

## CHAPTER III

### EXPERIMENTAL

#### 3.1 Chemicals

All chemicals are purchased from commercial sources and used as received, unless noted otherwise.

1. Cardanol : Obtained locally
2. Sodium sulfate (anhydrous) : Merck
3. Triethylamine : Merck
4. Potassium hydroxide : Merck
5. Hydrochloric acid : Merck
6. Methylene chloride : Distilled from commercial grade (Lab-scan)
7. Hexane : Distilled from commercial grade (Lab-scan)
8. Ethyl acetate : Distilled from commercial grade (Lab-scan)
9. Tetrahydrofuran : Merck
10. Toluene : Carlo Erba
11. Silica gel : Merck
12. Benzidine : Fluka
13. 1,5-Diamino-naphthalene : Merck
14. *o*-Phenylenediamine : Fluka
15. *m*-Phenylenediamine : Aldrich
16. *p*-Phenylenediamine : Fluka
17. 2-Nitro-1,4-phenylenediamine : Fluka
18. 4-Amino-*N,N*-dimethylaniline : Fluka
19. 1-Naphthylamine : Merck
20. 4-Nitro-1-naphthylamine : Fluka
21.  $\beta$ -Naphthol : Lab-scan
22. Methanol : Merck
23. Ethanol : Merck
24. Sodium nitrite : Merck
25. Base gasoline 91 : Veerasuwan oil refinery
26. Commercial gasoline 91 : PTT Public Company Limited

27. Deuterated chloroform : Merck

### 3.2 Analytical instruments

FT-IR spectra were recorded on a Nicolet Fourier Transform Infrared Spectrophotometer: Impact 410 (Nicolet Instruments Technologies, Inc. WI, USA). Infrared spectra were recorded between  $400\text{ cm}^{-1}$  to  $4,000\text{ cm}^{-1}$  in transmittance mode.

$^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra were obtained in deuterated chloroform ( $\text{CDCl}_3$ ) using Varian Mercury NMR spectrometer operated at 400 MHz for  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$  nuclei (Varian Company, CA, USA). Chemical shifts ( $\delta$ ) are reported in parts per million (ppm) relative to the residual  $\text{CHCl}_3$  peak (7.26 ppm for  $^1\text{H}$ -NMR and 77.0 ppm for  $^{13}\text{C}$ -NMR). Coupling constants ( $J$ ) are reported in Hertz (Hz).

Mass spectra were recorded on Mass Spectrometer: Waters Micromass Quattro micro API ESCi (Waters, MA, USA). Samples were dissolved in ethyl acetate and 50  $\mu\text{L}$  was directly injected into Mass Spectrometer (Compound **14**, **17** and **18**), and mass spectra of azo dyes **6**, **7**, **9**, **10**, **11**, **12** and **13** were recorded by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) by using dithranol as a matrix.

Absorption spectra and quantities of azo dyes in gasoline fuel were measured using a Perkin-Elmer (Lambda 2) UV/Vis spectrophotometer and  $\text{CH}_2\text{Cl}_2$  was used as solvent.

Colorimetric of azo dye blend in base gasoline 91 was determined by colorimeter CR 300.

Sulfur content in dyed and undyed gasoline fuel was determined using Outokumpu (X-MET820) automatic sulfur content.

Distillation of dyed and undyed gasoline fuel was carried out with a AD 86-5G 2 (ISL) automatic distillation apparatus.

Benzene and aromatic compounds in dyed and undyed gasoline fuel were observed by GC-Reformulyzer AC 6890N.

Research octane number of dyed and undyed gasoline was measured by CFR (Waukesha) automatic research octane number.

Water content in dyed and undyed gasoline was determined using 831 Karl Fischer Coulometer.

Vapor pressure of dyed and undyed gasoline was measured with MINIVAP VPS (Grabner).

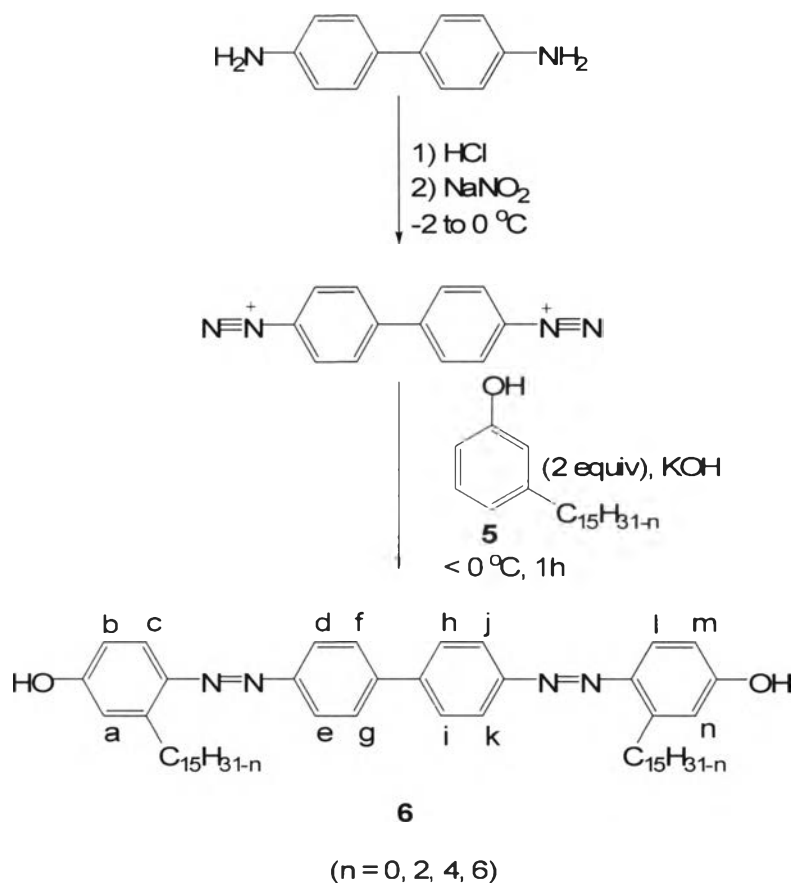
Silver strip corrosion of dyed and undyed gasoline was determined by Copper Corrosion Bath (Stanhope seta).

Solvent washed gum of dyed and undyed gasoline was observed using Existent Gum (Stanhope seta).

### 3.3 Experimental procedure

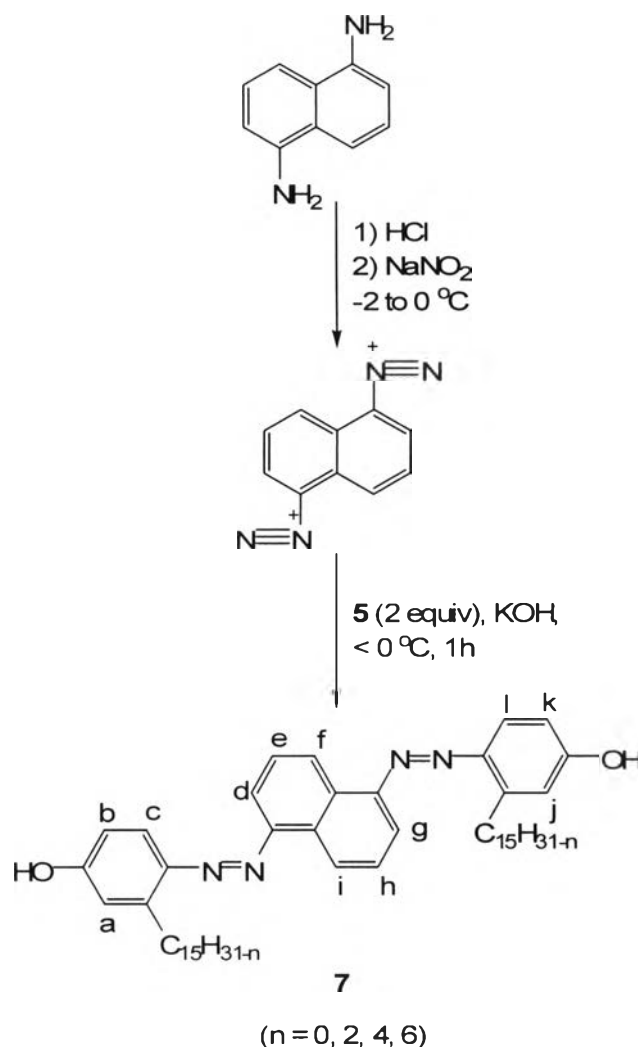
#### Part 1: Synthesis of azo dyes

##### 3.3.1 Azo dye 6 from cardanol and benzidine diamine



Following a previously published procedure [3], benzidine (0.184 g, 1.00 mmol) was added to 50% v/v aqueous solution of concentrated hydrochloric acid (1.2 mL) and the resulting reaction mixture was vigorously stirred in an ice bath. After the mixture was cooled to  $-2$  to  $0$  °C, a solution of sodium nitrite (0.138 g, 2.00 mmol) in distilled water (1.0 mL) was added dropwise into the reaction mixture while the reaction temperature was kept below  $0$  °C. Meanwhile, a 3-alkylphenoxide solution was prepared by dissolving potassium hydroxide (0.112 g, 2.00 mmol) in methanol (1.0 mL), then cooling the solution to  $0$  °C, and adding cardanol (0.608 g, 2.00 mmol) with continuous stirring to give a reddish-brown oil. Then diazonium salt solution was added dropwise to the cooled 3-alkylphenoxide solution. The reaction mixture was left stirring at a temperature below  $0$  °C for 1 h and the azo dye product was extracted with toluene. The toluene layer was separated, washed repeatedly with distilled water (4 x 20 mL) and dried over  $\text{Na}_2\text{SO}_4$ , and then evaporated to dryness. The resulting crude product was purified by column chromatography on a silica gel [1% TEA in hexane/ethyl acetate (4:1)] to give a yellow gummy solid (0.010 g, 1%);  $^1\text{H-NMR}$ :  $\delta$  (ppm) 0.84–0.90 (m, 6H,  $-\text{CH}_3$ ), 1.23–2.80 (m, 44H,  $-\text{CH}_2-$ ), 3.13 (t,  $J = 7.6$  Hz, 4H, Ar- $\text{CH}_2-$ ), 4.94–5.82 (m, 6H,  $-\text{CH}=\text{CH}-$ ), 6.74 (dd,  $J = 8.8$  Hz, 2.6 Hz, 2H,  $\text{H}_b, \text{H}_m$ ), 6.81 (d,  $J = 2.6$  Hz, 2H,  $\text{H}_a, \text{H}_n$ ), 7.74 (d,  $J = 8.8$  Hz, 2H,  $\text{H}_c, \text{H}_l$ ), 7.80 (d,  $J = 8.4$  Hz, 4H,  $\text{H}_f, \text{H}_g, \text{H}_h, \text{H}_i$ ), 7.97 (d,  $J = 8.4$  Hz, 4H,  $\text{H}_d, \text{H}_e, \text{H}_j, \text{H}_k$ ) (**Figure A-1**);  $^{13}\text{C-NMR}$ :  $\delta$  (ppm) 13.8, 14.1, 22.6, 22.8, 25.55, 25.62, 27.2, 29.0, 29.25, 29.31, 29.39, 29.44, 29.6, 29.67, 29.72, 29.8, 31.4, 31.5, 31.8, 32.0 ( $-\text{CH}_3, -\text{CH}_2-$ ), 113.8, 114.7, 116.4, 117.2, 123.3, 126.8, 127.5, 127.7, 127.96, 128.11, 129.3, 129.8, 129.9, 130.1, 130.4, 136.8, 141.9, 144.8, 146.1 (C=C in aromatic ring and side chain), 152.4, 158.4 (C-OH) (**Figure A-2**);  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3399 (O-H st), 3009 (=C-H st), 2852, 2924 (C-H st), 1467, 1583 (C=C st) (**Figure A-3**); MALDI-MS obsd 808.674; calcd avg mass 808.612–814.612 [ $\text{M} = \text{C}_{54}\text{H}_{78-n}\text{N}_4\text{O}_2$ ;  $n = 0, 2, 4, 6$ ] (**Figure A-4**);  $\lambda_{\text{abs}}$  ( $\text{CH}_2\text{Cl}_2$ ) 387 nm,  $\epsilon$  ( $\text{CH}_2\text{Cl}_2$ ) 10950,  $\epsilon$  (Toluene) 10193 (**Figure B-1**).

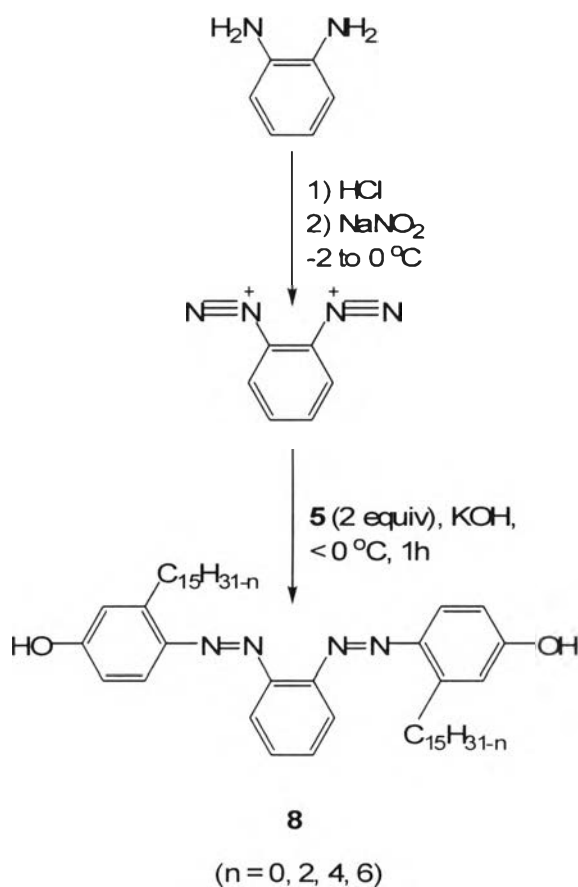
### 3.3.2 Azo dye 7 from cardanol and 1,5-diamino-naphthalene



According to the above-mentioned synthesis of compound 6 using 1,5-diamino-naphthalene (0.158 g, 1.00 mmol) as an aniline derivative, the reaction mixture was extracted with tetrahydrofuran and saturated ammonium chloride solution. The organic layer was separated, washed repeatedly with distilled water (4 x 20 mL) and dried over  $\text{Na}_2\text{SO}_4$ , and then evaporated to dryness. The resulting crude product was purified by column chromatography (silica, 1% TEA in  $\text{CH}_2\text{Cl}_2$ ) to give a yellow gummy solid (0.04 g, 5%);  $^1\text{H-NMR}$ :  $\delta$  (ppm) 0.77–0.83 (m, 6H,  $-\text{CH}_3$ ), 1.18–2.74 (m, 46H,  $-\text{CH}_2-$ ), 3.13 (t,  $J = 7.6$  Hz, 4H, Ar- $\text{CH}_2-$ ), 4.88–5.77 (m, 6H,  $-\text{CH}=\text{CH}-$ ), 6.73 (dd,  $J = 8.7$  Hz, 2.6 Hz, 2H,  $\text{H}_b, \text{H}_k$ ), 6.77 (d,  $J = 2.6$  Hz, 2H,  $\text{H}_a, \text{H}_j$ ), 7.64 (t,  $J = 7.9$  Hz, 2H,  $\text{H}_e, \text{H}_h$ ), 7.78 (d,  $J = 7.4$  Hz, 2H,  $\text{H}_f, \text{H}_i$ ), 7.83 (d,  $J = 8.7$  Hz, 2H,  $\text{H}_c, \text{H}_l$ ), 9.03 (d,  $J = 8.4$  Hz, 2H,  $\text{H}_d, \text{H}_g$ ) (**Figure A-5**);  $^{13}\text{C-NMR}$ :  $\delta$  (ppm) 13.8, 14.1, 22.6,

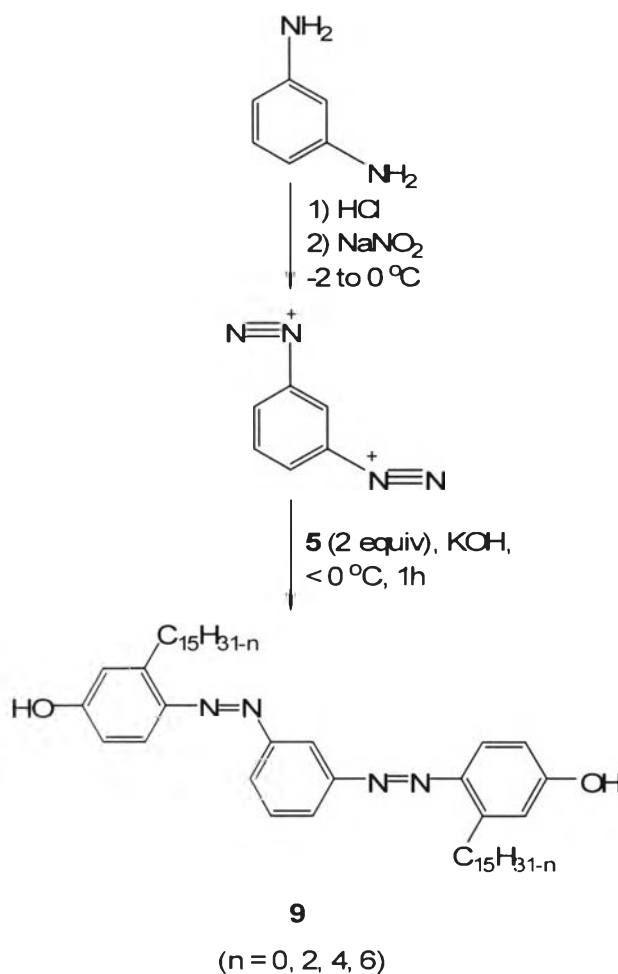
22.8, 25.5, 25.6, 27.2, 28.92, 28.96, 29.25, 29.4, 29.5, 29.6, 29.66, 29.71, 29.8, 31.5, 31.8, 32.1 (-CH<sub>3</sub>, -CH<sub>2</sub>-), 112.1, 113.8, 114.7, 116.4, 117.6, 126.1, 126.4, 126.8, 127.5, 127.9, 128.1, 129.3, 129.8, 129.9, 130.1, 130.4, 132.3, 136.8, 145.5, 146.3, 147.9 (C=C in aromatic ring and side chain), 158.3 (C-OH) (**Figure A-6**);  $\nu_{\max}$  (cm<sup>-1</sup>): 3426 (O-H st), 3009 (=C-H st), 2852, 2924 (C-H st), 1468, 1593 (C=C st) (**Figure A-7**); MALDI-MS obsd 782.547; calcd avg mass 782.597–788.597 [M = C<sub>52</sub>H<sub>76-n</sub>N<sub>4</sub>O<sub>2</sub>; n = 0, 2, 4, 6] (**Figure A-8**);  $\lambda_{\text{abs}}$  (CH<sub>2</sub>Cl<sub>2</sub>) 402 nm,  $\epsilon$  (CH<sub>2</sub>Cl<sub>2</sub>) 8086,  $\epsilon$  (Toluene) 9275 (**Figure B-2**).

### 3.3.3 Azo dye 8 from cardanol and *o*-phenylenediamine



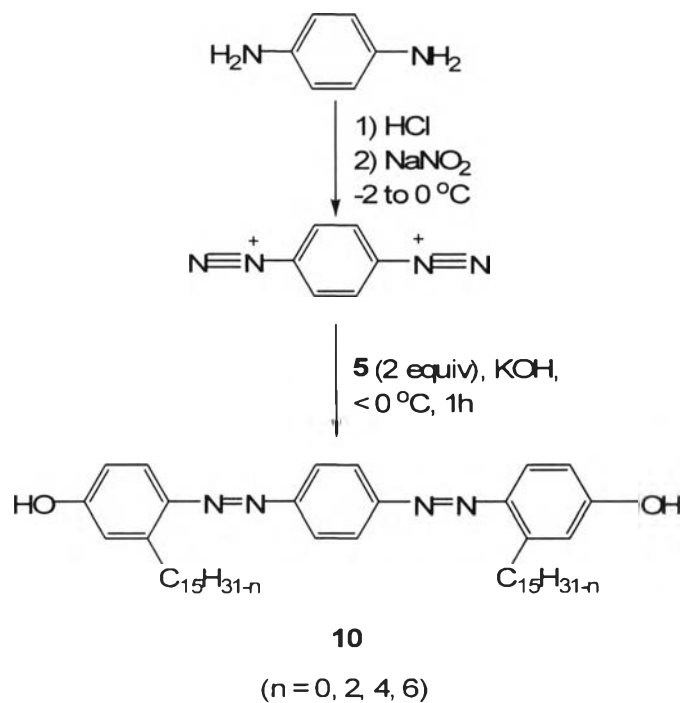
According to the above-mentioned synthesis of compound **6** using *o*-phenylenediamine (0.108 g, 1.00 mmol) as an aniline derivative, working up with toluene and dried over Na<sub>2</sub>SO<sub>4</sub>, afforded an orange oil. An attempt to couple *o*-phenylenediamine with cardanol failed to give compound **8**.

### 3.3.4 Azo dye 9 from cardanol and *m*-phenylenediamine



According to the above-mentioned synthesis of compound **6** using *m*-phenylenediamine (0.108 g, 1.00 mmol) as an aniline derivative, working up with toluene and dried over Na<sub>2</sub>SO<sub>4</sub>, afforded an orange oil. Purification by column chromatography failed to isolate from the reaction crude. Due to the complication of the spectrum of the crude sample, peak integration of the target compound could not be elucidated. <sup>1</sup>H-NMR spectrum of the reaction crude indicated the formation of compound **9** in the aromatic region at δ 6.81, 7.61, 7.74, 7.94, 8.34 ppm (**Figure A-9**); MALDI-MS obsd 733.160; calcd avg mass 732.581–738.581 [M = C<sub>48</sub>H<sub>74-n</sub>N<sub>4</sub>O<sub>2</sub>; n = 0, 2, 4, 6] (**Figure A-10**).

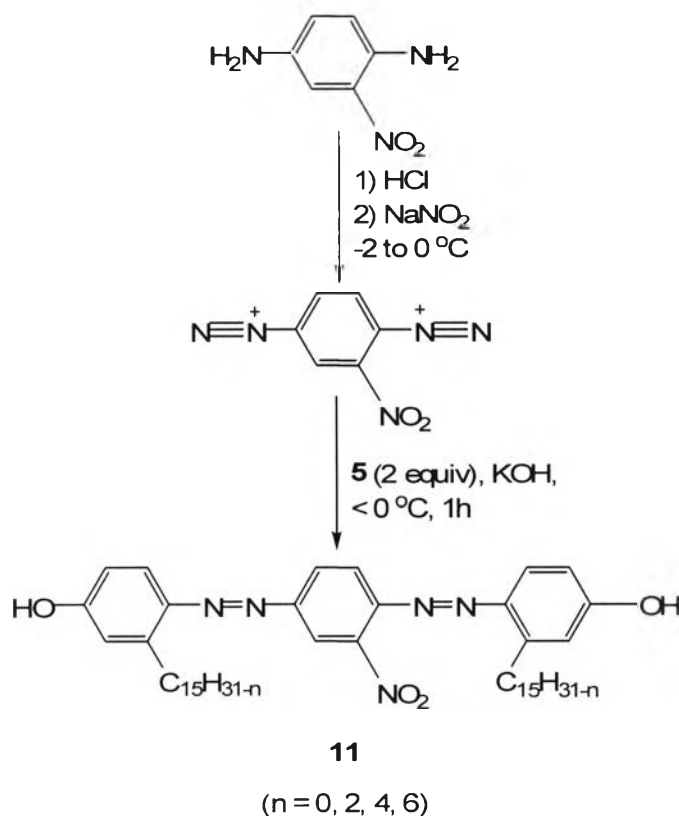
### 3.3.5 Azo dye **10** from cardanol and *p*-phenylenediamine



Following a published method [40], a mixture of *p*-phenylenediamine (0.108 g, 1.00 mmol) in 6% v/v aqueous solution of concentrated hydrochloric acid (5.4 mL) was reacted with a solution of sodium nitrite (0.160 g, 2.00 mmol) in distilled water (6.0 mL) at below  $-2\text{ }^{\circ}\text{C}$ . The resulting diazonium salt was coupled by adding into an alkaline solution. The alkaline solution was prepared by dissolving potassium hydroxide (0.112 g, 2.00 mmol) in ethanol (4.0 mL), then cooling the solution to  $0\text{ }^{\circ}\text{C}$  and adding cardanol (0.608 g, 2.00 mmol) with continuous stirring. The reaction mixture was left stirring at below  $-2\text{ }^{\circ}\text{C}$  for 1 h and then extracted with toluene (30 mL). The organic layer was separated, washed repeatedly with distilled water (4 x 20 mL) and dried over  $\text{Na}_2\text{SO}_4$ , and then evaporated to dryness. The resulting crude product led to an orange oil. Purification by column chromatography failed to give pure compound **10**. Due to the complication of the spectrum of the crude sample, peak integration of the target compound could not be elucidated.  $^1\text{H-NMR}$  spectrum of crude product in the aromatic region at  $\delta$  6.67, 6.74, 7.59, 7.73 ppm indicated that compound **10** was occurred while the starting material remained (**Figure A-11**); MALDI-MS obsd 733.064; calcd avg mass 732.581–738.581 [ $\text{M} = \text{C}_{48}\text{H}_{74-n}\text{N}_4\text{O}_2$ ;  $n = 0, 2, 4, 6$ ] (**Figure A-12**).



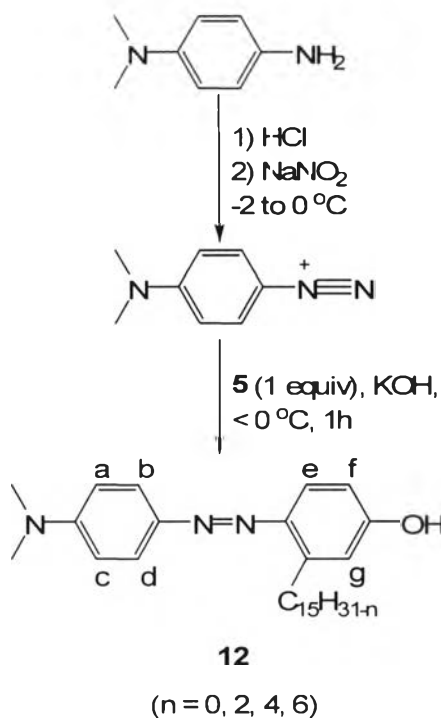
### 3.3.6 Azo dye **11** from cardanol and 2-nitro-1,4-phenylenediamine



Following a published method [40], a mixture of 2-nitro-1,4-phenylenediamine (0.153 g, 1.00 mmol) in 6% v/v aqueous solution of concentrated hydrochloric acid (5.4 mL) was reacted with a solution of sodium nitrite (0.160 g, 2.00 mmol) in distilled water (6.0 mL) at below  $-2\text{ }^{\circ}\text{C}$ . The resulting diazonium salt was coupled by adding into an alkaline solution. The alkaline solution was prepared by dissolving potassium hydroxide (0.112 g, 2.00 mmol) in ethanol (4.0 mL), then cooling the solution to  $0\text{ }^{\circ}\text{C}$  and adding cardanol (0.608 g, 2.00 mmol) with continuous stirring. The reaction mixture was left stirring at below  $-2\text{ }^{\circ}\text{C}$  for 1 h and then extracted with toluene (30 mL). The organic layer was separated, washed repeatedly with distilled water (4 x 20 mL) and dried over  $\text{Na}_2\text{SO}_4$ , and then evaporated to dryness. The resulting crude product led to an orange crude. Purification by column chromatography failed to give pure **11**. Due to the complication of the spectrum of the crude sample, peak integration of the target compound could not be elucidated.  $^1\text{H-NMR}$  spectrum of crude product in the aromatic region at  $\delta$  6.71, 6.78, 6.88, 7.66, 7.96, 8.70 ppm indicated that compound **11** was occurred while the starting material remained (**Figure A-13**); MALDI-MS

obsd 781.572; calcd avg mass 777.566–783.566 [ $M = C_{48}H_{73-n}N_5O_4$ ;  $n = 0, 2, 4, 6$ ] (Figure A-14).

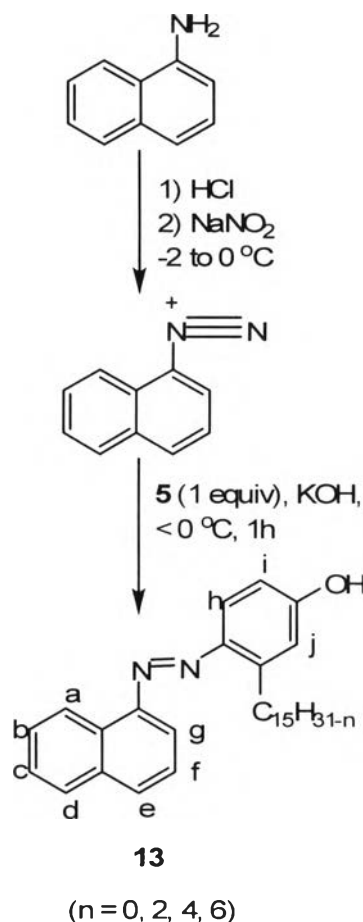
### 3.3.7 Azo dye 12 from cardanol and 4-amino-*N,N*-dimethyl-aniline



The coupling reaction was performed in the same manner as described for compound **6** using 4-amino-*N,N*-dimethyl-aniline (2.72 g, 20.0 mmol) as an aniline starting material with the cardanol:aromatic amine ratio of 1:1. After working up with tetrahydrofuran and saturated ammonium chloride solution, the organic layer was separated, washed repeatedly with distilled water (4 x 20 mL) and dried over  $Na_2SO_4$ , and then evaporated to dryness. The resulting crude product was purified by column chromatography (silica, 1% TEA in  $CH_2Cl_2$ ) gave a yellow oil (0.67 g, 8%);  $^1H$ -NMR:  $\delta$  (ppm) 0.78–0.84 (m, 3H, - $CH_3$ ), 1.19–2.74 (m, 22H, - $CH_2$ -), 2.94–2.98 (m, 6H, - $CH_3$ ), 4.88–5.77 (m, 4H, - $CH=CH$ -) 6.59 (dd,  $J = 8.7$  Hz, 2.3 Hz, 1H,  $H_f$ ), 6.65 (d,  $J = 2.3$  Hz, 1H,  $H_g$ ), 6.68 (d,  $J = 9.0$  Hz, 2H,  $H_a, H_c$ ), 7.51 (d,  $J = 8.7$  Hz, 1H,  $H_e$ ), 7.76 (d,  $J = 9.0$  Hz, 2H,  $H_b, H_d$ ) (Figure A-15);  $^{13}C$ -NMR:  $\delta$  (ppm) 14.1, 14.2, 22.7, 25.6, 27.3, 28.99, 29.30, 29.4, 29.5, 29.6, 29.7, 29.8, 31.5, 31.8, 31.9, 40.4, 60.7 (- $CH_3$ , - $CH_2$ -), 111.8, 113.7, 116.4, 116.9, 124.5, 129.3, 129.9, 130.5, 144.2, 144.8, 151.9 (C=C in aromatic ring and side chain), 157.5 (C-OH) (Figure A-16);  $\nu_{max}$  ( $cm^{-1}$ )

<sup>1</sup>): 3340 (O-H st), 3007 (=C-H st), 2854, 2925 (C-H st), 1517, 1600 (C=C st) (**Figure A-17**); MALDI-MS obsd 450.555; calcd avg mass 445.356–451.356 [ $M = C_{29}H_{45-n}N_3O$ ;  $n = 0, 2, 4, 6$ ] (**Figure A-18**);  $\lambda_{\text{abs}}$  ( $CH_2Cl_2$ ) 407 nm,  $\epsilon$  ( $CH_2Cl_2$ ) 18894,  $\epsilon$  (Toluene) 19227 (**Figure B-3**).

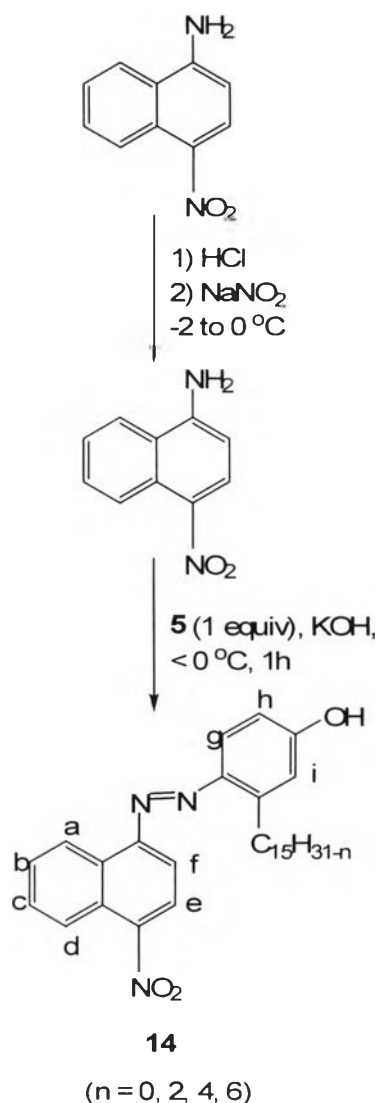
### 3.3.8 Azo dye **13** from cardanol and 1-naphthylamine



The coupling reaction was performed in the same manner as described for compound **6** using 1-naphthylamine (0.143 g, 1.00 mmol) as an aniline starting material with the cardanol:aromatic amine ratio of 1:1. Working up with  $CH_2Cl_2$  and dried over  $Na_2SO_4$ , followed by column chromatography (silica, 1% TEA in  $CH_2Cl_2$ ) gave a yellow oil (0.28 g). According to the <sup>1</sup>H-NMR data, the yield of azo dye **13** was equivalent to 62%. <sup>1</sup>H-NMR spectrum of a mixture compound indicated the compound **13**:starting material (cardanol) with a ratio of 1:2.2 (31% purity); <sup>1</sup>H-NMR:  $\delta$  (ppm) 0.87–0.93 (m, 3H, -CH<sub>3</sub>), 1.25–2.83 (m, 20H, -CH<sub>2</sub>-), 2.55 (t, 2H,  $J = 7.6$ , Ar-CH<sub>2</sub>-), 4.85–5.87 (m, 4H, -CH=CH-), 6.79 (d,  $J = 2.4$  Hz, 1H, H<sub>i</sub>), 6.82 (d,  $J = 2.4$

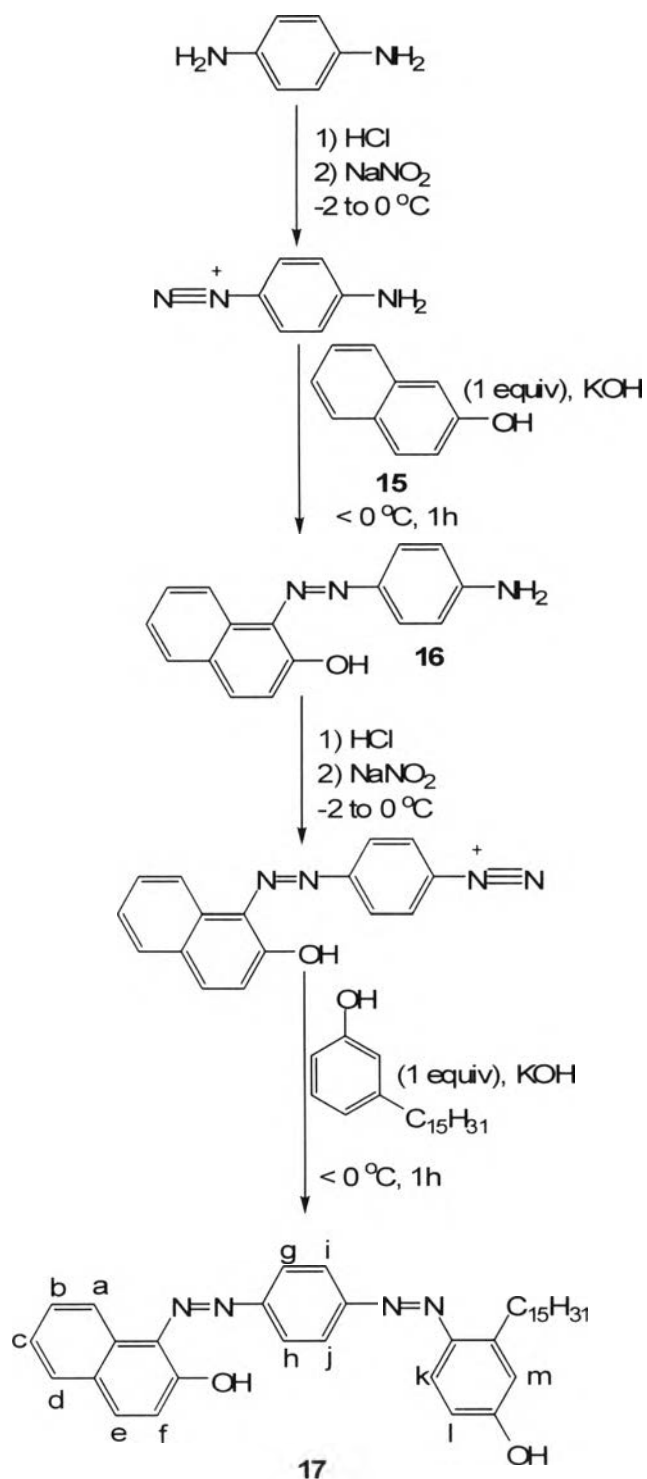
Hz, 1H, H<sub>j</sub>), 7.53–7.65 (m, 3H, H<sub>b</sub>, H<sub>c</sub>, H<sub>d</sub>), 7.76 (d,  $J = 7.2$  Hz, 1H, H<sub>a</sub>), 7.87 (d,  $J = 8.8$  Hz, 1H, H<sub>h</sub>), 7.91–7.95 (m, 2H, H<sub>e</sub>, H<sub>f</sub>), 8.96 (d,  $J = 8.4$  Hz, 1H, H<sub>g</sub>) (**Figure A-19**); due to the complication of the spectrum, <sup>13</sup>C-NMR data of compound **13** could not be elucidated; MALDI-MS obsd 453.293; calcd avg mass 452.330–458.330 [ $M = C_{31}H_{42-n}N_2O$ ;  $n = 0, 2, 4, 6$ ] (**Figure A-20**);  $\lambda_{\text{abs}}$  (CH<sub>2</sub>Cl<sub>2</sub>) 379 nm,  $\epsilon$  (CH<sub>2</sub>Cl<sub>2</sub>) 1721,  $\epsilon$  (Toluene) 1605 (**Figure B-4**).

### 3.3.9 Azo dye **14** from cardanol and 4-nitro-1-naphthylamine

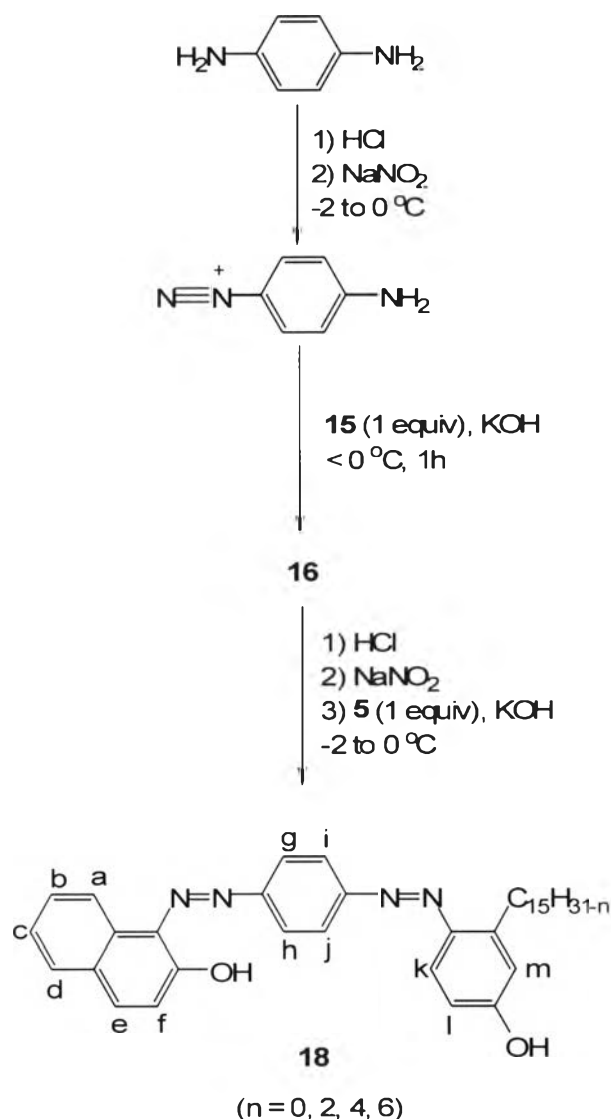


The coupling reaction was performed in the same manner as described for compound **6** using 4-nitro-1-naphthylamine (0.188 g, 1.00 mmol) with the cardanol:aromatic amine ratio of 1:1. After working up with CH<sub>2</sub>Cl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub> layer was washed repeatedly with distilled water (4 x 20 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>, followed by column chromatography (silica, 1% TEA in CH<sub>2</sub>Cl<sub>2</sub>), affording a yellow gummy solid (0.32 g). According to the <sup>1</sup>H-NMR data, the yield of azo dye **14** was equivalent to 64%. <sup>1</sup>H-NMR spectrum of a mixture compound indicated the compound **14**:starting material (cardanol) with a ratio of 1:0.9 (53% purity); <sup>1</sup>H-NMR: δ (ppm) 0.83–0.89 (m, 3H, -CH<sub>3</sub>), 1.26–2.83 (m, 20H, -CH<sub>2</sub>-), 3.18 (t, *J* = 7.6 Hz, 2H, Ar-CH<sub>2</sub>-), 4.94–5.84 (m, 4H, -CH=CH-), 6.80 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H, H<sub>h</sub>), 6.85 (d, *J* = 2.4 Hz, 1H, H<sub>i</sub>), 7.68 (d, *J* = 8.0 Hz, 1H, H<sub>a</sub>), 7.73–7.84 (m, 2H, H<sub>b</sub>, H<sub>c</sub>), 7.90 (d, *J* = 8.8 Hz, 1H, H<sub>e</sub>), 8.31 (d, *J* = 8.4 Hz, 1H, H<sub>d</sub>), 8.64 (d, *J* = 8.4 Hz, 1H, H<sub>f</sub>), 9.06 (d, *J* = 8.4 Hz, 1H, H<sub>e</sub>) (**Figure A-21**); due to the complication of the spectrum, <sup>13</sup>C-NMR data of compound **14** could not be elucidated; ESI-MS obsd 497.373, calcd 497.315–503.315 [(M-H)<sup>+</sup>; M = C<sub>31</sub>H<sub>41-n</sub>N<sub>3</sub>O<sub>3</sub>; n = 0, 2, 4, 6] (**Figure A-22**); λ<sub>abs</sub> (CH<sub>2</sub>Cl<sub>2</sub>) 407 nm, ε (CH<sub>2</sub>Cl<sub>2</sub>) 2831, ε (Toluene) 2795 (**Figure B-5**).

### 3.3.10 Azo dye 17 from hydrogenated cardanol, *p*-phenylenediamine and $\beta$ -naphthol



Following a published method [40], a mixture of *p*-phenylenediamine (0.108 g, 1.00 mmol) in 6% v/v aqueous solution of concentrated hydrochloric acid (2.7 mL) was reacted with a solution of sodium nitrite (0.080 g, 1.0 mmol) in distilled water (3.0 mL) at below  $-2\text{ }^{\circ}\text{C}$ . The resulting diazonium salt was coupled by adding into an alkaline solution. The alkaline solution was prepared by dissolving potassium hydroxide (0.056 g, 1.0 mmol) in ethanol (2.0 mL), then cooling the solution to  $0\text{ }^{\circ}\text{C}$  and adding  $\beta$ -naphthol (0.144 g, 1.00 mmol) with continuous stirring. The reaction mixture was left stirring at below  $-2\text{ }^{\circ}\text{C}$  for 1 h. The crude mixture was extracted with  $\text{CH}_2\text{Cl}_2$  and the organic layer was separated and dried over  $\text{Na}_2\text{SO}_4$ . After removal of the solvent, the mixture was purified by column chromatography on silica gel [1% TEA in hexane/ethyl acetate (1:1)] to give an orange solid (0.250 g). Then this solid (0.250 g) was diazotized and coupled further with cardanol by the same manner as mentioned above, using the azo dye (**16**) and cardanol (0.304 g, 1.00 mmol). The resulting crude was extracted with  $\text{CH}_2\text{Cl}_2$ . The  $\text{CH}_2\text{Cl}_2$  layer was separated, washed repeatedly with distilled water (4 x 20 mL) and dried over  $\text{Na}_2\text{SO}_4$ , and then evaporated to dryness. The resulting crude product was purified by column chromatography on silica gel [1% TEA in  $\text{CH}_2\text{Cl}_2$  and then 1% TEA in ethyl acetate/ $\text{CH}_2\text{Cl}_2$  (1:4)] to give **17** as a red solid (0.05 g, 9%);  $^1\text{H-NMR}$ :  $\delta$  (ppm) 0.82–0.87 (m, 3H,  $-\text{CH}_3$ ), 1.21–1.71 (m, 26H,  $-\text{CH}_2-$ ), 3.11 (t,  $J = 7.8\text{ Hz}$ , 2H, Ar- $\text{CH}_2-$ ), 6.74 (dd,  $J = 8.8\text{ Hz}$ , 2.8 Hz, 1H,  $\text{H}_l$ ), 6.80 (d,  $J = 2.8\text{ Hz}$ , 1H,  $\text{H}_m$ ), 6.83 (d,  $J = 9.2\text{ Hz}$ , 1H,  $\text{H}_f$ ), 7.39–7.42 (m, 1H,  $\text{H}_d$ ), 7.54–7.58 (m, 2H,  $\text{H}_b$ ,  $\text{H}_c$ ), 7.70–7.73 (m, 2H,  $\text{H}_e$ ,  $\text{H}_k$ ), 7.81 (d,  $J = 8.8\text{ Hz}$ , 2H,  $\text{H}_i$ ,  $\text{H}_j$ ), 7.98 (d,  $J = 8.8\text{ Hz}$ , 2H,  $\text{H}_g$ ,  $\text{H}_h$ ), 8.54 (d,  $J = 8.4\text{ Hz}$ , 1H,  $\text{H}_a$ ) (**Figure A-23**);  $^{13}\text{C-NMR}$ :  $\delta$  (ppm) 14.1, 22.7, 29.1, 29.2, 29.4, 29.5, 29.66, 29.72, 29.99, 30.03, 30.2, 31.4, 31.5, 31.9, 32.1 ( $-\text{CH}_3$ ,  $-\text{CH}_2-$ ), 113.9, 116.5, 117.1, 118.5, 122.0, 124.3, 125.3, 126.2, 128.1, 128.7, 128.8, 128.9, 129.1, 130.7, 133.4, 141.2, 144.6, 145.0, 146.2, 151.5 (C=C in aromatic ring), 158.9, 174.7 (C-OH) (**Figure A-24**);  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3367 (O-H st), 3026 (=C-H st), 2849, 2921 (C-H st), 1498, 1578 (C=C st) (**Figure A-25**); ESI-MS obsd 579.578, calcd 578.362 [(M+H) $^+$ ; M =  $\text{C}_{37}\text{H}_{46}\text{N}_4\text{O}_2$ ] (**Figure A-26**);  $\lambda_{\text{abs}}$  ( $\text{CH}_2\text{Cl}_2$ ) 513 nm,  $\epsilon$  ( $\text{CH}_2\text{Cl}_2$ ) 31948,  $\epsilon$  (Toluene) 31780 (**Figure B-6**).

3.3.11 Azo dye **18** from cardanol, *p*-phenylenediamine and  $\beta$ -naphthol

The coupling reaction is performed in the same manner as that described for compound **17** using natural cardanol (0.304 g, 1.00 mmol) instead of hydrogenated cardanol. Compound **18** was obtained as a red solid (0.15 g, 26%);  $^1\text{H-NMR}$ :  $\delta$  (ppm) 0.77–0.85 (m, 3H,  $-\text{CH}_3$ ), 1.18–2.74 (m, 20H,  $-\text{CH}_2-$ ), 3.05 (t,  $J = 7.6$  Hz, 2H, Ar- $\text{CH}_2-$ ), 4.87–5.76 (m, 4H,  $-\text{CH}=\text{CH}-$ ), 6.67 (dd,  $J = 8.8$  Hz, 2.8 Hz, 1H,  $\text{H}_i$ ), 6.73 (d,  $J = 2.8$  Hz, 1H,  $\text{H}_m$ ), 6.77 (d,  $J = 9.6$  Hz, 1H,  $\text{H}_f$ ), 7.32–7.36 (m, 1H,  $\text{H}_d$ ), 7.48–7.53 (m, 2H,  $\text{H}_b, \text{H}_c$ ), 7.64–7.67 (m, 2H,  $\text{H}_k, \text{H}_e$ ), 7.75 (d,  $J = 8.4$  Hz, 2H,  $\text{H}_i, \text{H}_j$ ), 7.92 (d,  $J = 8.4$  Hz, 2H,  $\text{H}_g, \text{H}_h$ ), 8.48 (d,  $J = 8.4$  Hz, 1H,  $\text{H}_a$ ) (**Figure A-27**);  $^{13}\text{C-NMR}$ :  $\delta$  (ppm) 14.1, 22.6, 22.8, 25.6, 27.2, 28.97, 29.26, 29.4, 29.5, 29.6, 29.7, 29.8, 31.5, 31.8, 32.0 ( $-\text{CH}_3, -\text{CH}_2-$ ), 113.9, 116.4, 117.2, 118.5, 121.9, 124.3, 125.3, 126.3, 128.1, 128.8,



129.2, 129.95, 130.38, 130.7, 133.4, 141.4, 144.7, 144.9, 146.2, 151.5 (C=C in aromatic ring and side chain), 158.7, 175.0 (C-OH) (**Figure A-29**);  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3350 (O-H st), 3008 (=C-H st), 2852, 2922 (C-H st), 1497, 1578 (C=C st) (**Figure A-30**); ESI-MS obsd 579.383, calcd 572.362–578.362 [(M+H)<sup>+</sup>; M = C<sub>37</sub>H<sub>46-n</sub>N<sub>4</sub>O<sub>2</sub>; n = 0, 2, 4, 6] (**Figure A-31**);  $\lambda_{\text{abs}}$  (CH<sub>2</sub>Cl<sub>2</sub>) 516 nm,  $\epsilon$  (CH<sub>2</sub>Cl<sub>2</sub>) 31345,  $\epsilon$  (Toluene) 31406 (**Figure B-7**).

## **Part 2: Physical properties of azo dyes in gasoline 91**

### **3.3.12 Preparation of stock solution of azo dye 18**

A 200 ppm stock azo dye solution was prepared by dissolving 2 mg of compound **18** in base gasoline 91 and the volume was made up to 10 mL in a volumetric flask.

### **3.3.13 Quantitative determination of azo dye 18 in gasoline 91**

A standard calibration solution of **18** in base gasoline 91 was prepared at concentration of 0–10 ppm by pipetting the stock solution into a 10 mL volumetric flask and the volume was made up with base gasoline 91. The volume of the stock solution used to prepare each calibration solution is shown in Table 3-1.

**Table 3-1** Preparation of calibration solution of azo dye **18** in base gasoline 91.

Concentration (ppm)	Volume of 200 ppm stock solution (mL)
0.0	0.00
2.0	0.10
4.0	0.20
6.0	0.30
8.0	0.40
10	0.50

Absorption spectra of each calibration solution were recorded by a UV/Vis spectrophotometer (**Figure B-9**). The calibration curve was plotted between absorbance (y-axis) and the concentration (x-axis) of azo dye **18** in base gasoline 91 (**Figure B-10**).

#### 3.3.14 Colorimetric analysis of azo dye **18** in gasoline 91

Solutions of azo dye **18** in base gasoline 91 at concentration of 4–8 ppm was prepared by pipetting the 200 ppm stock solution into a 10 mL volumetric flask and the volume was made up with base gasoline 91. The volume of the stock solution used to prepare each solution is shown in Table 3-2.

**Table 3-2** Preparation of solution of azo dye **18** in base gasoline 91.

Concentration (ppm)	Volume of 200 ppm stock solution (mL)
4.0	0.20
5.0	0.25
6.0	0.30
7.0	0.35
8.0	0.40

Then these solution and commercial gasoline 91, obtained from PTT Public Company Limited, were measured by colorimeter to find the concentration of azo dye **18** in base gasoline 91 that gives the most similar color as the commercial gasoline 91.

### 3.3.15 Colorimetric analysis of crude of azo dye **18** in gasoline **91**

A 1000 ppm stock solution of azo dye crude was prepared by dissolving 10 mg of crude of compound **18** in base gasoline **91** and the volume was made up to 10 mL in a volumetric flask. Then the solution of crude of azo dye **18** in base gasoline **91** at concentration of 10–20 ppm was prepared by pipetting the 1000 ppm stock solution into a 10 mL volumetric flask and the volume was made up with base gasoline **91**. The volume of the stock solution used to prepare each solution is shown in Table 3-3.

**Table 3-3** Preparation of solution of crude of azo dye **18** in base gasoline **91**.

Concentration (ppm)	Volume of 1000 ppm stock solution (mL)
10	0.10
11	0.11
12	0.12
13	0.13
14	0.14
15	0.15
16	0.16
17	0.17
18	0.18
19	0.19
20	0.20

Then these solution and commercial gasoline **91** were measured by colorimeter to find the concentration of crude of azo dye **18** in base gasoline **91** that gives the most similar color as the commercial gasoline **91**.

### 3.3.16 Effect of azo dye 18 on the physical properties of gasoline 91

Physical properties of dyed (6 ppm), crude of dyed (18 ppm) and undyed gasoline 91 were investigated according to the ASTM method described in Table 3-4.

**Table 3-4** ASTM testing methods of gasoline 91.

Test items	Test method ASTM
Vapor pressure @ 37.8 °C, kPa	D 5191
Distillation	D 86-01
Solvent Washed Gum, mg/100 mL	D 381
Silver strip corrosion	D 130
Sulfur, %wt.	D 5453
Water, %wt.	D 6304
Benzene, %vol.	D 5580
Aromatics, %vol.	D 5580
Research Octane Number; RON	D 2699

### Part 3: Stability test of azo dye 18 in gasoline 91

The stability test was performed with the base gasoline 91 dyed containing compound **18** in the concentration of 6 ppm on a UV-visible spectrophotometer. The three 6 ppm solutions of compound **18** in base gasoline 91 were prepared by diluting the 200-ppm stock solution of azo dye **18** (1.5 mL) with the base gasoline 91 in three 50-mL volumetric flasks. A portion of each 50-mL solution (5 mL) was placed into 3 sealed vials and stored for 3 months in an ambient environment. Each three solutions were directly taken from the vials to the UV-visible measurement ( $\lambda_{\text{abs}} = 509 \text{ nm}$ ) after 1, 2 and 3 months. The quantity of azo dye **18** in the blended gasoline was determined by the calibration equation.