Near-Infrared Fluorescent 2,3-Dicyanopyrazines

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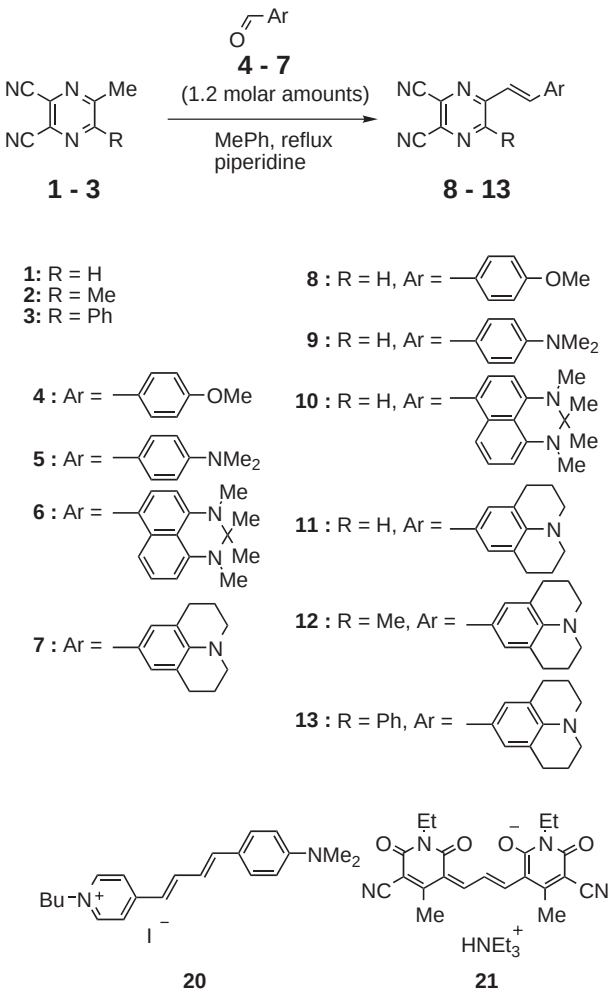
Novel non-ionic near-infrared (NIR) fluorescent 2,3-dicyanopyrazines were prepared. 5-[6-(9-julolidyl)-1,3,5-hexatrienyl]- and 5-[8-(9-julolidyl)-1,3,5,7-octatetraenyl]-2,3-dicyanopyrazines showed fluorescence maxima (F_{max}) at 716 and 751 nm with fluorescence quantum yields (Φ_f) 0.12 and 0.03 in toluene, respectively. MO calculations showed that these compounds have an intramolecular charge-transfer chromophoric system from the julolidyl to dicyanopyrazine moieties. The calculations also showed that since the HOMO energy level was unstabilized, and at the same time, the LUMO energy level was stabilized by expanding the conjugated system at 5-position, NIR fluorescent derivatives were obtained. They showed clear positive solvatochromism in the fluorescence spectroscopy. The fluorescence intensity drastically decreased in polar solvents.

NIR fluorescent compounds have potential applications as fluorescent labeling reagents and biological probes.[1] However, most typical NIR fluorescent dyes, such as cyanines, squaryliums, and croconiums, are ionic. Therefore, it is important to find non-ionic NIR fluorescent dyes. To our knowledge, only pyrone and quaterrylenetetracarboxylic bisimide derivatives have been reported as non-ionic NIR fluorescent dyes.[2] In our previous paper, some 2,3-dicyano-6*H*-1,4-diazepines have been also reported to show NIR fluorescence.[3] However, the Φ_f is low. For example, the Φ_f of 2,3-dicyano-5-[6-(9-julolidyl)ethenyl]-6*H*-1,4-diazepine is 0.19 in chloroform.[3] To increase the Φ_f values, we thought that six-membered 2,3-dicyanopyrazines should show more intense fluorescence than seven-membered 2,3-dicyano-6*H*-1,4-diazepines. 2,3-Dicyanopyrazines have been prepared by Matsuoka et al.,[4–6] however, no NIR fluorescent derivatives have been obtained so far. We report herein the synthesis and properties of novel non-ionic NIR fluorescent 2,3-dicyanopyrazines.

Results and Discussion

Synthesis. 2,3-Dicyanopyrazines **8**–**13** were synthesized as shown in Scheme 1. 2,3-Dicyano-5-methylpyrazines **1**–**3** were allowed to react with aromatic aldehydes **4**–**7** in refluxing toluene in the presence of piperidine to give **8**–**13** in low to moderate yields.

When compound **1** was allowed to react with conjugated aldehyde **14** under the same conditions, both the ethenyl **11** and 1,3-butadienyl **15** derivatives were produced depending on the reaction conditions as shown in Scheme 2 and Table 1. The former product came from 1,4-addition followed by elimination of acetaldehyde. The latter one was produced by 1,2-addition followed by dehydration. Though compounds **11** and **15** showed different hue on silica gel TLC (**11**: red, **15**: reddish purple), they could not be separated by chromatography due to similar R_f value (SiO_2, CH_2Cl_2, **11**: 0.68, **15**: 0.70). When the reaction was carried out at low temperature in polar solvent, the product yields increased. In addition, the product distribution of **15** also increased. Finally, it was found that the reaction of **1** with **14** in acetonitrile at 0 °C preferentially provided **15** (Run 5).



Scheme 1.

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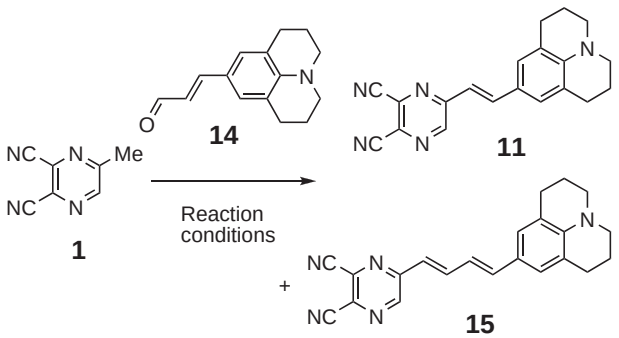
1,3,5-Hexatrienyl and 1,3,5,7-octatetraenyl derivatives **18** and **19** were obtained by the reaction of **1** with the corresponding aldehydes **16** and **17** in acetonitrile at 0 °C in the presence of piperidine in 24 and 20% yields, respectively, as shown in Scheme 3.

UV–Vis Absorption and Fluorescence Spectra. The UV–vis absorption and fluorescence spectra of **8**–**13**, **15**, **18**, and **19** in toluene are summarized in Table 2. The UV–vis absorption maxima (λ_{max}) of **11**, **12**, and **13** were observed at around 530 nm with molar absorption coefficients (ε) ca. 40000 $dm^3\,mol^{-1}\,cm^{-1}$, with no remarkable difference among them (Runs 4–6). Their F_{max} were observed at around 615 nm. 6-Unsubstituted and 6-methyl derivatives **11** and **12** showed more intense fluorescence than the 6-phenyl derivative **13**. The λ_{max} of **8**, **9**, **10**, and **11** were observed in the range of 400–549 nm with ε values 11000–42000 $dm^3\,mol^{-1}\,cm^{-1}$ (Runs 1–4). Their F_{max} were observed in the range of 487 to 669 nm. Stronger electron-donating ability of the aryl moiety lead to a larger bathochromic shift in λ_{max} and F_{max}. Julolidyl derivative **11** showed both the largest bathochromic and most intense fluorescence among the aryl derivatives **8**, **9**, **10**, and **11**. It was concluded that the julolidyl moiety at the 5-position and unsubstituted one at the 6-position is important to obtain bathochromic and intensely fluorescent 2,3-dicyanopyrazine derivatives.



Scheme 2.

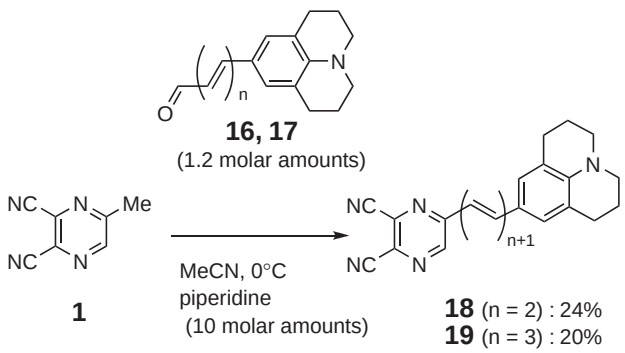
Table 1. Reaction of **1** with **14**[a)]

Run	Reaction conditions			Yield[b)]/%	
	Solvent	Temperature/°C	Time/h	**11**	**15**
1	MePh	reflux	24	9	8
2	MePh	25	72	1	4
3	CH_2Cl_2	25	24	18	27
4	MeCN	25	24	10	53
5	MeCN	0	72	0	53
6	EtOH	25	24	0	8

a) The reaction was carried out with 2,3-dicyano-5-methylpyrazine (**1**, 0.1 mmol) and **14** (0.12 mmol) in the presence of piperidine (1.0 mmol) in solvent (10 mL). b) Determined by ^{1}H NMR spectrum.

The UV–vis absorption and fluorescence spectra of **11**, **15**, **18**, and **19** in toluene are shown in Fig. 1. Both the λ_{max} and F_{max} showed bathochromic shift as the number of ethylene units increased. The 1,3,5-hexatrienyl and 1,3,5,7-octatetraenyl derivatives **18** and **19** showed the F_{max} at 716 and 751 nm, respectively. As expected, the Φ_f of 2,3-dicyanopyrazine derivative **12** (0.73) was much greater than the corresponding 2,3-dicyano-6*H*-1,4-diazepine derivative 2,3-dicyano-5-methyl-7-[2-(9-julolidyl)ethenyl]-6*H*-1,4-diazepine (Φ_f: 0.03, λ_{max}: 500 nm, F_{max}: 599 nm in toluene). It is clear that NIR fluorescent 2,3-dicyanopyrazine derivatives **18** ($\Phi_f = 0.12$) and **19** (0.03) have more intense fluorescence than the corresponding tri- and tetraenyl 2,3-dicyano-6*H*-1,4-diazepine derivatives.

Figure 2a shows the effect of solvent on the λ_{max} and F_{max} of **11**, **15**, **18**, and **19**. The solvatochromism of cationic and



Scheme 3.

Table 2. Observed and Calculated UV–Vis Absorption and Fluorescence Spectra and Dipole Moment of **8**–**13**, **15**, **18**, and **19**

Run	Compd	Obsrd[a)]			Calcd[b)]			
		λ_{max}/nm ($\varepsilon_{max}/M^{-1}\,cm^{-1}$)	F_{max}/nm	Φ_f[c)]	λ_{max}/nm (f[d)])	CI component	μ_g	μ_{ex}
1	**8**	400 (11000)	487	0.02	352 (1.12)	HOMO to LUMO (83%)	8.35	13.74
2	**9**	484 (36000)	580	0.92	356 (1.14)	HOMO to LUMO (83%)	9.38	15.54
3	**10**	549 (36000)	669	0.33	389 (1.10)	HOMO to LUMO (87%)	13.22	23.35
4	**11**	523 (42000)	617	0.70	374 (1.26)	HOMO to LUMO (82%)	11.10	20.69
5	**12**	524 (35000)	601	0.73	367 (1.19)	HOMO to LUMO (78%)	11.14	19.77
6	**13**	534 (38000)	621	0.45	375 (1.13)	HOMO to LUMO (80%)	11.48	19.76
7	**15**	561 (34000)	669	0.35	389 (1.64)	HOMO to LUMO (79%)	11.36	21.82
8	**18**	573 (42000)	716	0.12	402 (2.01)	HOMO to LUMO (76%)	11.55	22.42
9	**19**	583 (39000)	751	0.03	412 (2.40)	HOMO to LUMO (72%)	11.69	22.71

a) Measured at the concentration of 1×10^{-5} mol dm^{-3} in toluene at 25 °C. b) MOPAC program. c) Determined using quinine sulfate in 0.1 mol dm^{-3} sulfuric acid as a reference ($\Phi_f = 0.55$, $F_{max} = 366$ nm) at 25 °C. d) Oscillator strength.

anionic dyes **20** and **21** are also shown in Figs. 2b and 2c, respectively. The λ_{max} of **11**, **15**, **18**, **19**, **20**, and **21** were scarcely affected by the solvents. Meanwhile, clear positive solvatochromism was observed in the F_{max} of **11**, **15**, **18**, **19**, and **20**. The F_{max} of 2,3-dicyanopyrazines **11**, **15**, **18**, and **19** was more sensitive to the polarity of the solvent than that of the cationic dye **20**. The effect of solvent on relative fluorescence intensity (RFI) is shown in Fig. 2d. The RFI of **11**, **15**, **18**, and **19** drastically decreased in polar solvents, due to intermolecular interactions between the fluorophore and solvent.

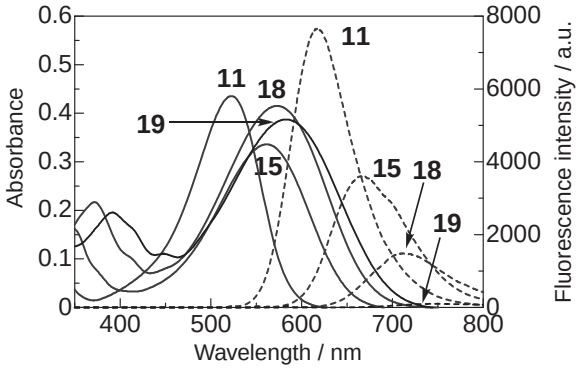


Fig. 1. UV–vis absorption and fluorescence spectra of **11**, **15**, **18**, and **19**. Solid and dotted lines represent UV–vis absorption and fluorescence spectra, respectively. Measured at the concentration of 1×10^{-5} mol dm^{-3} in toluene at 25 °C.

MO Calculations. To explain the UV–vis absorption spectra of 2,3-dicyanopyrazines, semi-empirical MO calculations were carried out. Geometry optimization was performed using the AM1 method. Then, the UV–vis absorption spectra were analyzed by the INDO/S method. The first absorption band was attributed to HOMO–LUMO transition as shown in Table 2.

The graphic view of HOMO and LUMO, and difference in electron density accompanied by the first excitation of **18** are shown in Figs. 3a and 3b, respectively. The optimized structure of **18** was planar. The electron densities in HOMO and LUMO of **18** are located at the julolydyl and dicyanopyrazyl moieties, respecively. The difference in electron density also indicated that compound **18** has an intramolecular charge-transfer chromophoric system from the julolidyl to dicyanopyrazine moieties.

The calculated LUMO and HOMO energy levels of **8**–**13**, **15**, **18**, and **19** are shown in Fig. 4. In the cases of **11**, **12**, and **13**, no remarkable difference in both the LUMO and HOMO energy levels was observed, resulting in similar λ_{max} (**11**: 523 nm, **12**: 524 nm, **13**: 534 nm). In the cases of **8**, **9**,

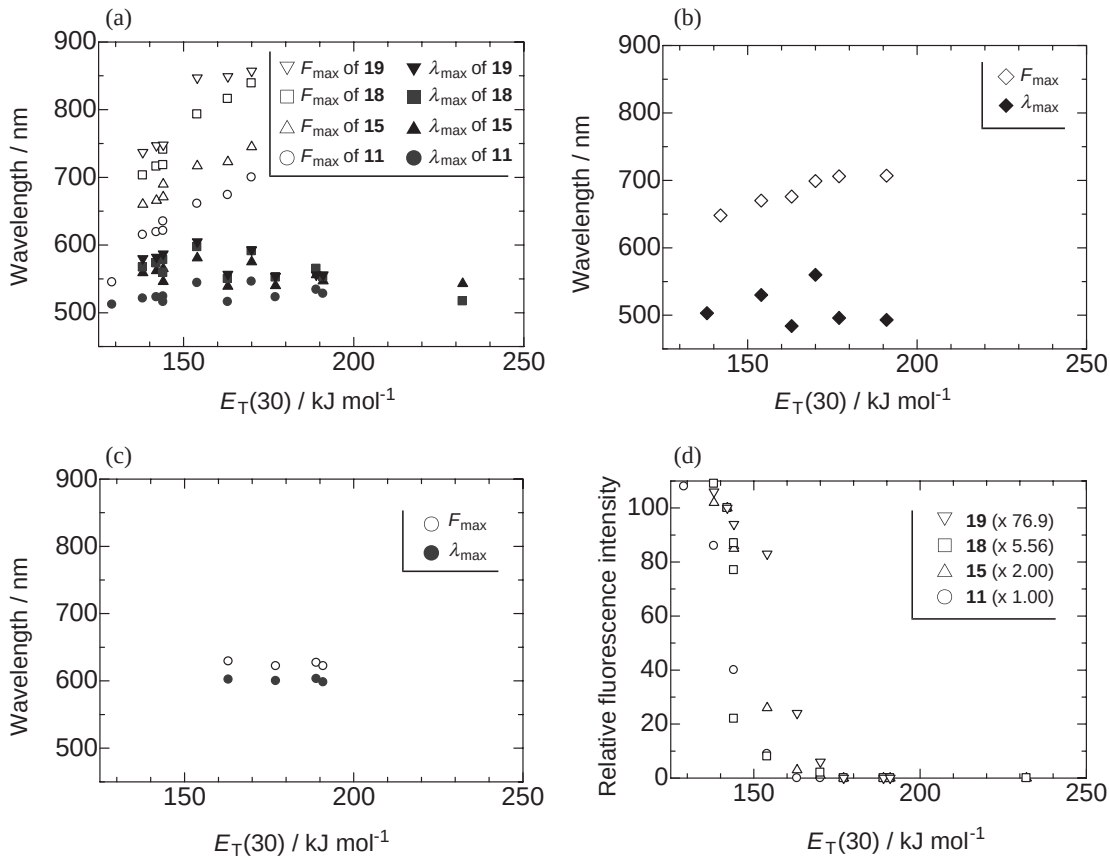


Fig. 2. Solvent effect in **11**, **15**, **18**, **19**, **20**, and **21**: (a) change in λ_{max} and F_{max} of **11**, **15**, **18**, and **19**, (b) change in λ_{max} and F_{max} of **20**, (c) change in λ_{max} and F_{max} of **21**, and (d) change in relative fluorescence intensity of **11**, **15**, **18**, and **19**.[a] $E_T(30)$ (kJ mol^{-1})[b]: cyclohexane (129), *p*-xylene (138), toluene (142), benzene (144), diethyl ether (144), chlorobenzene (154), ethyl acetate (163), dichloromethane (170), acetone (177), DMSO (189), acetonitrile (191), methanol (232). a) Relative flurecemce intenseity (RFI) in tolune: 100. b) Reference 7.

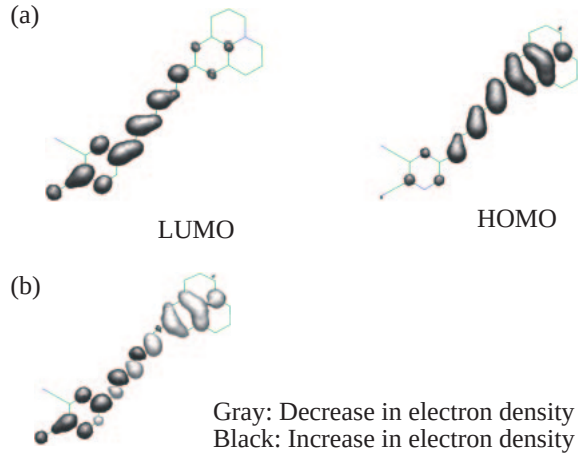


Fig. 3. Graphic view of **18**: (a) HOMO and LUMO, and (b) difference in electron density accompanied by first excitation.

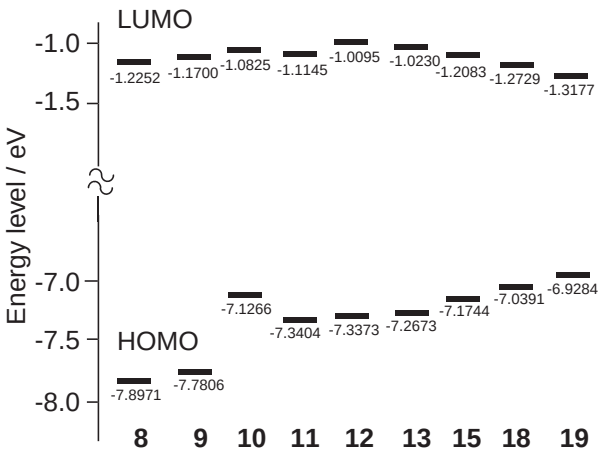


Fig. 4. Calculated HOMO and LUMO energy levels of **8**–**13**, **15**, **18**, and **19**.

10, and **11**, though no marked difference was observed in LUMO energy level; however, the HOMO energy level was higher with increasing electron-donating ability of the aryl moiety. In other words, λ_{max} showed a greater bathochromic shift in the following order: **10** (549 nm) > **11** (523 nm) > **9** (484 nm) > **8** (400 nm). In the cases of **11**, **15**, **18**, and **19**, with expansion of conjugated system, the HOMO energy level became higher, and at the same time, the LUMO energy level became lower, resulting in bathochromic shift (**11**: 523 nm, **15**: 561 nm, **18**: 573 nm, **19**: 583 nm).

The calculated ground (μ_g) and excited state dipole moment (μ_{ex}) of 2,3-dicyanopyrazine derivatives are shown in Table 2. In all cases, μ_{ex} was larger than μ_g. This result is reasonable for neutral organic compounds. Clear positive solvatochromism was observed in the F_{max} of **11**, **15**, **18**, and **19** as shown in Fig. 2a, whereas their λ_{max} was scarcely affected by the polarity of solvents. Generally, the time-scale for fluorescence spectroscopy is much longer than that for absorption spectroscopy. Therefore, the excited state energy of the molecule in a polar solvent is stabilized due to solvent relaxation, resulting in positive solvatochromism in the fluorescence spectroscopy.

Conclusion

Novel non-ionic NIR fluorescent 2,3-dicyanopyrazines were obtained. They showed more intense fluorescence than 2,3-dicyano-6*H*-1,4-diazepine derivatives. The F_{max} showed clear positive solvatochromism. Their fluorescence intensity drastically decreased in polar solvents. MO calculations supported the formation of NIR fluorescent derivatives.

Experimental

Instruments. Melting points were measured with a Yanagimoto MP-52 micro-melting-point apparatus. NMR spectra were obtained by a Varian Inova 400 and 500 spectrometers. Mass spectra were taken on a Jeol MStation 700 spectrometer. IR spectra were recorded on a Perkin-Elmer 2000 FT-IR spectrometer. UV–vis absorption and fluorescence spectra were taken on Hitachi U-3500 and F-4500 spectrophotometers, respectively.

Materials. 2,3-Dicyano-5-methylpyrazines **1**,[4] **2**,[4] and **3**,[8] perimidine-6-carbaldehyde (**6**),[9] julolidine-9-carbaldehyde (**7**),[10] 2,3-dicyano-5-(4-methoxystyryl)pyrazine (**8**),[5] 2,3-dicyano-5-[4-(dimethylamino)styryl]pyrazine (**9**),[4] 2,3-dicyano-5-[2-(9-julolidyl)-ethenyl]pyrazine (**11**),[6] 3-(9-julolidyl)propenal (**14**),[11] 5-(9-julolidyl)-2,4-pentadienal (**16**),[11] and 7-(9-julolidyl)-2,4,6-heptatrienal (**17**)[11] were prepared as described in the literature.

Synthesis of 5-Substituted 2,3-Dicyanopyrazines 10, 12, and 13. To a toluene solution (20 mL) of 2,3-dicyano-5-methylpyrazines **1**–**3** (2.0 mmol) were added aromatic aldehydes **4**–**7** (2.4 mmol) and a few drops of piperidine. The mixture was refluxed for 24 h. After the reaction was complete, the solvent was removed in vacuo. The product was purified by silica gel column chromatography (CH_2Cl_2) and recrystallized from a toluene–dichloromethane mixed solution.

2,3-Dicyano-5-[2-(1,2,2,3-tetramethyl-9-perimidyl)ethenyl]-pyrazine (10). Yield 11%; mp 238–240 °C; ^{1}H NMR ($CDCl_3$) δ 1.56 (s, 6H), 2.99 (s, 3H), 3.11 (s, 3H), 6.60 (d, J = 8.3 Hz, 1H), 6.69 (d, J = 8.1 Hz, 1H), 7.00 (d, J = 15.4 Hz, 1H), 7.51 (t, J = 8.1 Hz, 1H), 7.66 (d, J = 8.3 Hz, 1H), 7.92 (d, J = 8.1 Hz, 1H), 8.58 (s, 1H), 8.75 (d, J = 15.4 Hz, 1H); EI-MS (70 eV) m/z (rel intensity) 380 (M^+; 29), 365 (100), 350 (26); IR (KBr) 2232 cm^{-1}. HR-MS (+EI) Found: m/z 380.1713, Calcd for $C_{23}H_{20}N_6$: 380.1749.

2,3-Dicyano-5-[2-(9-julolidyl)ethenyl]-6-methylpyrazine (12). Yield 13%; mp > 300 °C; ^{1}H NMR ($CDCl_3$) δ 1.99 (quin, J = 5.9 Hz, 4H), 2.70 (s, 3H), 2.78 (t, J = 5.9 Hz, 4H), 3.30 (t, J = 5.9 Hz, 4H), 6.83 (d, J = 15.1 Hz, 1H), 7.12 (s, 2H), 7.99 (d, J = 15.1 Hz, 1H); EI-MS (70 eV) m/z (rel intensity) 341 (M^+; 100), 340 (36); IR (KBr) 2227 cm^{-1}. HR-MS (+EI) Found: m/z 341.1656, Calcd for $C_{21}H_{19}N_5$: 341.1640.

2,3-Dicyano-5-[2-(9-julolidyl)ethenyl]-6-phenylpyrazine (13). Yield 18%; mp > 300 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (quin, J = 6.0 Hz, 4H), 2.71 (t, J = 6.0 Hz, 4H), 3.26 (t, J = 6.0 Hz, 4H), 6.91 (d, J = 14.9 Hz, 1H), 6.96 (s, 2H), 7.53–7.56 (m, 3H), 7.69–7.71 (m, 2H), 7.99 (d, J = 14.9 Hz, 1H); EI-MS (70 eV) m/z (rel intensity) 403 (M^+; 100), 402 (22); IR (KBr) 2224 cm^{-1}. HR-MS (+EI) Found: m/z 403.1831, Calcd for $C_{26}H_{21}N_5$: 403.1797.

Synthesis of 5-Substituted 2,3-Dicyanopyrazines 15, 18, and 19. To an acetonitrile solution (10 mL) of 2,3-dicyano-5-methylpyrazine (**1**) (14.4 mg, 0.1 mmol) were added aldehydes **14**, **16**, and **17** (0.12 mmol) and piperidine (1.0 mmol) at 0 °C. Then, the mixture was stirred (**15**: 72 h, **18**: 96 h, **19**: 48 h). After the reaction

was complete, the solvent was removed in vacuo. The product was purified by silica-gel column chromatography (CH_2Cl_2) and recrystallized from a toluene–dichloromethane mixed solution.

2,3-Dicyano-5-[4-(9-julolidyl)-1,3-butadienyl]pyrazine (15). Yield 24%; mp 250–252 °C (dec); ^{1}H NMR ($CDCl_3$) δ 1.97 (quin, $J = 6.0$ Hz, 4H), 2.75 (t, $J = 6.0$ Hz, 4H), 3.25 (t, $J = 6.0$ Hz, 4H), 6.49 (d, $J = 15.0$ Hz, 1H), 6.80 (dd, $J = 15.0$ and 11.0 Hz, 1H), 6.94 (d, $J = 15.0$ Hz, 1H), 6.98 (s, 2H), 7.80 (dd, $J = 15.0$ and 11.0 Hz, 1H), 8.56 (s, 1H); EI-MS (70 eV) m/z (rel intensity) 353 (M^+; 100), 224 (13), 196 (11); IR (KBr) 2227 cm^{-1}. HR-MS (+EI) Found: m/z 353.1617, Calcd for $C_{22}H_{19}N_5$: 353.1640.

2,3-Dicyano-5-[6-(9-julolidyl)-1,3,5-hexatrienyl]pyrazine (18). Yield 20%; mp 248–251 °C (dec); ^{1}H NMR ($CDCl_3$) δ 1.95 (quin, $J = 6.0$ Hz, 4H), 2.73 (t, $J = 6.0$ Hz, 4H), 3.21 (t, $J = 6.0$ Hz, 4H), 6.46 (dd, $J = 14.7$ and 10.7 Hz, 1H), 6.48 (d, $J = 14.7$ Hz, 1H), 6.65–6.73 (m, 2H), 6.87 (dd, $J = 14.7$ and 10.7 Hz, 1H), 6.92 (s, 2H), 7.73 (dd, $J = 14.7$ and 10.7 Hz, 1H), 8.56 (s, 1H); EI-MS (70 eV) m/z (rel intensity) 379 (M^+; 100); IR (KBr) 2229 cm^{-1}. HR-MS (+EI) Found: m/z 379.1765, Calcd for $C_{24}H_{21}N_5$: 379.1797.

2,3-Dicyano-5-[6-(9-julolidyl)-1,3,5,7-octatetraenyl]pyrazine (19). Yield 20%; mp 258–261 °C (dec); ^{1}H NMR ($CDCl_3$) δ 1.96 (quin, $J = 6.1$ Hz, 4H), 2.74 (t, $J = 6.1$ Hz, 4H), 3.20 (t, $J = 6.1$ Hz, 4H), 6.37 (dd, $J = 14.3$ and 11.6 Hz, 1H), 6.46 (dd, $J = 14.3$ and 11.6 Hz, 1H), 6.52 (d, $J = 14.3$ Hz, 1H), 6.55–6.68 (m, 3H), 6.82 (dd, $J = 14.3$ and 11.6 Hz, 1H), 6.90 (s, 2H), 7.72 (dd, $J = 14.9$ and 11.6 Hz, 1H), 8.58 (s, 1H); EI-MS (70 eV) m/z (rel intensity) 405 (M^+; 100); IR (KBr) 2232 cm^{-1}. HR-MS (+EI) Found: m/z 405.1933, Calcd for $C_{26}H_{23}N_5$: 405.1953.

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