Electronic Supplementary Material (ESI) for RSC Advances

Supplementary Information

New unsymetrical alkyltetrazines; original synthesis, fluorescence and electrochemical behaviour

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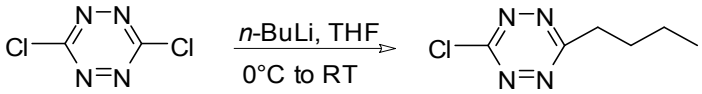
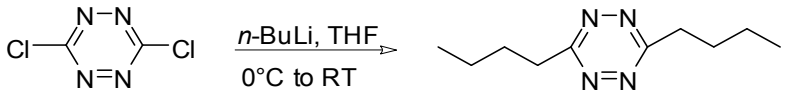
Synthetic procedures

Preparation of 3-butyl-6-chloro--*s*-tetrazine **2a**



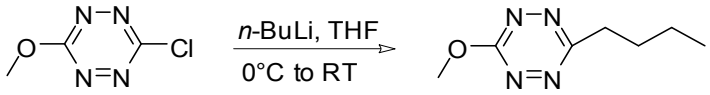
3,6-dichloro-*s*-tetrazine **1a** (0.1g, 0.66mmol) was dissolved in dry THF (10mL). The solution was cooled to 0℃ in an ice-bath, and then *n*-BuLi (0.05g, 0.78mmol) was added dropwise. The mixture was allowed to reach room temperature and stirred for 30min. The solvent was removed under reduced pressure, and the crude mixture purified by column chromatography (petroleum ether - dichloromethane 1:1 v/v). 0.04g of 3-butyl-6-chloro--*s*-tetrazine was obtained (yield: 35%).
^{1}H NMR (400MHz, $CDCl_3$): δ 1.01 (t, *J*=7.3 Hz, 3H), 1.56 (m, 2H), 1.93 (m, 2H), 4.66 (t, *J*=6.9 Hz, 2H) ppm.
^{13}C NMR (100MHz, $CDCl_3$): δ 13.8, 19.1, 30.6, 70.9, 164.3, 166.9 ppm.

Preparation of 3,6-dibutyl-*s*-tetrazine **3a**



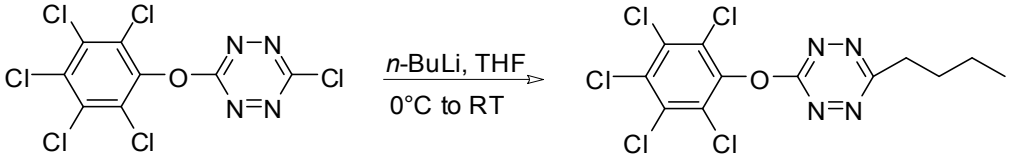
3,6-dichloro-*s*-tetrazine **1a** (0.1g, 0.66mmol) was dissolved in dry THF (10ml). The solution was cooled to 0℃ in an ice-bath, and then *n*-BuLi (0.10g, 1.56mmol) was added dropwise. The mixture was allowed to reach room temperature and stirred for 30min. The solvent was removed under reduced pressure, and the crude mixture purified by column chromatography (petroleum ether - dichloromethane 1:1 v/v). 0.02g of 3,6-dibutyl-*s*-tetrazine was obtained (yield: 15.6%).
^{1}H NMR ($CDCl_3$): δ 1.00 (t, *J*=7.3 Hz, 3H), 1.55 (m, 2H), 1.89 (m, 2H), 4.55 (t, *J*=6.4 Hz, 2H) ppm.
^{13}C NMR ($CDCl_3$): δ 13.8, 19.1, 30.8, 69.8, 166.2 ppm.

Preparation of 3-butyl-6-methoxy-*s*-tetrazine **2b**



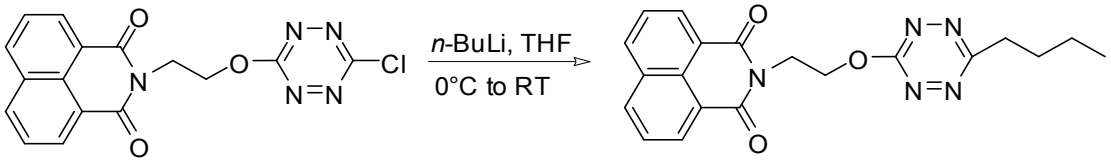
3-Chloro-6-methoxy-*s*-tetrazine **1b** (0.15g, 1.03mmol) was dissolved in dry THF (15ml). The solution was cooled to 0℃ in an ice-bath, and then *n*-BuLi (0.08g, 1.24mmol) was added dropwise. The mixture was allowed to reach room temperature and stirred for 30min. The solvent was removed under reduced pressure, and the crude mixture purified by column chromatography (petroleum ether - dichloromethane 1:1 v/v). 0.09g of 3-butyl-6-methoxy-*s*-tetrazine was obtained (yield: 52%).
^{1}H NMR (400MHz, $CDCl_3$): δ 0.99 (t, *J*=7.3 Hz, 3H), 1.55 (m, 2H), 1.89 (m, 2H), 4.23 (s, 3H), 4.55 (t, *J*=6.4 Hz, 2H) ppm.
^{13}C NMR (100MHz, $CDCl_3$): δ 13.8, 19.1, 30.7, 56.8, 69.9, 166.3 ppm.

Preparation of 3-butyl-6-(perchlorophenoxy)-*s*-tetrazine **2c**



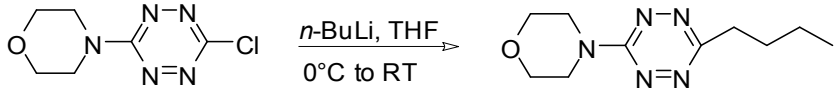
3-Chloro-6-(perchlorophenoxy)-*s*-tetrazine **1c** (0.1g, 0.26mmol) was dissolved in dry THF (15ml). The solution was cooled to 0℃ in an ice-bath, and then *n*-BuLi (0.02g, 0.31mmol) was added dropwise. The mixture was allowed to reach room temperature and stirred for 30min. The solvent was removed under reduced pressure, and the crude mixture purified by column chromatography (petroleum ether - dichloromethane 1:1 v/v). 0.03g 3-butyl-6-(perchlorophenoxy)-*s*-tetrazine was obtained (yield: 30%).
^{1}H NMR (400MHz, $CDCl_3$): δ 1.00 (t, *J*=7.3 Hz, 3H), 1.56 (m, 2H), 1.91 (m, 2H), 4.61 (t, *J*=6.8 Hz, 2H) ppm.
^{13}C NMR (100MHz, $CDCl_3$): δ 13.8, 19.1, 30.7, 70.7, 127.8, 132.5, 132.7, 145.1, 165.1, 167.4 ppm.

Preparation of -3-butyl-6-(naphtalimidylethoxy)-*s*-tetrazine **2d**



Chloro-naphthalimidylethoxy-*s*-tetrazine **1d** (0.053g, 0.15mmol) was dissolved in dry THF (15ml). The solution was cooled to 0℃ in an ice-bath, and then *n*-BuLi (0.01g, 0.18mmol) was added dropwise. The mixture was allowed to reach room temperature and stirred for 30min. The solvent was removed under reduced pressure, and the crude mixture purified by column chromatography (petroleum ether - dichloromethane 1:1 v/v). 0.01g of 3-butyl-6-(naphtalimidylethoxy)-*s*-tetrazine was obtained (yield: 18%).
^{1}H NMR (400MHz, $CDCl_3$): δ 1.01 (t, *J*=7.3 Hz, 3H), 1.55 (m, 2H), 1.89 (m, 2H), 4,57 (t, *J*=6.6 Hz, 2H), 4.75 (t, *J*=5.5 Hz, 2H), 4.97 (t, *J*=5.5 Hz, 2H), 7.76 (dd, *J*=8.2 and 7.3 Hz, 2H), 8.23 (dd, *J*=8.5 and 1.1 Hz, 2H), 8.58 (dd, *J*=7.3 and 0.9 Hz, 2H) ppm.
^{13}C NMR (100MHz, $CDCl_3$): δ: 13.9, 19.1, 30.8, 38.4, 66.6, 69.9, 122.5, 127.1, 128.4, 131.7, 134.4, 164.5, 165.9, 166.3 ppm.

Preparation of 3-butyl-6-(morpholin-4-yl)-*s*-tetrazine **2e**



3-chloro-6-(morpholin-4-yl)-*s*-tetrazine **1e** (0.1g, 0.5mmol) was dissolved in dry THF (15ml). The solution was cooled to 0℃ in an ice-bath, and then *n*-BuLi (0.032g, 0.5mmol) was dropped under 0℃. The mixture was stirring 30min at room temperature. The solution was cooled to 0℃ in an ice-bath, and then *n*-BuLi (0.01g, 0.18mmol) was added dropwise. The mixture was allowed to reach room temperature and stirred for 30min. The solvent was removed under reduced pressure, and the crude mixture purified by column chromatography

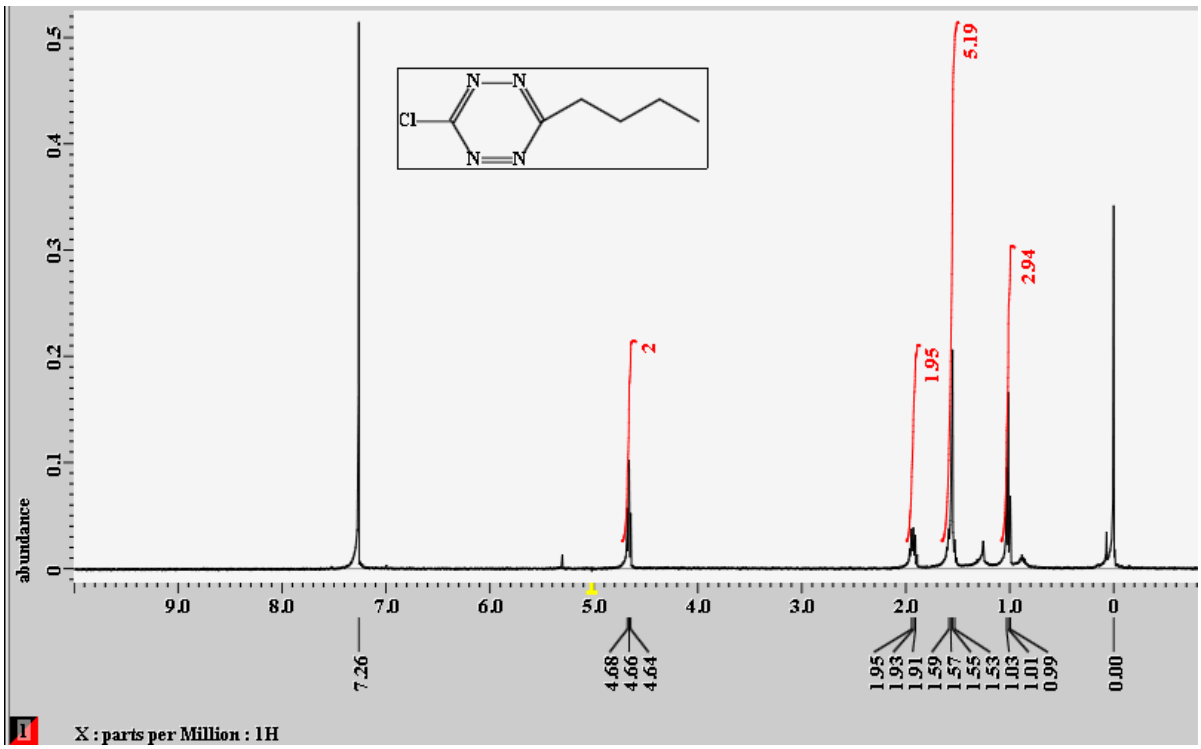
(petroleum ether - dichloromethane 1:1 v/v). 0.05g of 3-butyl-6-(morpholin-4-yl)-*s*-tetrazine was obtained (yield: 45%).

^{1}H NMR (400MHz, $CDCl_3$): δ 0.97 (t, *J*=7.6 Hz, 3H), 1.51 (m, 2H), 1.85 (m, 2H), 3.85 (m, 8H), 4.48 (t, *J*=6.6 Hz, 2H) ppm.

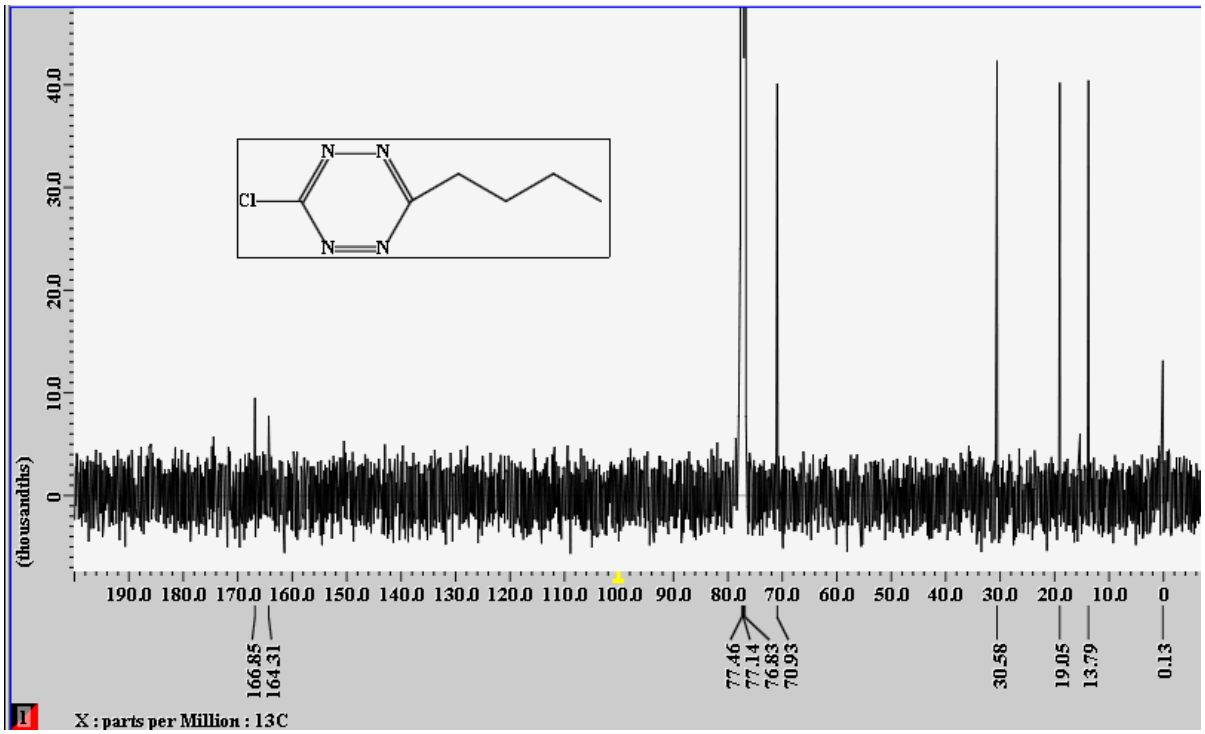
^{13}C NMR (100MHz, $CDCl_3$): δ 13.9, 19.1, 30.9, 44.3, 66.5, 69.0, 161.5, 164.5 ppm.

Tetrazine **2a**

^{1}H NMR

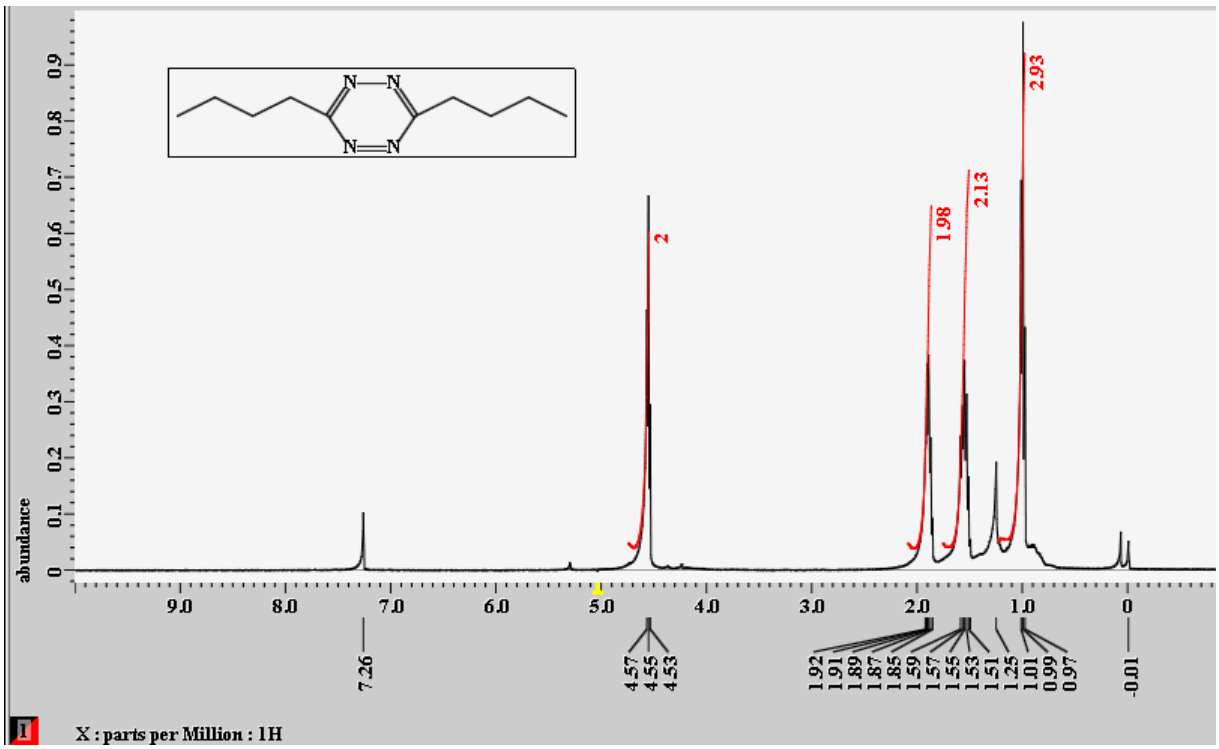


^{13}C NMR

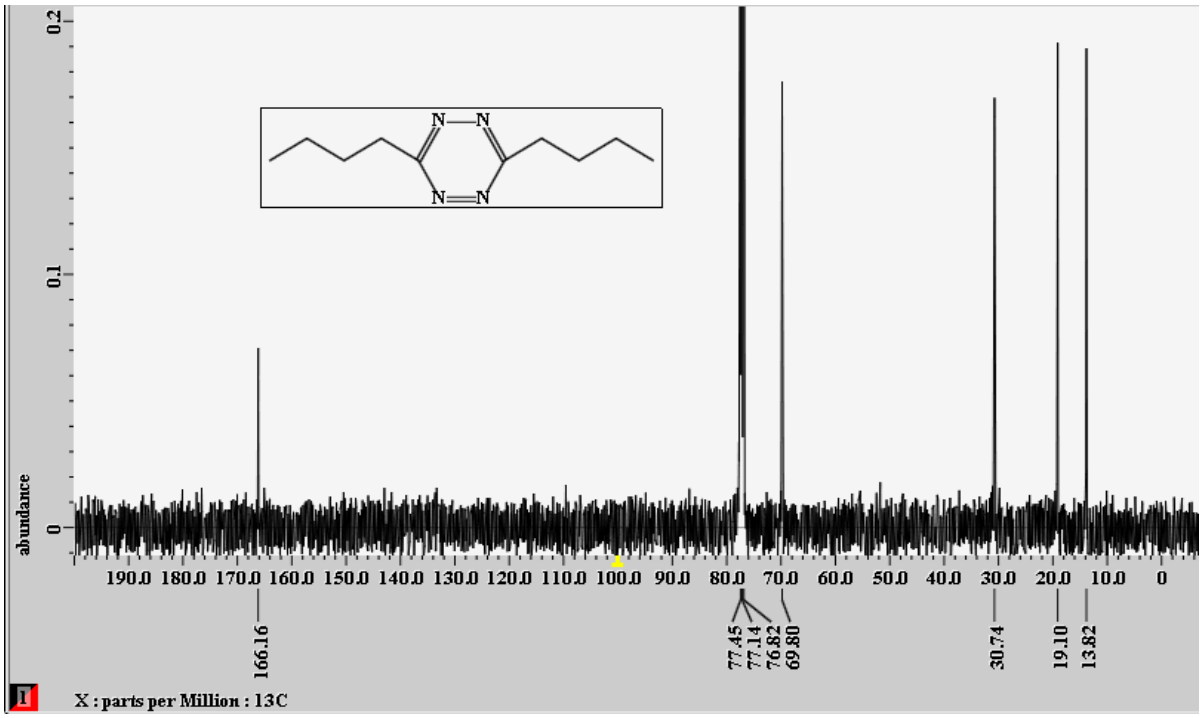


Tetrazine **3a**

^{1}H NMR

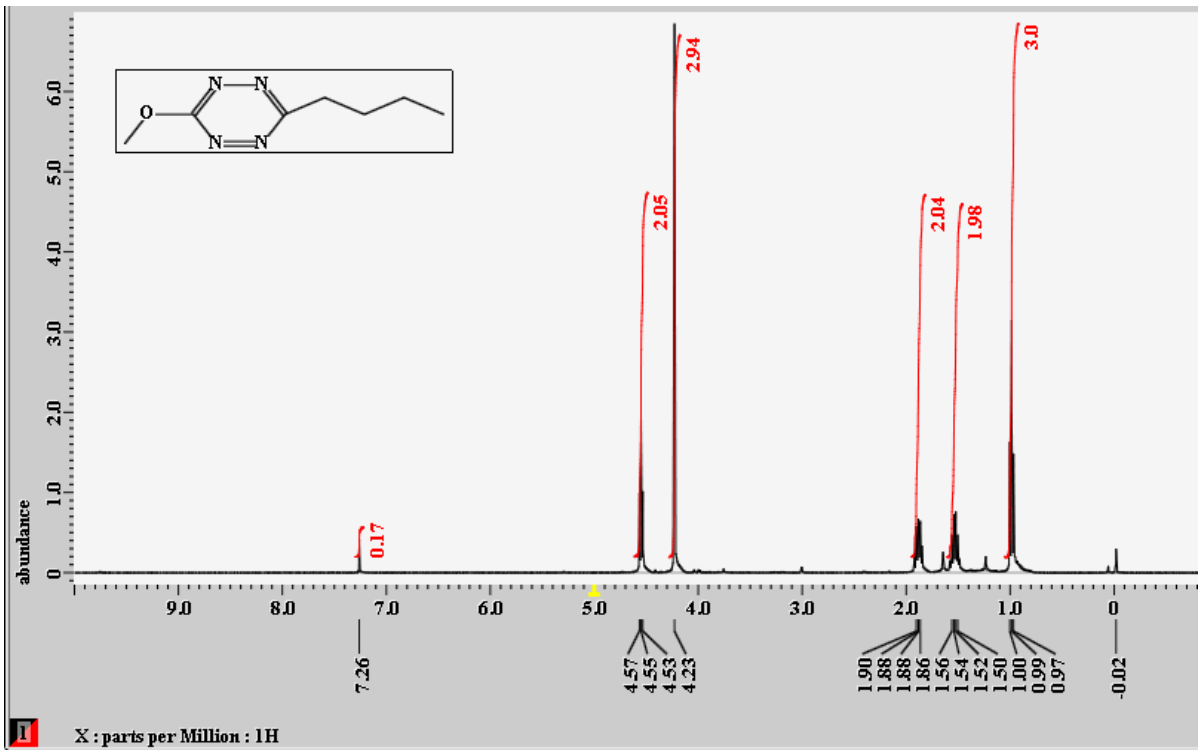


^{13}C NMR

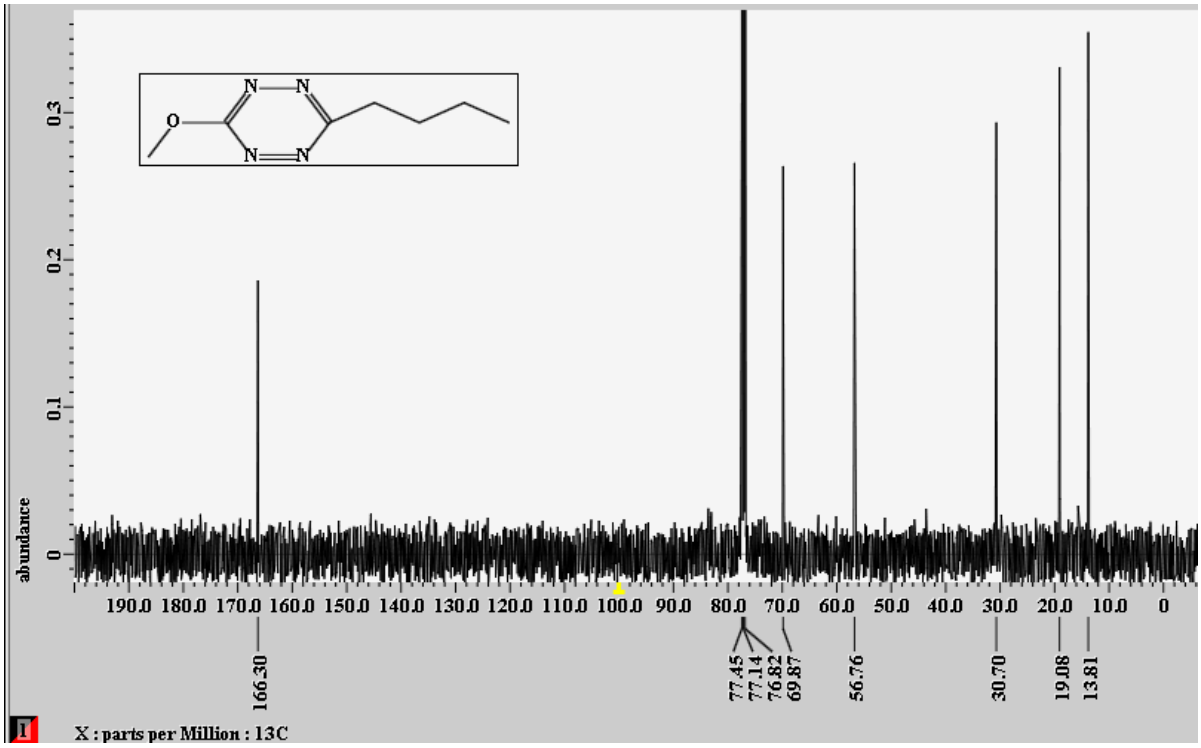


Tetrazine **2b**

^{1}H NMR

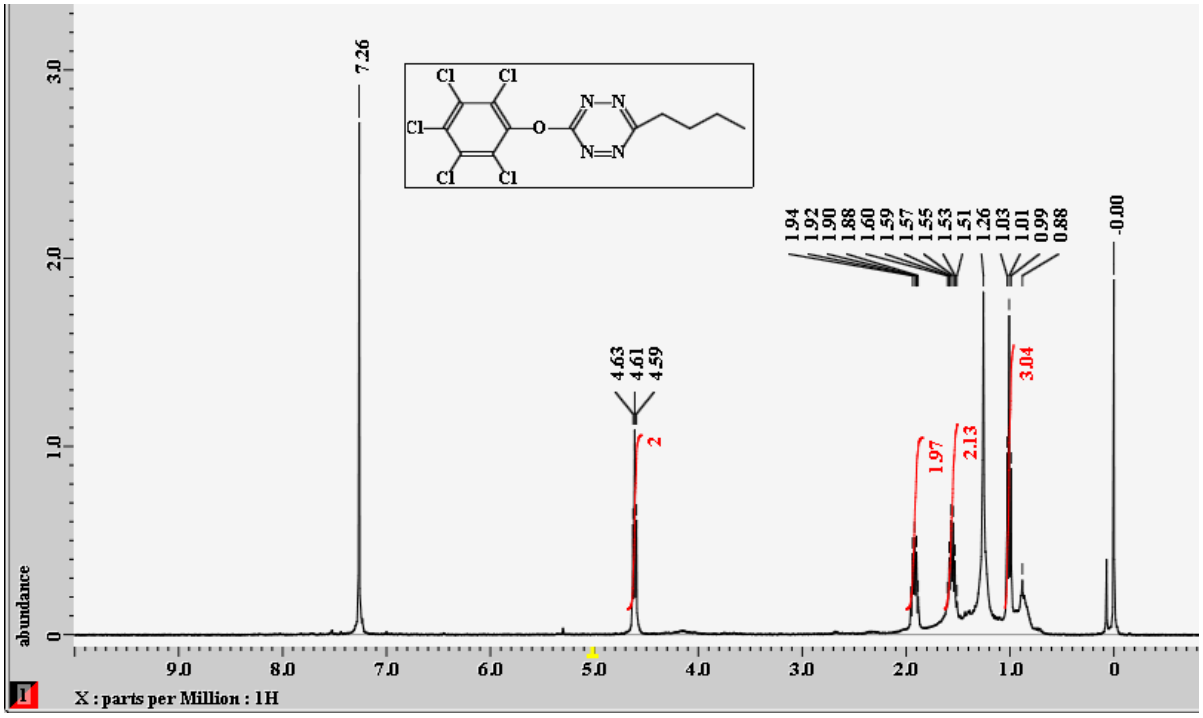


^{13}C NMR

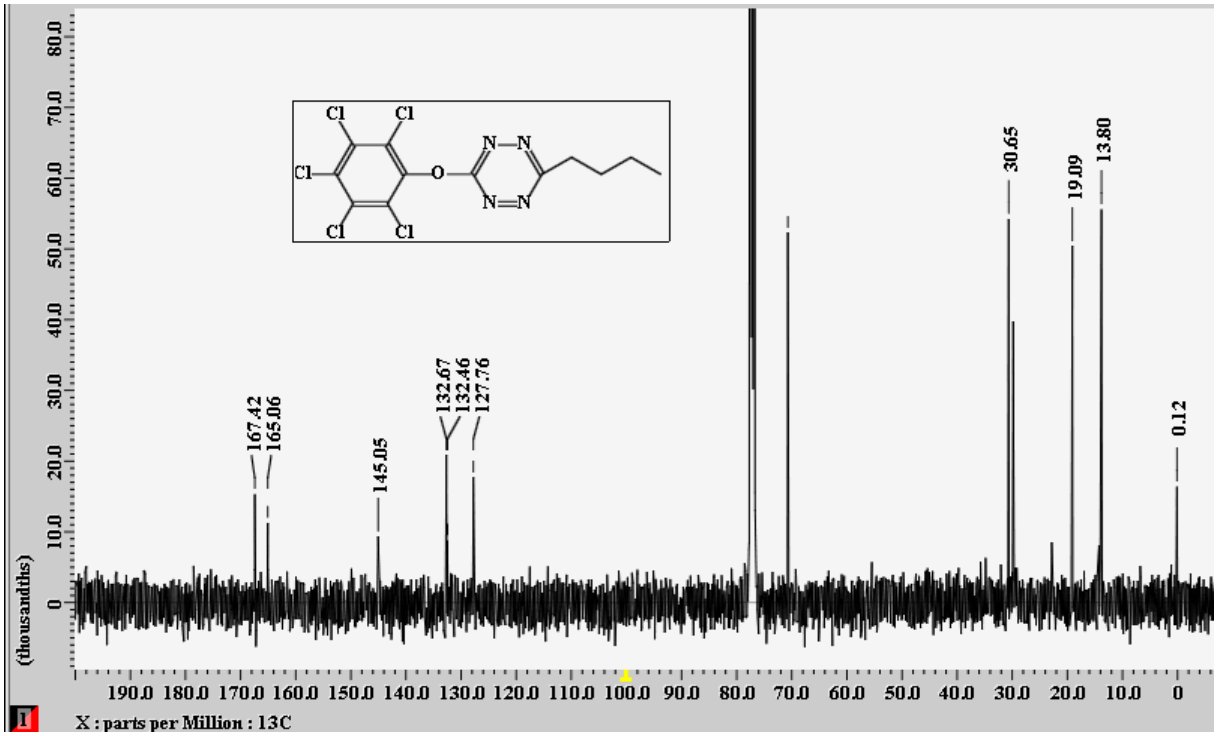


Tetrazine **2c**

^{1}H NMR

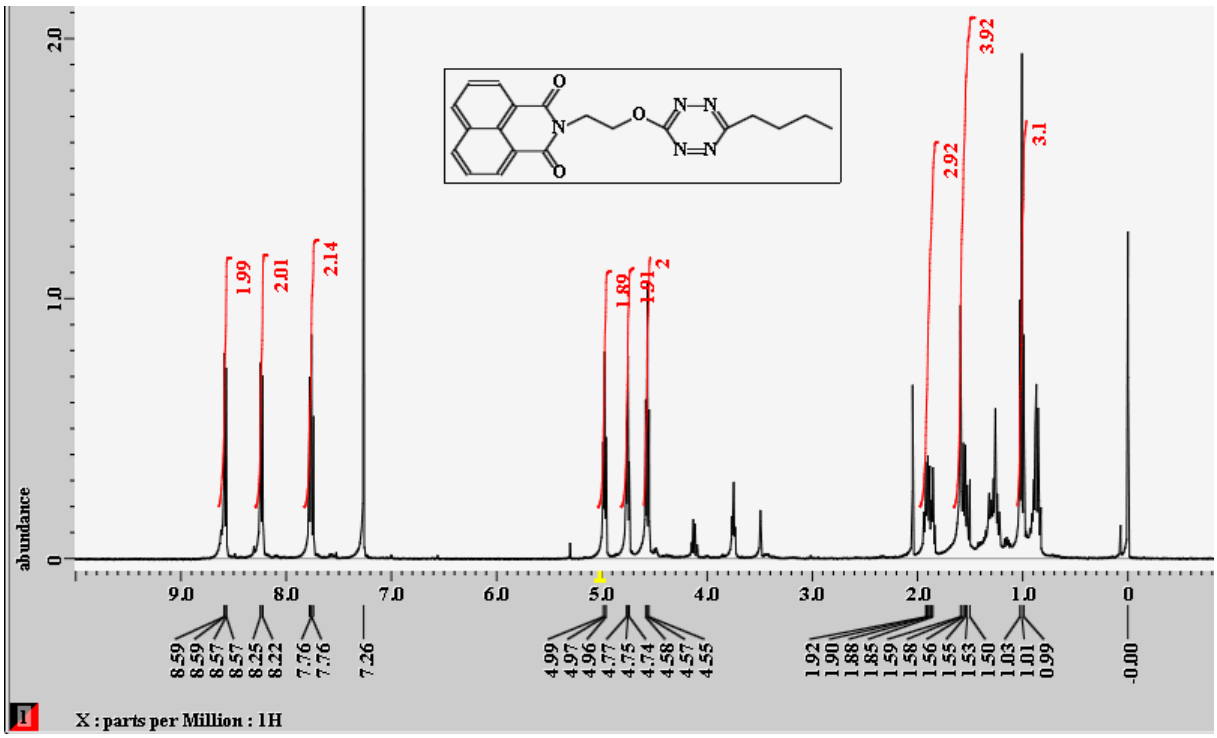


^{13}C NMR

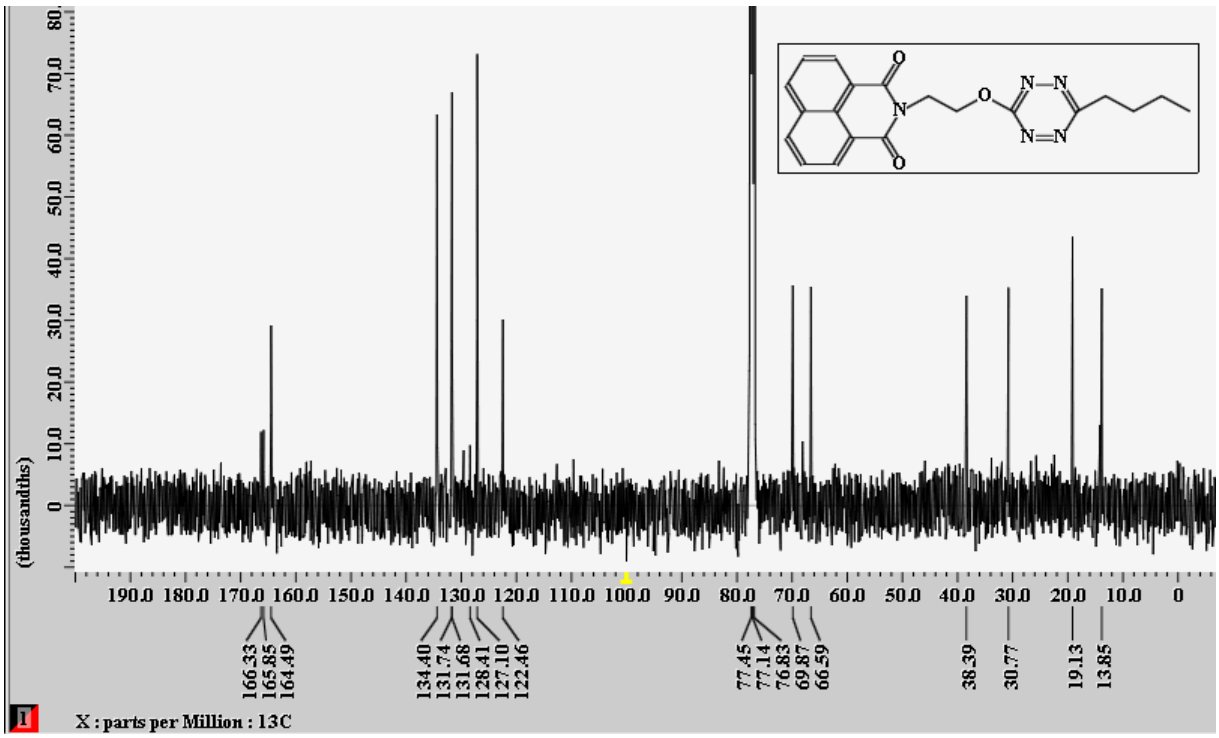


Tetrazine **2d**

^{1}H NMR

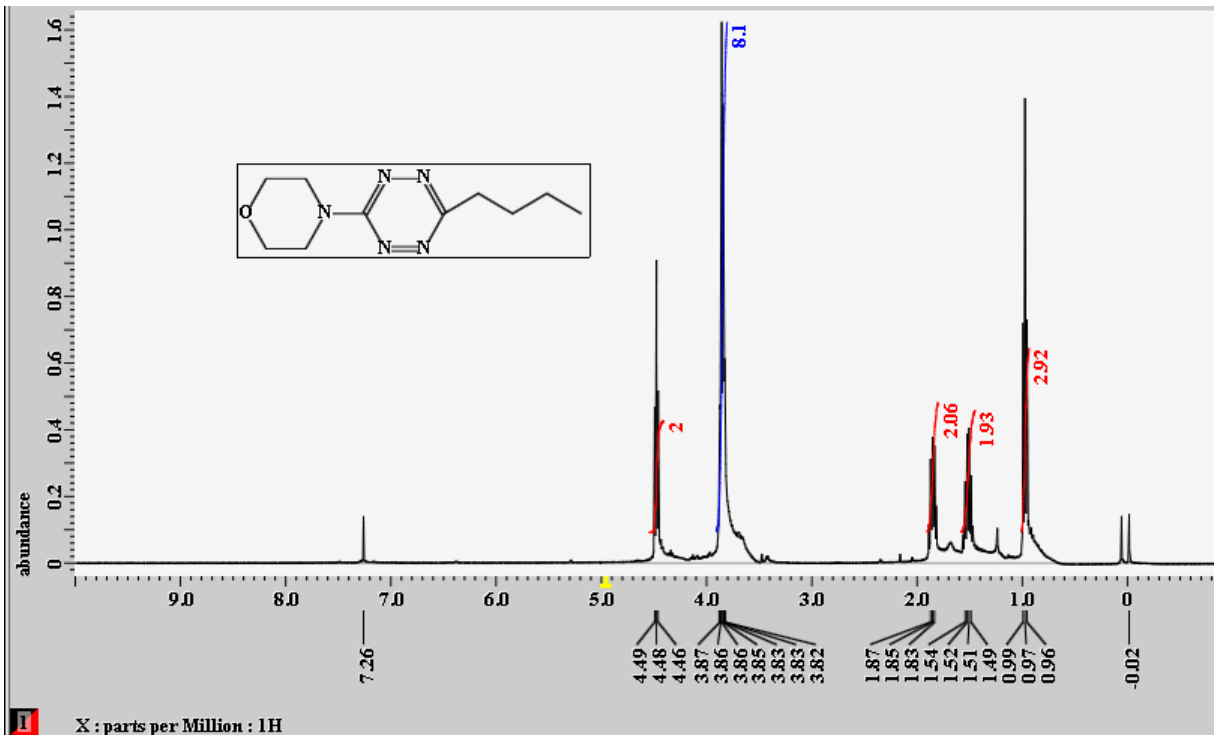


^{13}C NMR

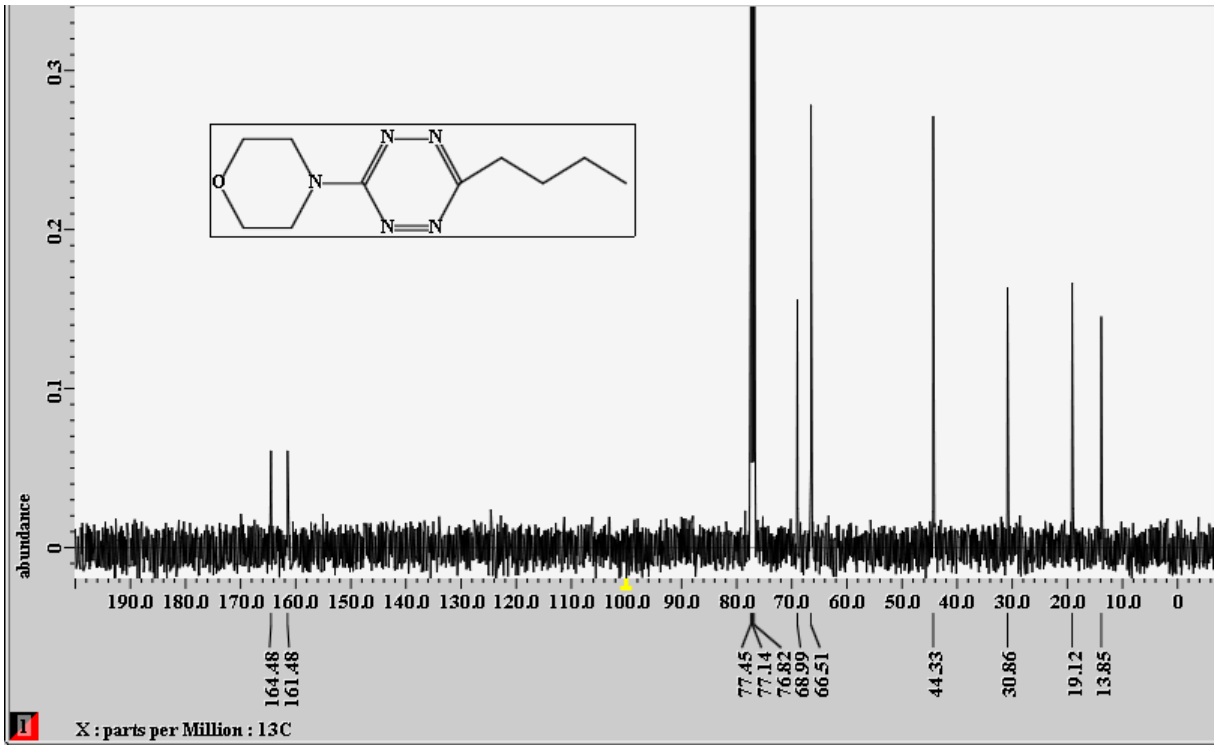


Tetrazine **2e**

^{1}H NMR



^{13}C NMR



Absorption and fluorescence spectroscopies

All solvents were of spectroscopic grade.

Steady-state spectroscopy

All spectroscopic experiments were carried out in DCM (spectroscopic grade from SDS) and at concentrations *ca.* 10 μmol.L^{-1} for absorption spectra, and *ca.* 1 μmol.L^{-1} for fluorescence spectra where only dilute solutions with an absorbance below 0.1 at the excitation wavelength λ_{ex} were used. UV/vis absorption spectra were recorded on a Varian Cary 500 spectrophotometer. Fluorescence emission and excitation spectra were measured on a SPEX fluorolog-3 (Horiba Jobin-Yvon). For emission fluorescence spectra, the excitation wavelengths were usually set equal to the maximum of the corresponding absorption spectra. Sulforhodamine 101 in ethanol (Φ_F = 0.9) was used for the determination of the relative fluorescence quantum yields.