under acidic conditions and subsequent reductive methylation of the generated secondary amines then successfully afforded CPZ dimer **1** (n=2). To demonstrate practicality of our synthetic platform, the synthetic protocol was applied to preparation of CPZ trimer **1** (n=3) (Chart 5). Following the substitution of chlorine of dimer **8** to pinacol borane, product **9** was coupled with bromide **3** and generated trimer **10** was converted to desired **1** (n=3) employing a procedure similar to that for the dimer.[23] These results clearly demonstrate that our synthetic platform enables facile access to the CPZ oligomers.

Finally, we examined whether photo-degradation of CPZ monomer generates dimer **1** (n=2). CPZ monomer **1** (n=1) dissolved in water was irradiated with UV for 2.5 h at room tem-

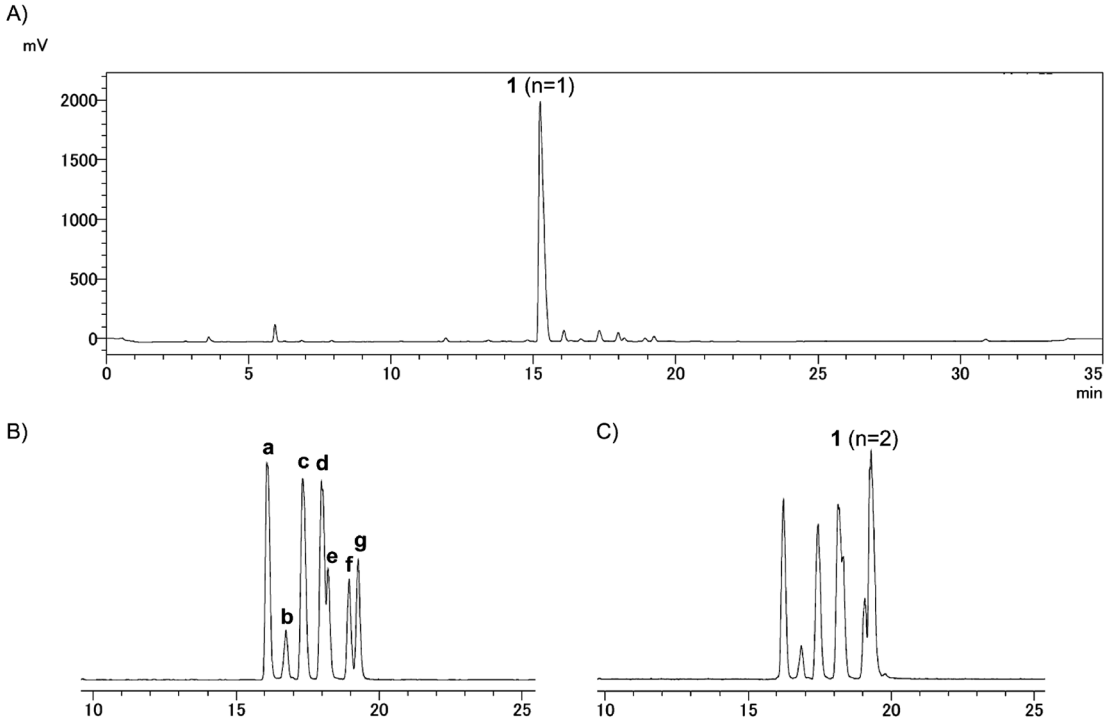


Fig. 2. LC/MS Profiles of a Mixture Obtained after UV-Irradiation of CPZ Monomer **1** (n=1)

Details of reaction conditions and LC/MS analysis are shown in Experimental. A) The photo-irradiation products detected by UV absorption at 250 nm; B) and C) The photo-irradiation products B) and its mixture with chemically synthesized dimer **1** (n=2) C) detected by MS (m/z=601, corresponding to [**1** (n=2)+H]$^+$). The retention time region in which peaks were detected is enlarged.

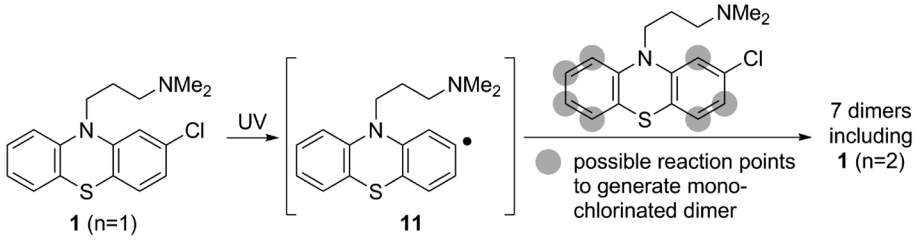


Chart 6. Photo-Induced Formation of Monochlorinated CPZ Dimer

perature. During the reaction, color of the reaction mixture was changed from colorless to brown. The obtained mixture was then analyzed using LC/MS (Fig. 2). Whereas substrate **1** (n=1) remained as the major component (Fig. 2A), generation of dimers was observed by detection using MS (m/z=601, corresponding to [**1** (n=2)+H]$^+$) (Fig. 2B). Unexpectedly, the masses of seven peaks identical to that of **1** (n=2) were detected (peaks **a** to **g** in Fig. 2B). Because CPZ has seven possible reactive points to react with photo-generated dechlorinated radical **11**[10,24] to generate the monochlorinated dimers (Chart 6), we speculated that radical **11** reacted with CPZ with low regioselectivity to generate all seven isomers. Finally, co-injection of the photo-degradation products with chemically synthesized dimer **1** (n=2) clarified that **1** (n=2) is one of the non-major photo-products corresponding to the peak **g** (Fig. 2C).

Conclusion

A chemical synthetic platform for preparation of CPZ oligomers **1**, which were reported as photo-degradation products of CPZ, was established. It was then unexpectedly clarified that the photo-generated monochlorinated CPZ dimer is not only **1** (n=2), but also a mixture of its isomers. This suggests that biological studies of the CPZ oligomers examined so far employed mixtures of the isomers; therefore, synthetic platforms, including ours reported in this paper, for preparation of each isomer should contribute to further biological and pharmacological study of photo-generated CPZ oligomers to elucidate which isomers are really responsible for the bioactivity and photo-toxicity.

Experimental

General Methods All reactions were carried out under a positive pressure of argon at room temperature unless otherwise noted. For column chromatography, silica Gel 60 N (spherical, neutral, Kanto Chemical Co., Inc., Japan) was employed. TLC was performed on precoated plates (0.25 nm, silica gel Merck Kiesegel 60F245). NMR spectra were recorded using a Bruker AV400N at 400 MHz frequency for ^{1}H and a JEOL JNM-AL300 at 300 or 75 MHz frequency for ^{1}H or ^{13}C, respectively. Chemical shifts are calibrated to the solvent signal. A Waters MICROMASS® LCT PRIMETM (electro-

spray ionization-time-of-flight (ESI-TOF)) was employed for measurement of high resolution (HR) mass spectra.[25] IR spectra were measured using a JASCO FT-IR 6200. Melting point was obtained on MICRO MELTING POINT APPARATUS (YANAGIMOTO, Japan) and was uncorrected. Elemental analysis was performed by CHN-CORDER (YANAGIMOTO). VL-30L (VILBER LOURMAT, 2×15 W, power=60 W, 365 nm tube) was employed for UV-irradiation experiment because UVA was widely used for photo-degradation of CPZ.[7–14] For LC/MS analysis (Shimadzu, Japan, Prominence-I LC-2030, LCMS-2020), a Cosmosil $5C_{18}$-AR-II analytical column (Nacalai Tesque, Japan, 4.6×250 mm, flow rate 1 mL min^{-1}) was employed, and eluting products were detected by UV at 250 nm and MS. A solvent system consisting of 0.1% (v/v) aq. trifluoroacetic acid (TFA) (solvent A) and 0.1% (v/v) TFA in MeCN (solvent B) was used for elution (flow rate: 1 mL/min; gradient of solvent A in solvent B: 30 to 65% over 30 min).

2-Chloro-10-(3-chloropropyl)-10*H*-phenothiazine (5) To a suspension of sodium hydride (NaH) (55% (w/w)), 542 mg, 12.4 mmol) in *N*,*N*-dimethylformamide (DMF) (4.1 mL) were added chlorophenothiazine **4** (1.00 g, 4.28 mmol) and 1-bromo-3-chloropropane (890 μL, 8.99 mmol) at 0°C, and the obtained suspension was stirred at room temperature for 2 h. After quenching by the addition of brine at 0°C, the mixture was extracted with Et_2O. Following to washing with water (3 times), the combined organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (hexane–EtOAc=100 : 1 (v/v)), and alkylated product **5** (1.23 g, 3.96 mmol, 93%) was obtained as pale yellow oil: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.20 (2H, quint, J=6.3 Hz), 3.63 (2H, t, J=6.3 Hz), 4.02 (2H, t, J=6.3 Hz), 6.84 (1H, d, J=1.8 Hz), 6.88–6.91 (2H, m), 6.94 (1H, td, J=7.5 and 1.2 Hz), 7.02 (1H, d, J=8.0 Hz), 7.12–7.19 (2H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 29.4, 42.2, 44.0, 115.8, 115.9, 122.5, 123.4, 124.1, 125.3, 127.5, 127.6, 128.0, 133.3, 144.2, 146.3; HR-MS ESI-TOF *m*/*z*: Calcd for $C_{15}H_{13}Cl_2NS$ (M^+) 309.0146. Found 309.0166; IR (neat) 749, 803, 854, 914, 1039, 1098, 1127, 1247, 1281, 1408, 1456, 1567, 1591, 2864, 2928, 2957, 3060 cm^{-1}.

***tert*-Butyl [3-(2-Chloro-10*H*-phenothiazin-10-yl)propyl]-(methyl)carbamate (2)** Chloride **5** (1.23 g, 3.96 mmol) and NaI (5.94 g, 39.6 mmol) in acetone (15 mL) were refluxed overnight. After addition of water, the reaction mixture was extracted with Et_2O. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was dissolved in tetrahydrofuran (THF) (5.3 mL), and methylamine in MeOH (40% (w/v), 10.6 mL, 137 mmol) was added to the THF solution. After stirring for 2 d, solvent was evaporated. To the resulting residue was added sat. aq. $NaHCO_3$, and the obtained mixture was extracted with EtOAc. Following to washing with brine, the combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. To the obtained residue in CH_2Cl_2 (14.3 mL) were added Et_3N (1.66 mL, 11.9 mmol) and Boc_2O (1.00 mL, 4.35 mmol) at 0°C, and the mixture was stirred at room temperature overnight. After addition of sat. aq. NH_3 followed by stirring for 30 min, water was added to the reaction mixture and it was extracted with CH_2Cl_2. The combined organic layer was washed with 5% (w/v) aq. $KHSO_4$ followed by brine. Then the resulting organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (hexane–EtOAc=70 : 1 then 10 : 1 (v/v)) and product **2** (1.43 g, 3.53 mmol, 89% over 3 steps) was obtained as a white solid: mp 88°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.41 (9H, br s), 2.01 (2H, quint, J=6.8 Hz), 2.79 (3H, s), 3.33 (2H, br s), 3.85 (2H, t, J=6.8 Hz), 6.82 (1H, d, J=2.0 Hz), 6.86 (1H, d, J=8.0 Hz), 6.89 (1H, dd, J=10.0, 1.8 Hz), 6.94 (1H, t, J=7.5 Hz), 7.03 (1H, d, J=8.0 Hz), 7.12–7.19 (2H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz, 50°C) δ: 25.5, 28.4, 34.6, 44.8, 46.5 (br), 79.4, 115.8, 115.9, 122.4, 123.0, 124.0 (br), 125.3 (br), 127.4, 127.6, 127.9, 133.3, 144.5, 146.5, 155.8 (br); *Anal.* Calcd for $C_{21}H_{25}ClN_2O_2S$: C, 62.29; H, 6.22; N, 6.92. Found: C, 62.10; H, 6.18; N, 6.80; IR (neat) 748, 1149, 1247, 1365, 1392, 1456, 1567, 1592, 1691, 2932, 2975 cm^{-1}.

7-Bromo-2-chloro-10-(3-chloropropyl)-10*H*-phenothiazine (6) NBS (4.12 g, 23.1 mmol) in THF (43.0 mL) was added to chlorophenothiazine **4** (5.00 g, 21.4 mmol) in THF (5.00 mL) at 0°C slowly, and the resulting mixture was stirred at room temperature for 3.5 h. To the mixture was added $Na_2S_2O_3$ until color of the mixture changes from green to yellow, and the resulting suspension was extracted with EtOAc after addition of water. The combined organic layer was washed with water (3 times) followed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The obtained solid was washed with water and then dissolved in EtOAc. The solution was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give 6.38 g of a crude brominated product. The obtained crude material (6.38 g, 20.4 mmol) was alkylated as similar to **4** in Chart 2, and product **6** (4.93 g, 12.7 mmol, 59% over 2 steps) was obtained as colorless oil: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.18 (2H, quint, J=6.3 Hz), 3.63 (2H, t, J=6.3 Hz), 3.99 (2H, t, J=6.3 Hz), 6.72 (1H, d, J=8.3 Hz), 6.84 (1H, d, J=2.0 Hz), 6.90 (1H, dd, J=8.3, 2.0 Hz), 7.01 (1H, d, J=8.0 Hz), 7.22–7.27 (2H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 29.2, 42.0, 44.0, 115.3, 116.0, 116.9, 122.9, 123.3, 127.6, 128.1, 129.9, 130.1, 133.5, 143.5, 145.9; HR-MS (ESI-TOF) *m*/*z*: Calcd for $C_{15}H_{12}BrCl_2NS$ (M^+) 386.9251. Found 386.9257; IR (neat) 754, 807, 852, 915, 1108, 1132, 1245, 1280, 1412, 1455, 1563, 1587, 2867, 2958, 3062 cm^{-1}.

***tert*-Butyl [3-(7-Bromo-2-chloro-10*H*-phenothiazin-10-yl)propyl](methyl)carbamate (3)** Substrate **6** (816 mg, 2.10 mmol) was converted to **3** as similar to conversion of **5** to **2**, and product **3** (782 mg, 1.62 mmol, 77% over 3 steps) was obtained as white amorphousness: ^{1}H-NMR ($CDCl_3$, 300 MHz, 50°C) δ: 1.39 (9H, s), 1.93 (2H, quint, J=6.8 Hz), 2.75 (3H, s), 3.27 (2H, t, J=6.8 Hz), 3.74 (2H, t, J=6.8 Hz), 6.61 (1H, d, J=8.4 Hz), 6.75 (1H, d, J=2.0 Hz), 6.82 (1H, dd, J=8.0, 1.8 Hz), 6.92 (1H, d, J=8.0 Hz), 7.13–7.21 (2H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz, 50°C) δ: 25.1, 28.2, 34.4, 44.7, 46.2 (br), 79.2, 115.0, 115.7, 116.6, 122.5, 122.9 (br), 127.2 (br), 127.8, 129.5, 129.8, 133.3, 143.4, 145.8, 155.4; HR-MS (ESI-TOF) *m*/*z*: Calcd for $C_{21}H_{24}BrClN_2NaO_2S$ ([M+Na]$^+$) 505.0328. Found 505.0319; IR (neat) 755, 805, 865, 928, 1051, 1107, 1150, 1246, 1393, 1455, 1562, 1587, 1689, 2872, 2932, 2973 cm^{-1}.

***tert*-Butyl Methyl{3-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-10*H*-phenothiazin-10-yl]propyl}carbamate (7)** Chloride **2** (1.43 g, 3.53 mmol), bis(pinacolato)diboron (1.79 g, 7.05 mmol), potassium acetate (416 mg, 4.24 mmol), $Pd(OAc)_2$ (15.9 mg, 71 μmol) and XPhos (67.3 mg, 134 μmol) were added to 1,4-dioxane (35.3 mL), and the obtained mixture was refluxed overnight. When remaining of the substrate had been observed by TLC (hexane–EtOAc=4 : 1 (v/v)), XPhos

(33.7 mg, 70.7 μmol) was added to the reaction mixture and it was refluxed for additional 8.5 h. After addition of water and EtOAc, the reaction mixture was filtered through cotton and the mixture was extracted with EtOAc. The combined organic layer was washed with sat. aq. $NaHCO_3$ followed by brine, filtered and concentrated *in vacuo*. The resulting residue was purified by column chromatography (hexane–EtOAc=20 : 1 then 5 : 1 (v/v)) and product **7** (1.57 g, 3.16 mmol, 90%) was obtained as pale yellow amorphousness: ^{1}H-NMR ($CDCl_3$, 300 MHz, 50°C) δ: 1.32 (12H, s), 1.42 (9H, s), 2.01 (2H, quint, J=6.7 Hz), 2.78 (3H, s), 3.33 (2H, t, J=6.7 Hz), 3.92 (2H, t, J=6.7 Hz), 6.79–6.91 (2H, m), 7.06–7.17 (3H, m), 7.29 (1H, s), 7.37 (1H, d, J=7.7 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz, 50°C) δ: 24.7, 25.4, 28.3, 34.3, 44.5, 46.4, 79.1, 83.6, 115.5, 121.1, 122.3, 125.2, 126.8, 127.1, 127.3, 129.0, 129.5, 144.6, 145.0, 155.7; HR-MS (ESI-TOF) m/z: Calcd for $C_{27}H_{37}BN_2NaO_4S$ ($[M+Na]^+$) 519.2465. Found 519.2446; IR (neat) 668, 684, 751, 823, 855, 966, 1051, 1109, 1145, 1247, 1353, 1410, 1456, 1552, 1593, 1697, 2869, 2934, 2976, 3061, 3479 cm^{-1}.

Di-*tert*-butyl {[8′-Chloro-10*H*,10′*H*-(2,3′-biphenothiazine)-10,10′-diyl]bis(propane-3,1-diyl)}bis(methylcarbamate) (8) K_2CO_3 (1.61 g, 11.7 mmol) and $Pd(PPh_3)_4$ (93.6 mg, 81.0 μmol) were added to a solution of substrates **3** (782 mg, 1.62 mmol) and **7** (883 mg, 1.78 mmol) in DME (51.4 mL) and H_2O (17.0 mL), and the obtained mixture was refluxed overnight. After addition of water and EtOAc, the reaction mixture was filtered through cotton and the mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The resulting residue was purified by column chromatography (hexane–EtOAc=6 : 1 then 4 : 1 (v/v)) and dimerized product **8** (1.10 g, 1.42 mmol, 88%) was obtained as yellow amorphousness: ^{1}H-NMR ($CDCl_3$, 300 MHz, 50°C) δ: 1.41 (9H, s), 1.44 (9H, s), 1.97–2.11 (4H, m), 2.78 (3H, s), 2.81 (3H, s), 3.35 (4H, t, J=6.7 Hz), 3.86 (2H, t, J=6.7 Hz), 3.94 (2H, t, J=6.7 Hz), 6.80–7.32, 13H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz, 50°C) δ: 25.4, 25.5, 28.3, 34.5, 44.7, 44.8, 46.3, 46.5, 79.2, 79.3, 113.7, 115.6, 115.7, 115.8, 120.8, 122.4, 122.5, 123.4, 124.6, 125.4, 125.6, 125.5, 125.8, 127.1, 127.4, 127.6, 127.9, 133.3, 135.8, 139.1, 143.6, 144.9, 145.6, 146.1, 155.6; HR-MS (ESI-TOF) m/z: Calcd for $C_{42}H_{49}ClN_4NaO_4S_2$ ($[M+Na]^+$) 795.2781. Found 795.2762; IR (neat) 732, 909, 1151, 1247, 1393, 1455, 1573, 1688, 2872, 2930, 2975 cm^{-1}.

3,3′-[8′-Chloro-10*H*,10′*H*-(2,3′-biphenothiazine)-10,10′-diyl]bis(*N*,*N*-dimethylpropan-1-amine) (1 (*n*=2)) Hydrogen chloride in 1,4-dioxane (4 M, 2.0 mL) was added to Boc derivative **8** (100 mg, 129 μmol), and the obtained mixture was stirred for 3 h. Following to addition of sat. aq. $NaHCO_3$, the mixture was extracted with CH_2Cl_2 and the obtained organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent *in vacuo*, the crude residue was dissolved in CH_2Cl_2 (3.0 mL). Then formalin (aq. formaldehyde, 37% (w/w), 137 μL, 5.16 mmol), $NaBH(OAc)_3$ (109 mg, 516 μmol), and AcOH (157 μL, 2.75 mmol) were added to the CH_2Cl_2 solution. Following to stirring for 14 h and subsequent addition of sat. aq. $NaHCO_3$, the mixture was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The product was purified by column chromatography ($CHCl_3$–MeOH–Et_3N=100 : 1 : 1 (v/v/v)), and CPZ dimer **1** (n=2) (68.4 mg, 114 μmol, 88% over 2 steps) was obtained as yellow amorphousness: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.91–2.02 (4H, m), 2.21 (6H, s), 2.23 (6H, s), 2.40 (2H, t, J=7.0 Hz), 2.41 (2H, t, J=7.0 Hz), 3.90 (2H, t, J=7.0 Hz), 3.97 (2H, t, J=7.0 Hz), 6.87–6.95 (5H, m), 6.99–7.07 (3H, m), 7.12–7.19 (3H, m), 7.30 (1H, d, J=2.2 Hz), 7.32 (1H, dd, J=8.2, 2.2 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 25.1, 25.3 45.5, 45.7, 113.8 115.6, 115.8, 120.7, 122.3, 122.5, 123.0, 124.1, 125.0, 125.1, 125.6, 125.9, 127.2, 127.4, 127.6, 127.9, 133.3, 135.7, 139.2, 143.7, 145.1, 145.7, 146.3; HR-MS (ESI-TOF) m/z: Calcd for $C_{34}H_{38}ClN_4S_2$ ($[M+H]^+$) 601.2226. Found 601.2207; IR (KBr) 751, 806, 931, 1039, 1105, 1132, 1168, 1219, 1244, 1457, 1573, 2765, 2940 cm^{-1}.

Di-*tert*-butyl {[8′-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-10*H*,10′*H*-(2,3′-biphenothiazine)-10,10′-diyl]-bis(propane-3,1-diyl)}bis(methylcarbamate) (9) Chloride **8** (200 mg, 259 μmol) was converted to **9** as similar to conversion of **2** to **7**, and product **9** (195 mg, 225 μmol, 87%) was obtained as yellow amorphousness: ^{1}H-NMR ($CDCl_3$, 300 MHz, 50°C) δ: 1.34 (12H, s), 1.41 (9H, s), 1.43 (9H, s), 2.06 (4H, quint, J=6.8 Hz), 2.78 (3H, s), 2.81 (3H, s), 3.25–3.45 (4H, m), 3.90–4.10 (4H, m), 6.82–7.45 (13H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz, 50°C) δ: 24.9, 25.6, 25.7, 28.5, 34.5, 34.6, 44.8, 46.6, 79.4, 83.9, 113.9, 115.8, 120.9, 121.2, 122.7, 125.7, 125.8, 127.0, 127.2, 127.6, 127.7, 129.2, 135.3, 139.6, 144.5, 144.6, 145.2, 145.8, 155.8; HR-MS (ESI-TOF) m/z: Calcd for $C_{48}H_{61}BN_4NaO_6S_2$ ($[M+Na]^+$) 887.4023. Found 887.4009; IR (KBr) 751, 809, 1147, 1249, 1356, 1393, 1417, 1458, 1578, 1693, 2929, 2977 cm^{-1}.

Di-*tert*-butyl [(10′-{3-[(*tert*-Butoxycarbonyl)(methyl)-amino]propyl}-8″-chloro-10*H*,10′*H*,10″*H*-[2,3′:8′,3″-terphenothiazine]-10,10″-diyl)bis(propane-3,1-diyl)]-bis(methylcarbamate) (10) Dimer **9** (100 mg, 116 μmol) was coupled with bromide **3** (46.8 mg, 96.7 μmol) to generate trimer **10** as similar to coupling of **7** and **3**. In this case $NaHCO_3$ was employed instead of K_2CO_3. Product **10** (90.6 mg, 79.3 μmol, 82%) was obtained as yellow amorphousness: ^{1}H-NMR ($CDCl_3$, 300 MHz, 50°C) δ: 1.41 (18H, s), 1.44 (9H, s), 1.95–2.18 (6H, m), 2.79 (3H, s), 2.81 (3H, s), 2.83 (3H, s), 3.22–3.45 (6H, m), 3.90 (2H, t, J=6.8 Hz), 3.90–4.25 (4H, m), 6.82–7.39 (19H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz, 50°C) δ: 24.9, 25.4, 25.6, 25.7, 28.5, 34.5, 34.7, 44.8, 44.9, 46.6, 79.4, 79.5, 114.0, 115.8, 115.9, 121.0, 121.1, 122.6, 122.7, 125.8, 125.9, 126.0, 127.3, 127.6, 127.7, 127.8, 128.1, 133.5, 135.6, 139.5, 143.9, 144.5, 144.8, 145.2, 145.6, 145.8, 146.3, 155.9; HR-MS (ESI-TOF) m/z: Calcd for $C_{63}H_{73}ClN_6NaO_6S_3$ ($[M+K]^+$) 1179.4079. Found 1179.4088; IR (KBr) 749, 808, 1150, 1247, 1394, 1457, 1569, 1692, 2930, 2975 cm^{-1}.

3,3′,3″-[8″-Chloro-10*H*,10′*H*,10″*H*-(2,3′:8′,3″-terphenothiazine)-10,10,′10″-triyl]tris(*N*,*N*-dimethylpropan-1-amine) (1 (*n*=3)) Boc derivative **10** (25.0 mg, 21.9 μmol) was converted to CPZ trimer **1** (n=3) as similar to conversion of **8** to **1** (n=2), and product **1** (n=3) (16.8 mg, 19.0 μmol, 87% over 2 steps) was obtained as yellow amorphousness: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.90–2.08 (6H, m), 2.21 (6H, s), 2.22 (6H, s), 2.23 (6H, s), 2.38–2.48 (6H, m), 3.91 (2H, t, J=6.8 Hz), 3.98 (2H, t, J=6.8 Hz), 4.00 (2H, t, J=7.2 Hz), 6.87–6.97 (6H, m), 7.00–7.09 (5H, m), 7.12–7.19 (4H, m), 7.29–7.36 (4H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 25.1, 25.3, 29.7, 45.6, 57.0, 57.2, 113.8, 115.7, 115.9, 120.7, 120.8, 122.3, 122.5, 123.0, 123.6, 124.0, 125.1, 125.2, 125.4, 125.6, 125.7, 125.8, 126.0, 127.2, 127.4, 127.6, 127.7, 127.9, 133.4, 135.3, 135.7, 139.4, 143.8, 144.3, 145.1, 145.5, 145.7, 146.3; HR-MS (ESI-TOF) m/z: Calcd

for $C_{51}H_{57}ClN_6S_3$ ($[M+2H]^+$) 442.1748. Found 442.1777; IR (KBr) 1039, 1457, 1569, 2768, 2819, 2856, 2926 cm^{-1}.

UV-Irradiation Experiment of CPZ Monomer 1 (n=1) A solution of CPZ monomer **1** (n=1) (7.0 mg, 22 μmol) in water (200 μL) was irradiated by UV (distance between the UV lamp and the reaction mixture: 3 cm) for 2.5 h at room temperature. The resulting mixture was analyzed with or without synthetic dimer **1** (n=2) using LC/MS. LC conditions were shown in the General methods section.

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Conflict of Interest The authors declare no conflict of interest.

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