Rigid tetrazine fluorophore conjugates with fluorogenic properties in inverse electron demand Diels-Alder reaction

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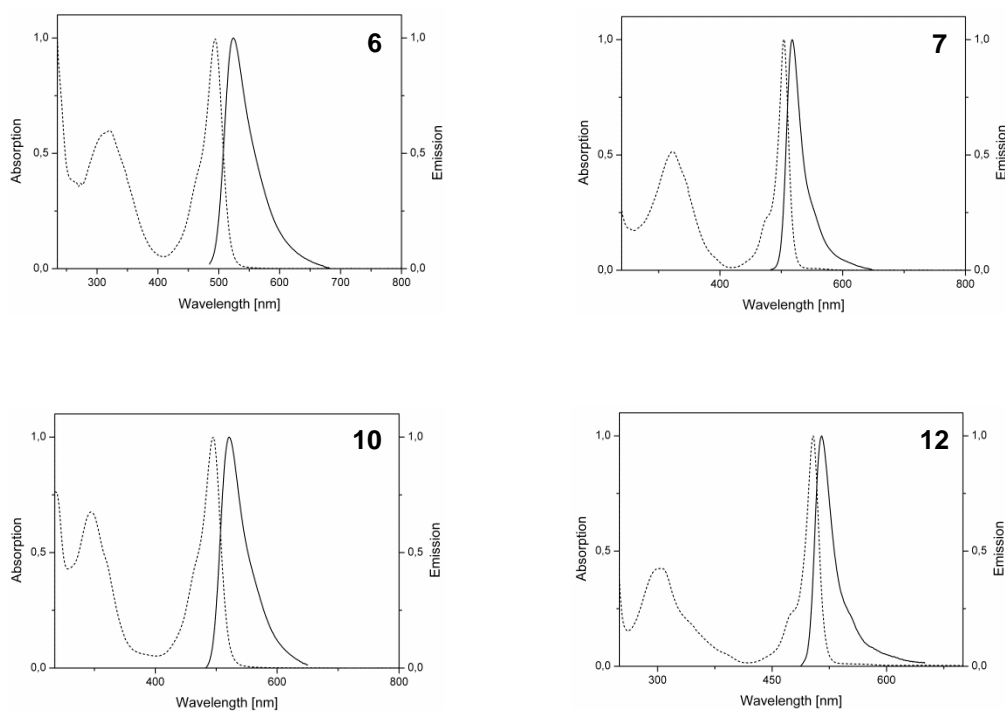
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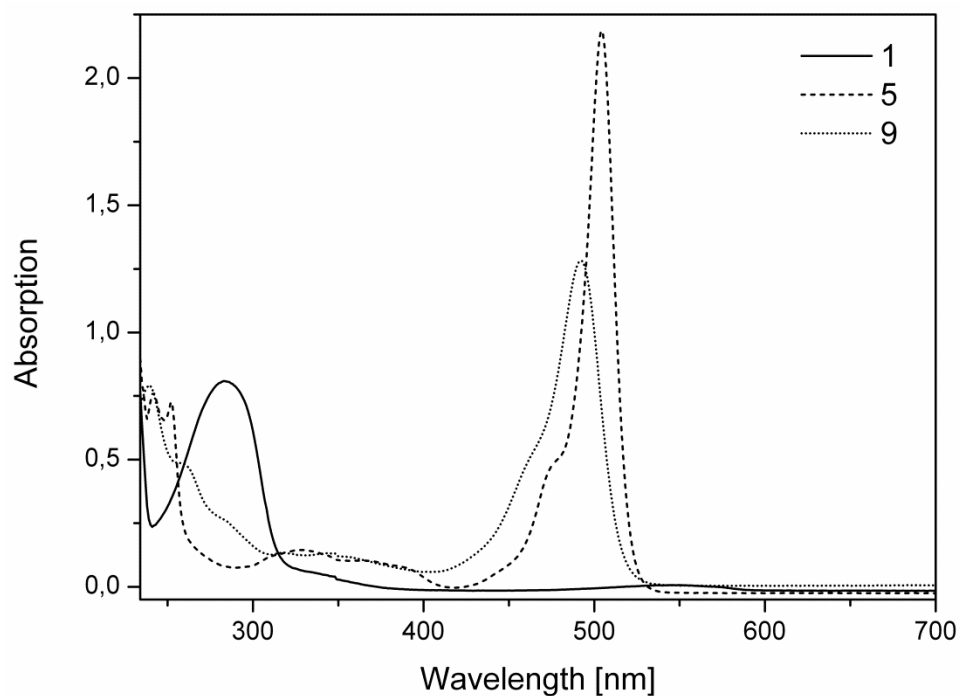## *Electronic Supplementary Information (ESI)*

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1 Normalized UV-Vis and Fluorescence spectra of compounds 6, 7,10 and 12
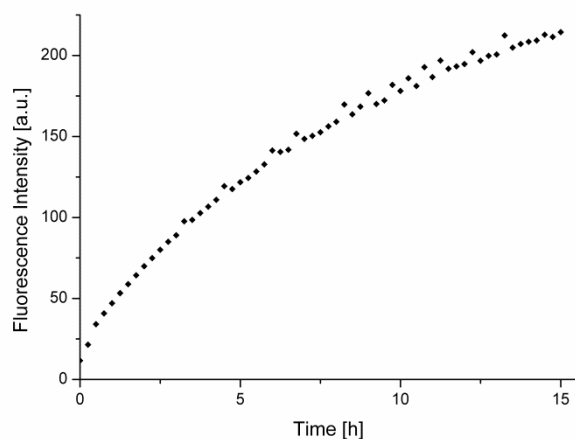

2 UV-Vis spectra of 1, 5, and 9

Equimolar solutions of **1**, **5** and **9** (30 µM) were measured in chloroform and phosphate buffer respectively. Absorption maxima of the displayed spectral range are as follows:

284 nm (**1**), 504 nm (**5**) and 492 nm (**9**).

## 3   Fluorescence timecourse of the reaction of 12 with TCO-5-OH

Compound **12** (1 µM) was reacted with a 2.5-fold molar excess of *trans*-cycloocten-5-ol in chloroform and the fluorescence peak intensity was monitored in 15 min intervals (irradiation @ 485 nm). A 18.5-fold increase in fluorescence was observed after 15 h (full conversion after 24 h).

4   Dependence of fluorescence emission of 12 on solvent polarity
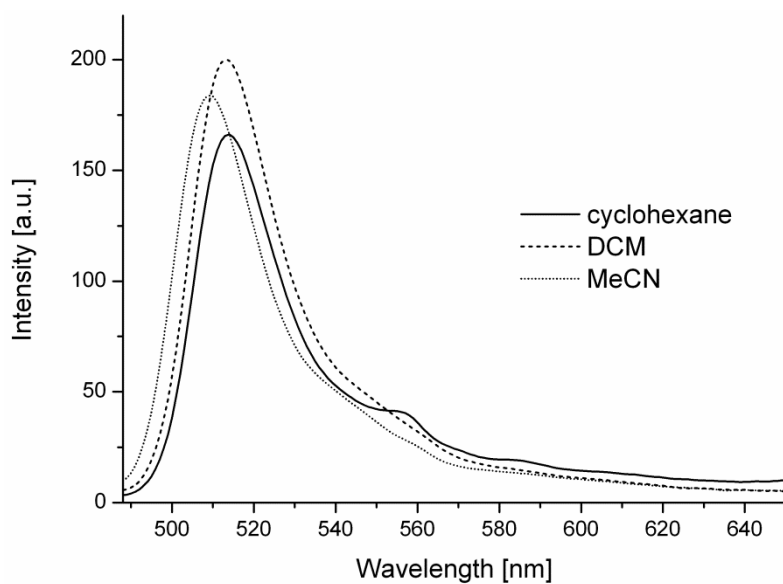
The fluorescence emission of 1 µM solutions of **12** in cyclohexane, dichloromethane and acetonitrile were measured in triplicate under irradiation @ 480 nm.

5   Transient Absorption spectra of 7
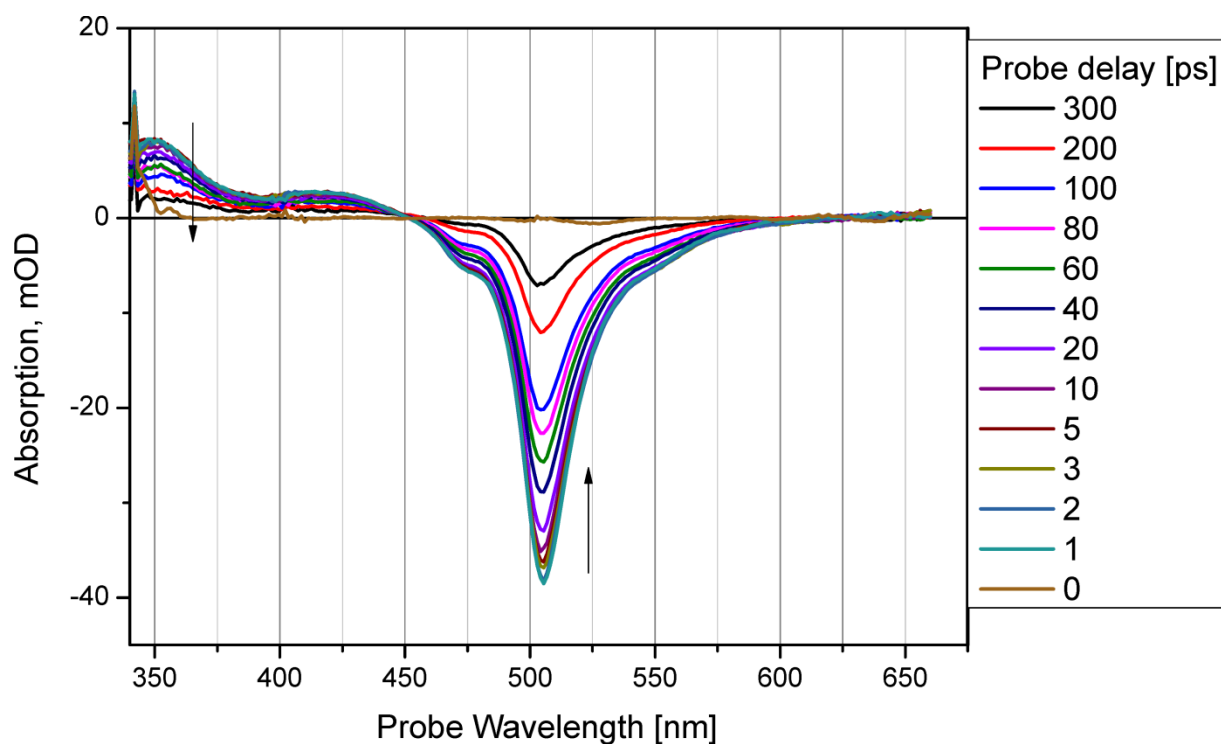
Transient absorption spectra of **7** selected for different probe delays. Arrows indicate the evolution of the probe delay. Transient spectra show one main negative feature at about 500 nm due to the ground state bleach and stimulated emission. A red shifted wing due to the stimulated emission can be observed at about 550 nm. An excited state absorption band is blue detuned from the maximum of the ground state bleach. The presence of an isobestic point at about 450 nm suggests that the stimulated emission as well as excited state absorption are originated from the same excited state, which decay back to the ground state.

6 Analysis of experimental data for agreement with distance dependence in RET

Förster resonance energy transfer (FRET) is often used as a molecular ruler, as it allows determining the distance between the donor and acceptor of a FRET-pair. The rate of the energy transfer between a donor and acceptor separated by the distance R is described by the following equation[1]:

$$k_T(R) = \frac{1}{\tau_D}\left(\frac{R_0}{R}\right)^6 \quad \text{, or} \quad k_T(R) = \frac{Q_D \kappa^2}{\tau_D R^6}\left(\frac{9000(ln10)}{128\pi^5 N n^4}\right)\int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \qquad (1)$$

Where, Q_D is the quantum yield of the donor D in absence of acceptor A, n is the refractive index of the medium, N is the Avogadro number, R is the distance between donor and acceptor and τ_D is the lifetime of the Donor D in absence of the acceptor A. The integral describes the spectral overlap between donor emission and acceptor absorption.

From both equations (1) follows the term (2) for the Förster radius R_0 respectively $R_0{}^6$.

$$R_0{}^6 = \frac{9000(ln10)Q_D\kappa^2}{128\pi^5 N n^4}\int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \qquad (2)$$

Considering that we assume resonance energy transfer (RET) as the dominating mechanism by which the BODIPY fluorescence within the tetrazine BODIPY conjugates is quenched, the following correlation between the energy transfer efficiency and the interchromophore distance must hold true:

$$E = \frac{1}{1+\left(\frac{R}{R_0}\right)^6} \qquad \text{with } R_0 = \text{Foerster radius,} \qquad (3)$$

$$R_0 = \frac{R}{\sqrt[6]{\frac{1}{E}-1}} \quad , \qquad (4)$$

$$E = 1 - \frac{I_{DA}}{I_D} \qquad (5)$$

with I_{DA} = Fluorescence intensity of donor in presence of acceptor and I_D = Fluorescence intensity of donor in absence of acceptor.

Equation (3) can be rewritten as equation (4). Combination of equation (4) and (5) leads to equation (6) which allows the calculation of the Förster radius R_0 from R and $\frac{I_{DA}}{I_D}$.

$$R_0 = \frac{R}{\sqrt[6]{\frac{1}{1-\frac{I_{DA}}{I_D}}-1}} \qquad (6)$$

The BODIPY tetrazine conjugates **7** and **12** possess the same relative orientation between donor (BODIPY) and acceptor (tetrazine). Due to the rigid molecular structure of both compounds the interchromophore distance within each molecule is fixed. Therefore we are able to calculate the

distance R between donor and acceptor from the molecular geometry† to $R = 12.3$ Å for compound **12**.

With $\left(\frac{I_{DA}}{I_D}\right)^{-1}$ reflecting the "turn on" (23.4 fold) of fluorescence, when compound **12** is fully converted to the product of inverse electron demand Diels-Alder reaction, we are able to calculate the Förster radius R_0 to 20.7 Å applying equation (6).
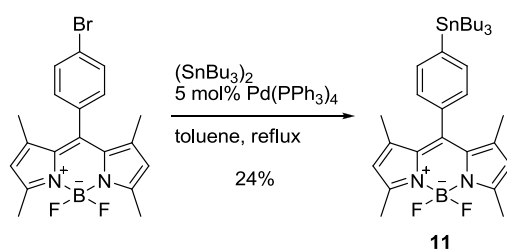
From equation (6) the "turn on" can be written as function of R as follows:

$$(I_{DA}/I_D)^{-1} = F(R) = \left(1 - \frac{1}{1+\left(\frac{R}{R_0}\right)^6} \right)^{-1} \qquad . \qquad (7)$$

With $R_0 = 20.7$ Å, calculated from compound **12** we can plot the "turn on" as function of R.
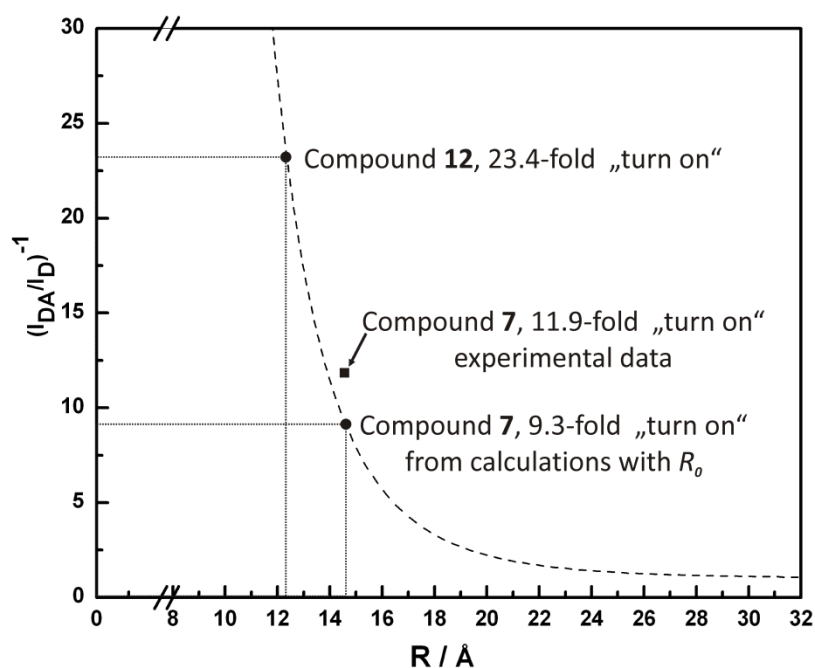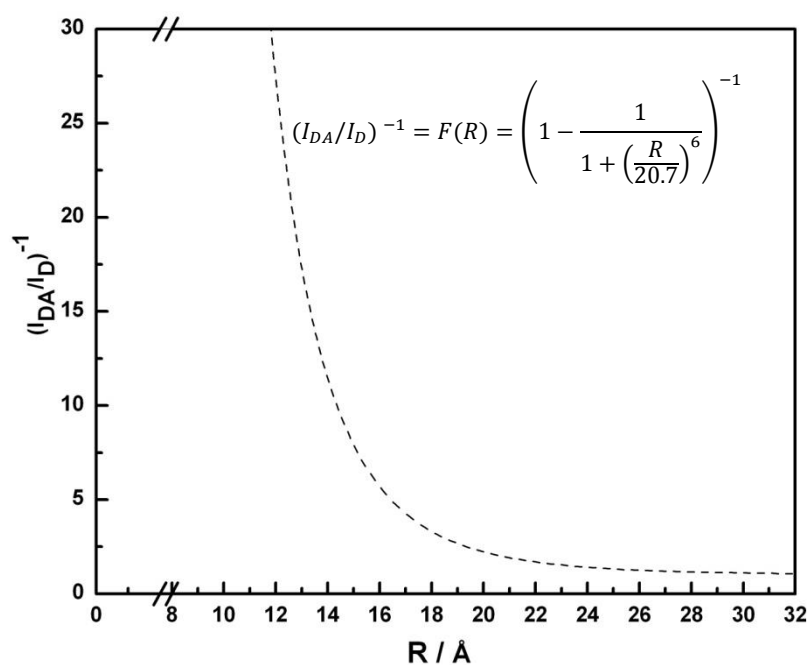
7 Synthesis of compound 11

8 Synthesis of compound 13
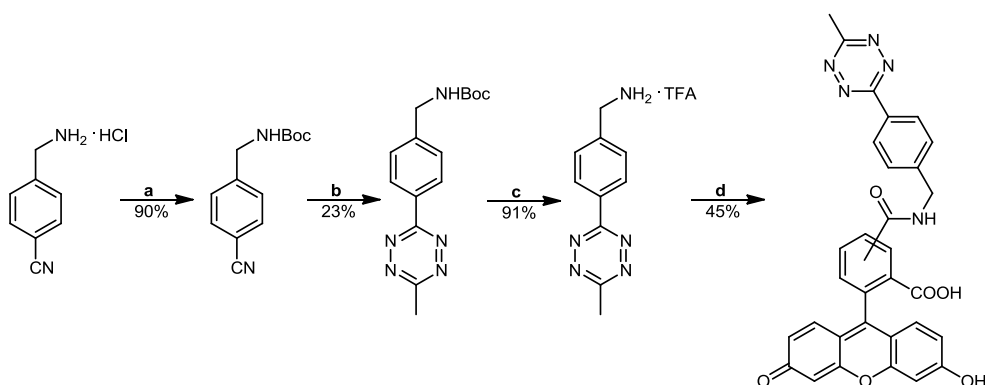
a[2]: 1) aq. NaOH, 2) Boc$_2$O; **b**[3]: 1) MeCN, N$_2$H$_4$.H$_2$O, 2) aq. NaNO$_2$/HCl; **c**: 50% TFA/DCM; **d**: Fluorescein-5/6-NHS-ester isomeric mixture, NEt$_3$, DMF.
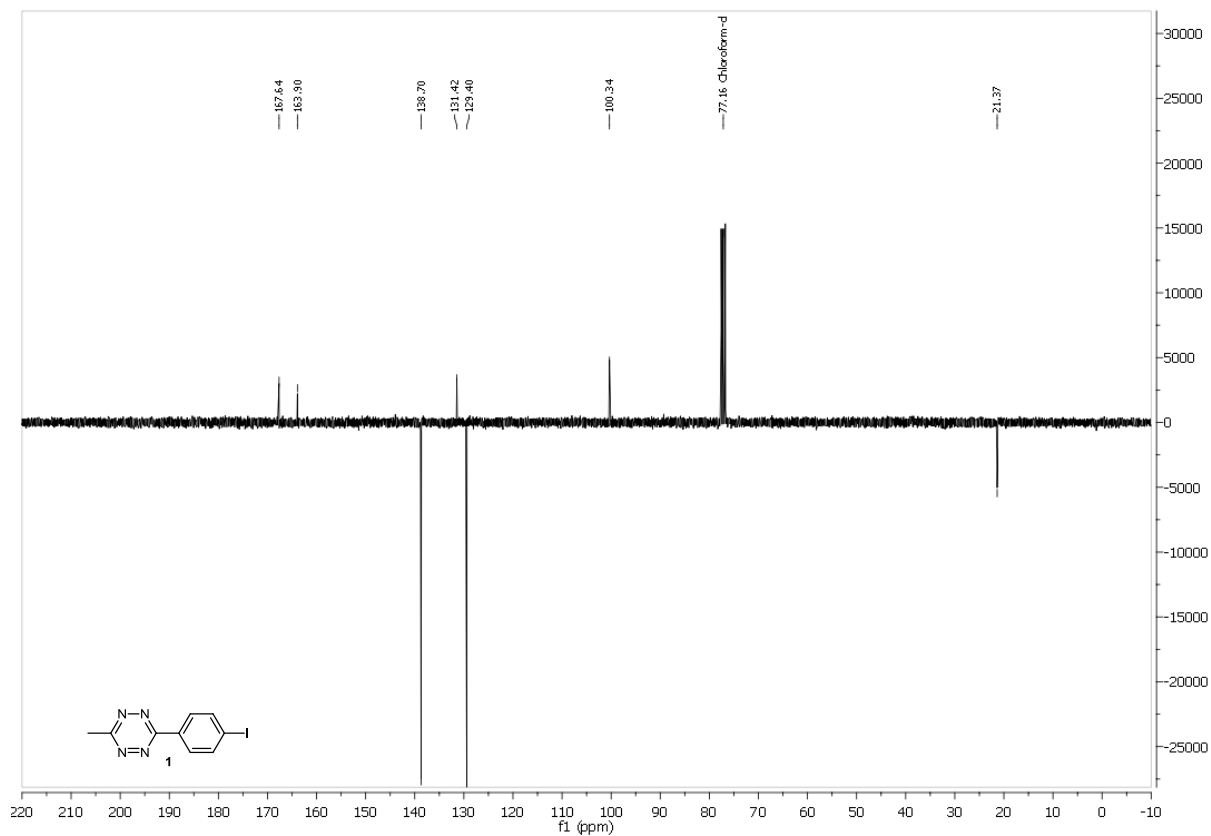
The synthetic steps shown above were performed combining literature known and standard procedures. The homologue compound with an H-atom at position 6 of the tetrazine ring has been described previously.[4]

9 NMR Spectra

^{1}H-NMR spectrum of compound **1** (CDCl$_3$)
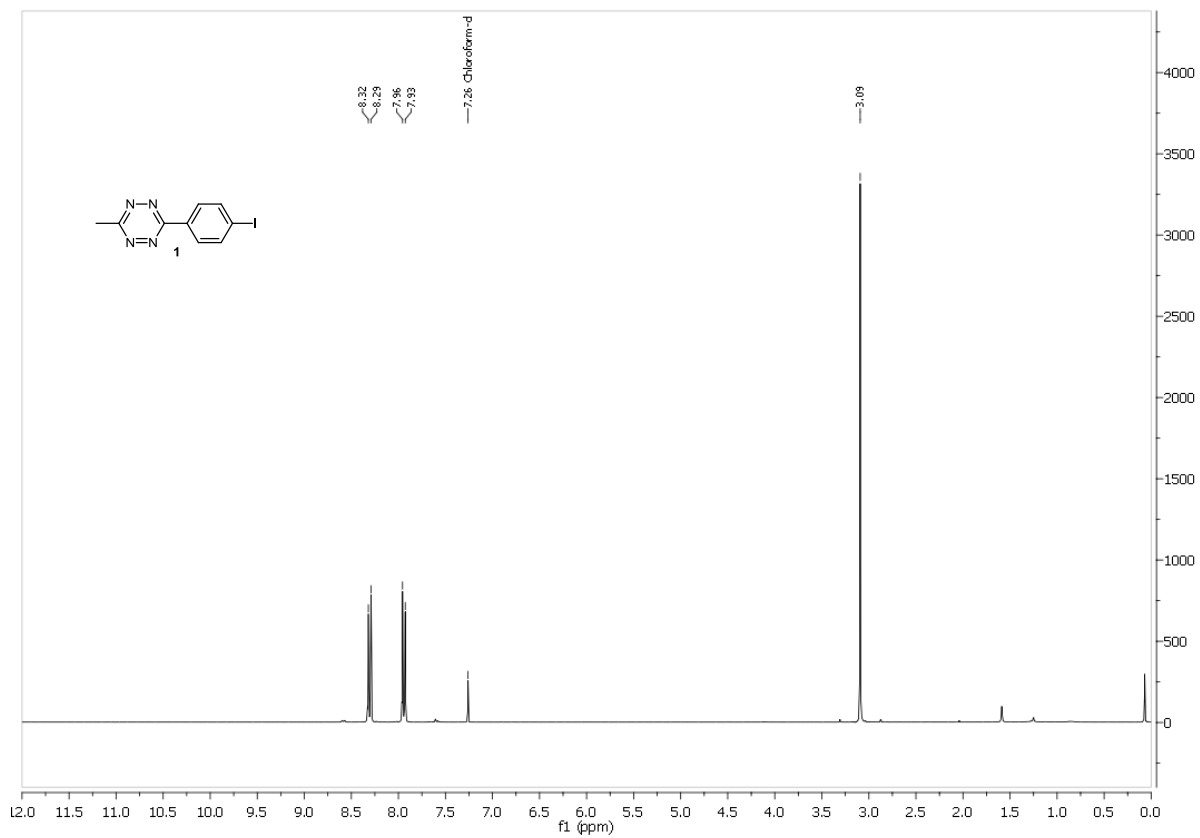
^{13}C-NMR spectrum of compound **1** (CDCl$_3$)

^{1}H-NMR spectrum of compound **2** (CDCl$_3$)
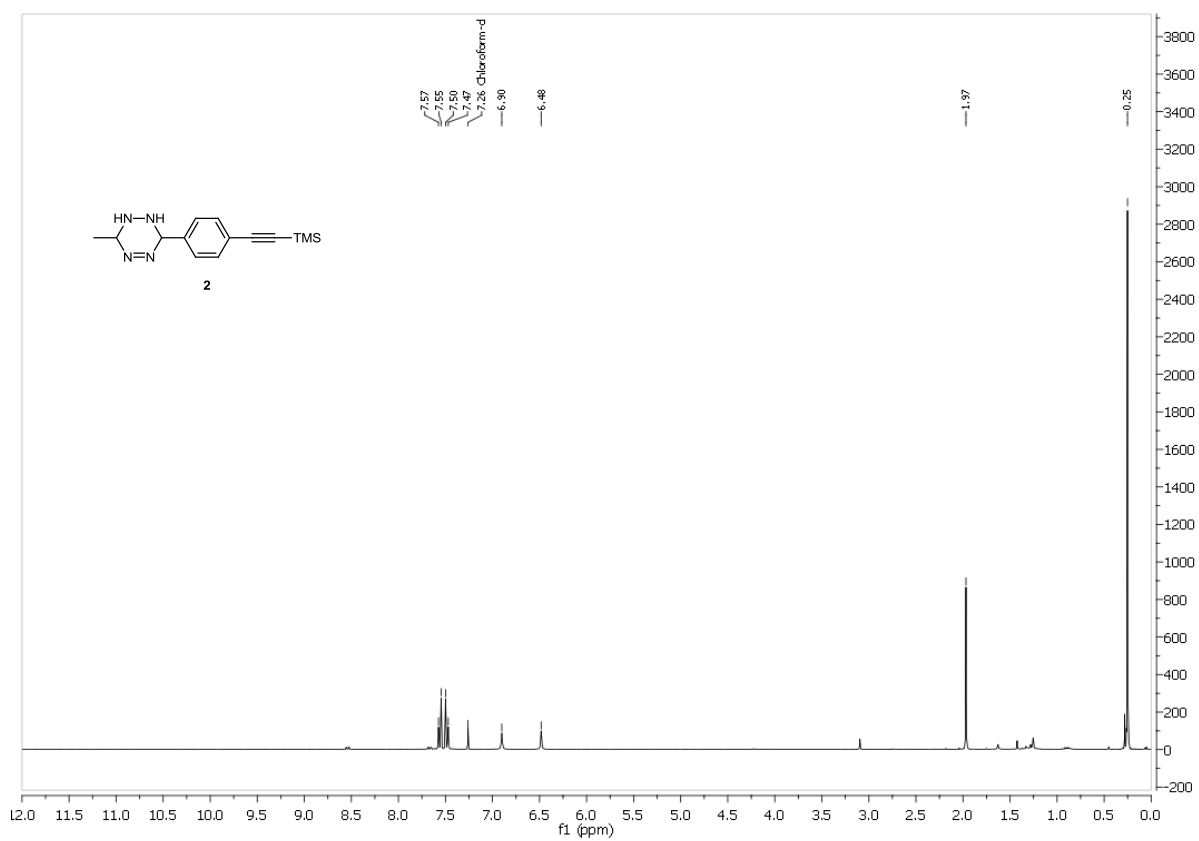
^{1}H-NMR spectrum of compound **3** (CDCl$_3$)
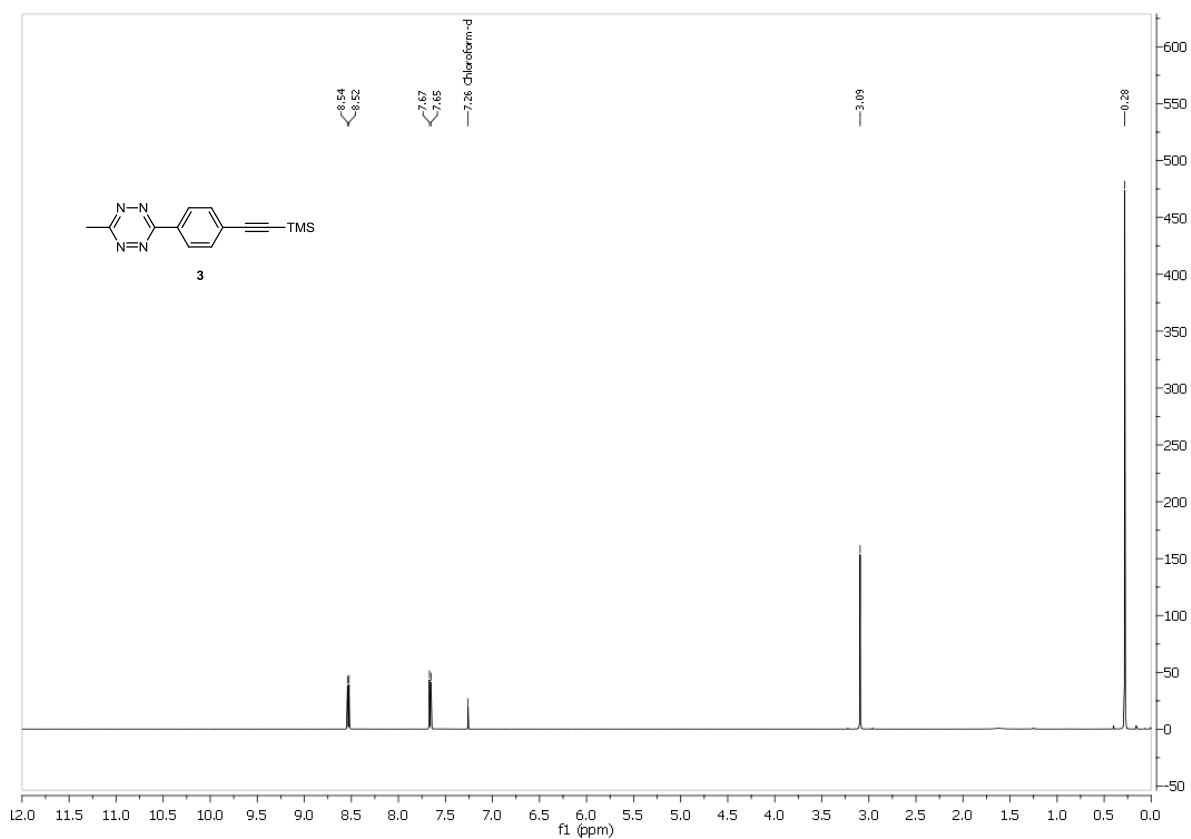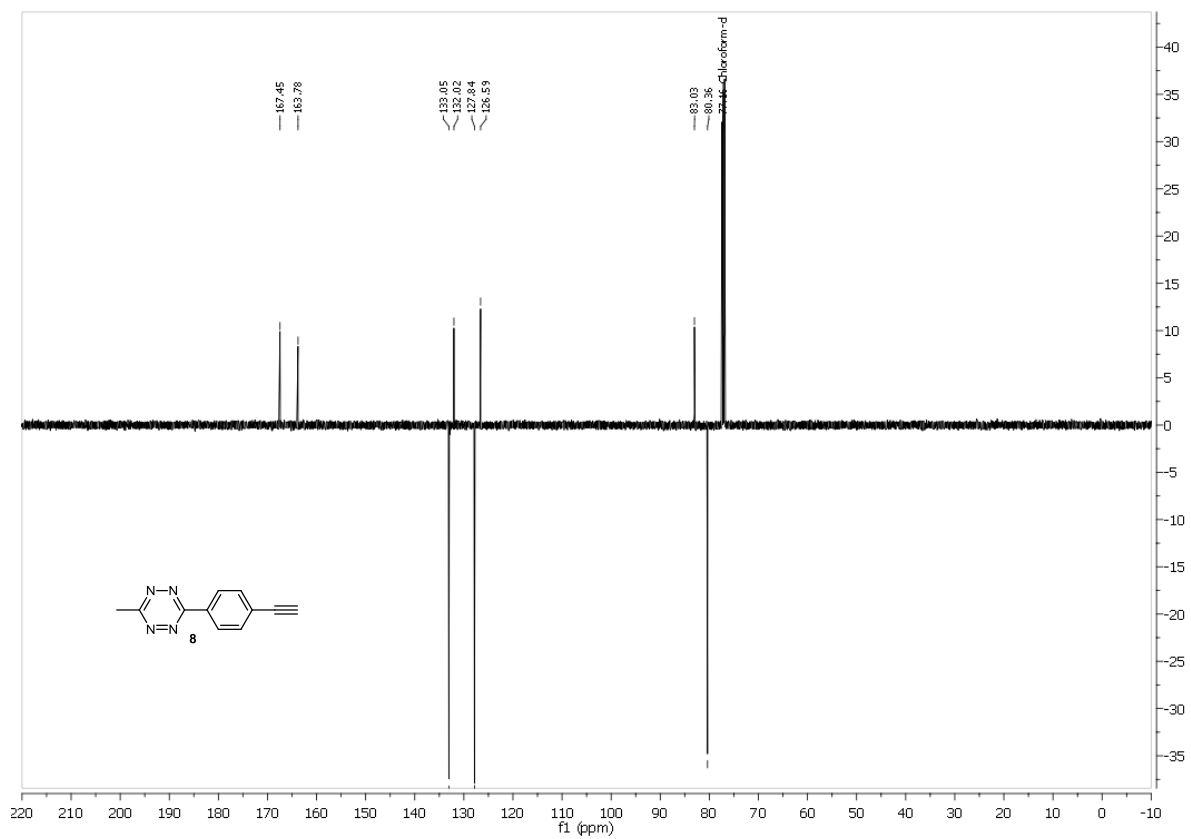

^{13}C-NMR spectrum of compound **3** (CDCl$_3$)
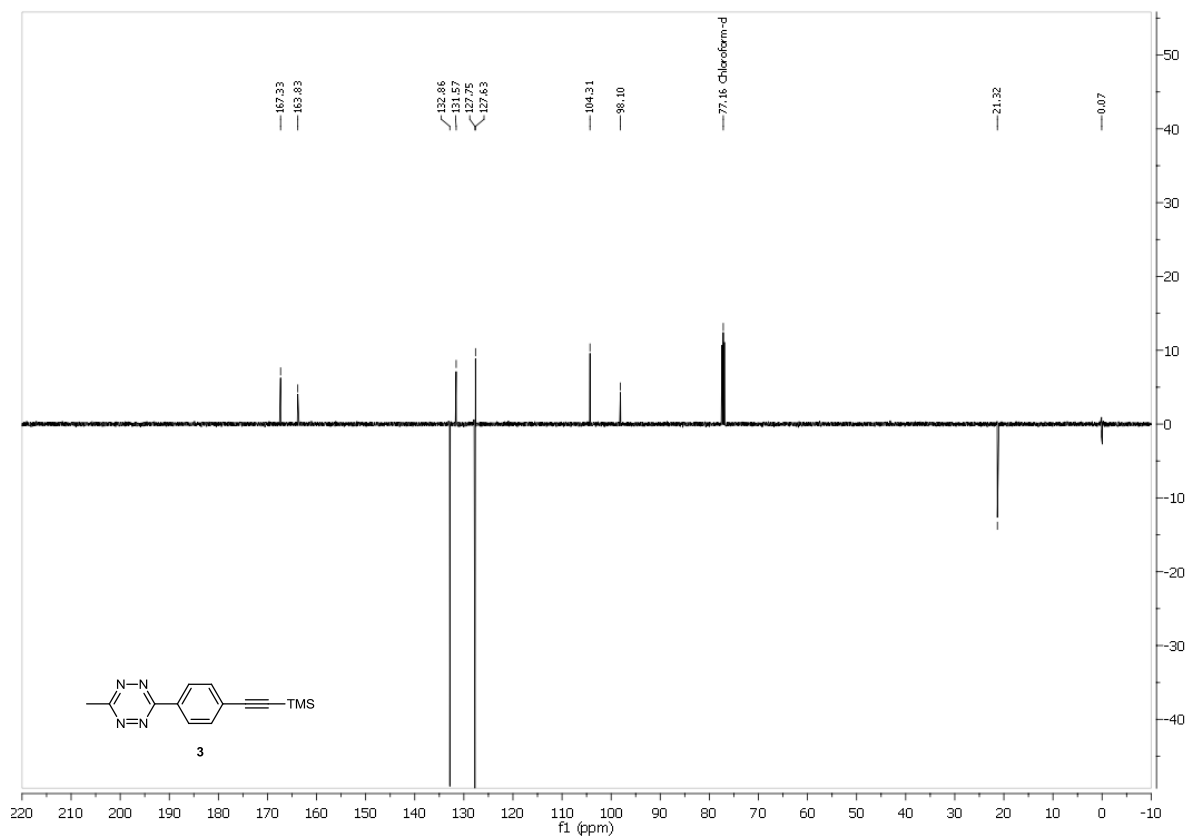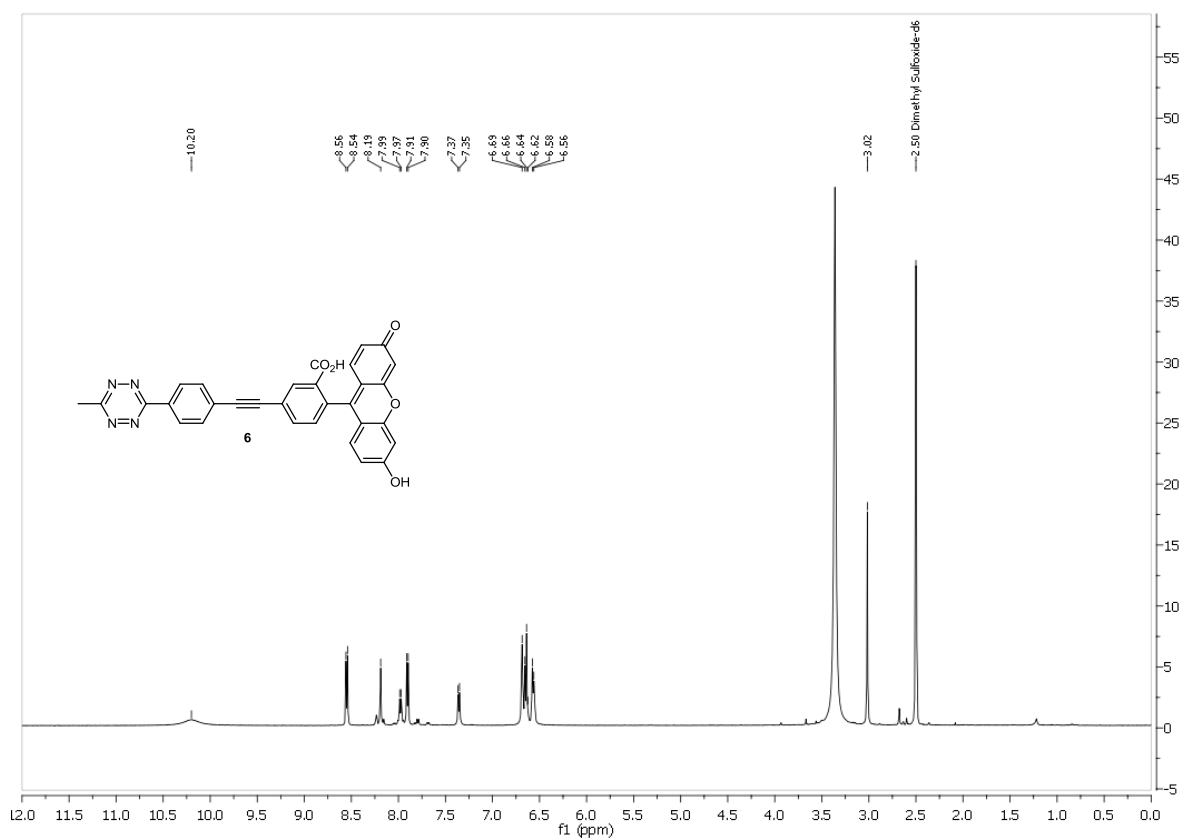
¹H-NMR spectrum of compound **8** (CDCl₃)
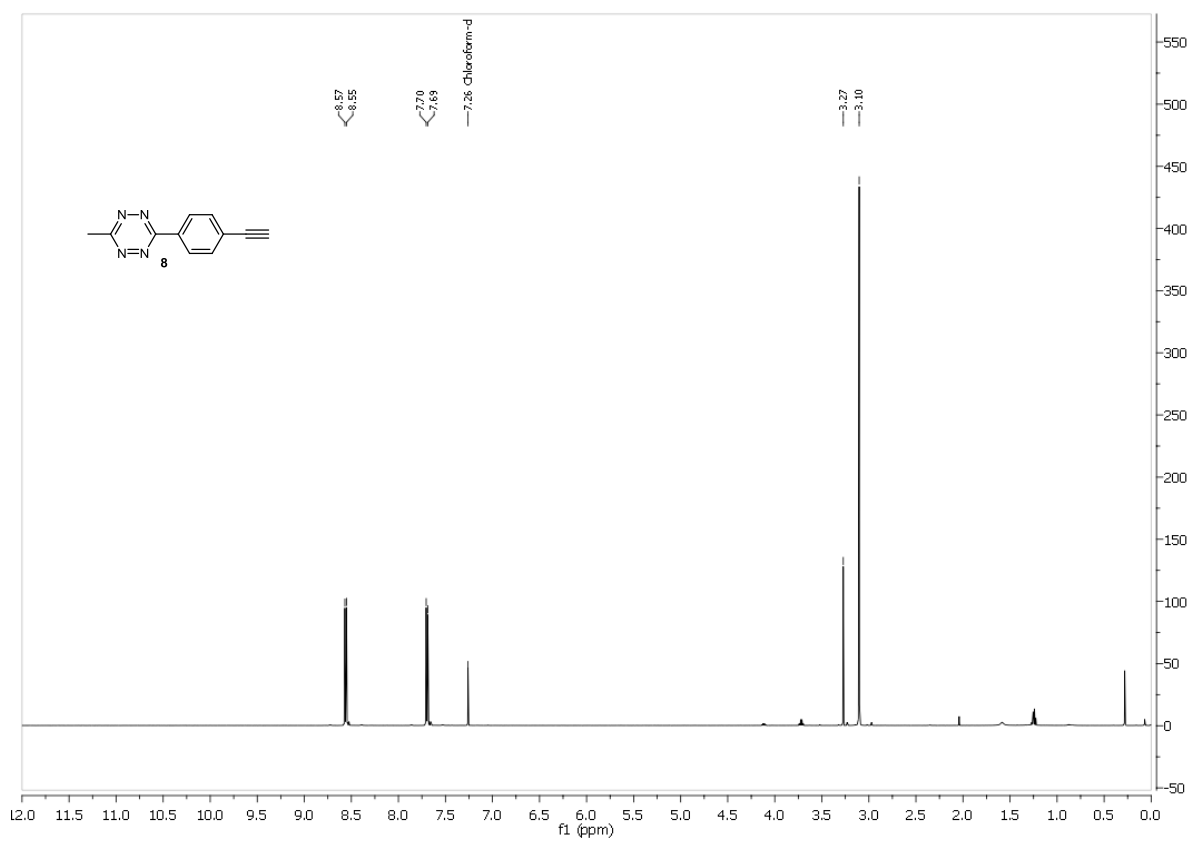
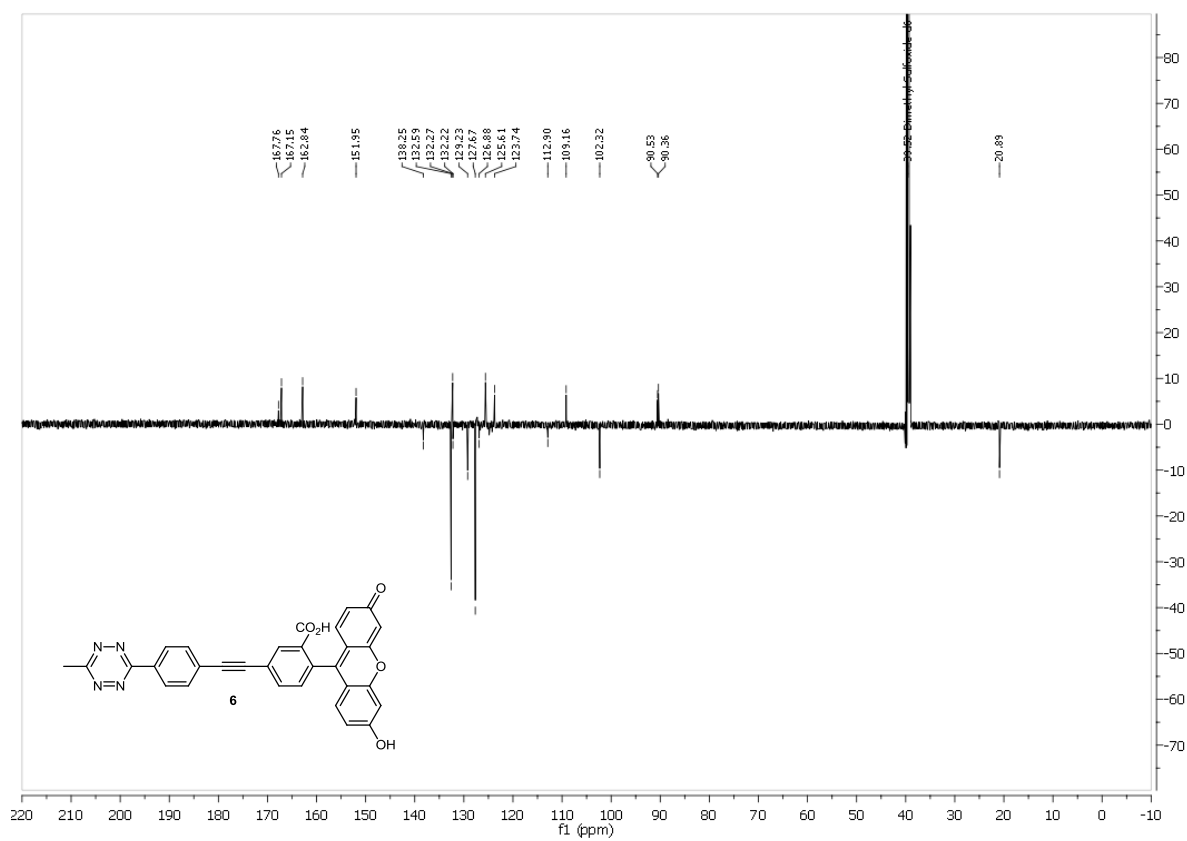¹³C-NMR spectrum of compound **8** (CDCl₃)
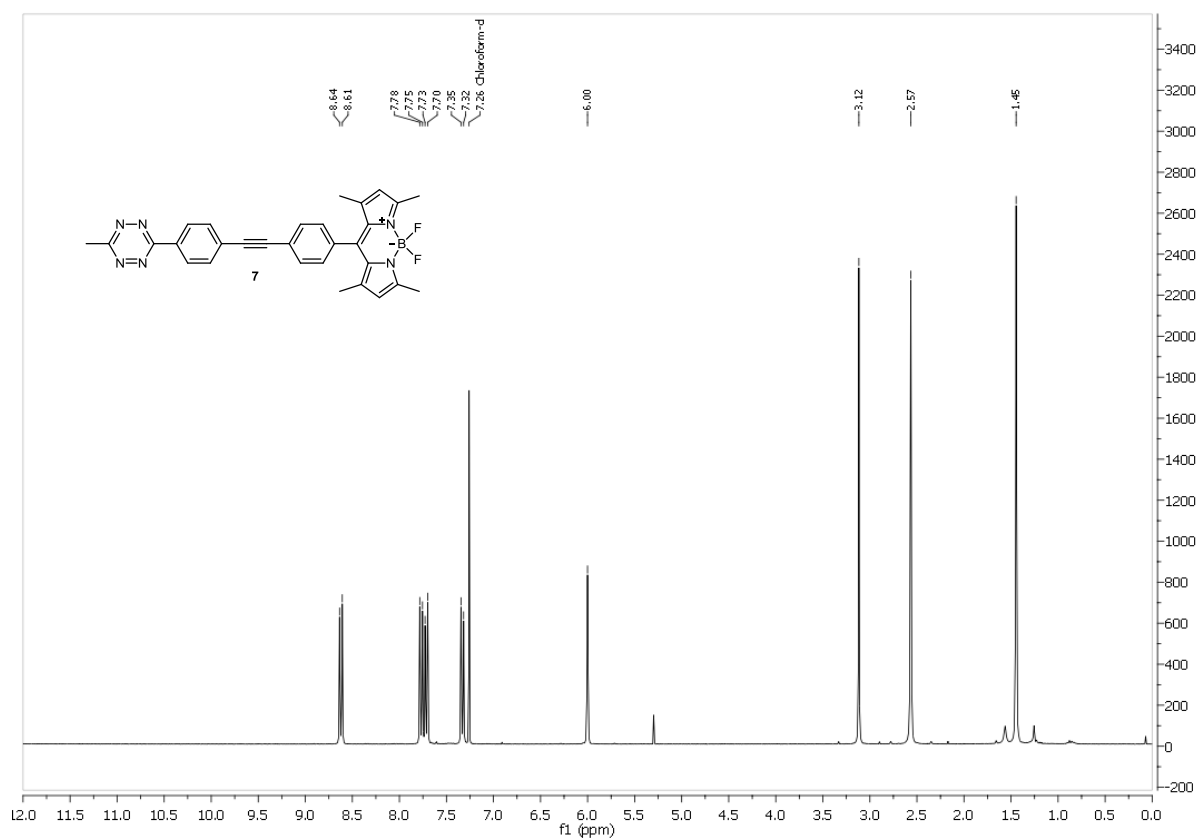
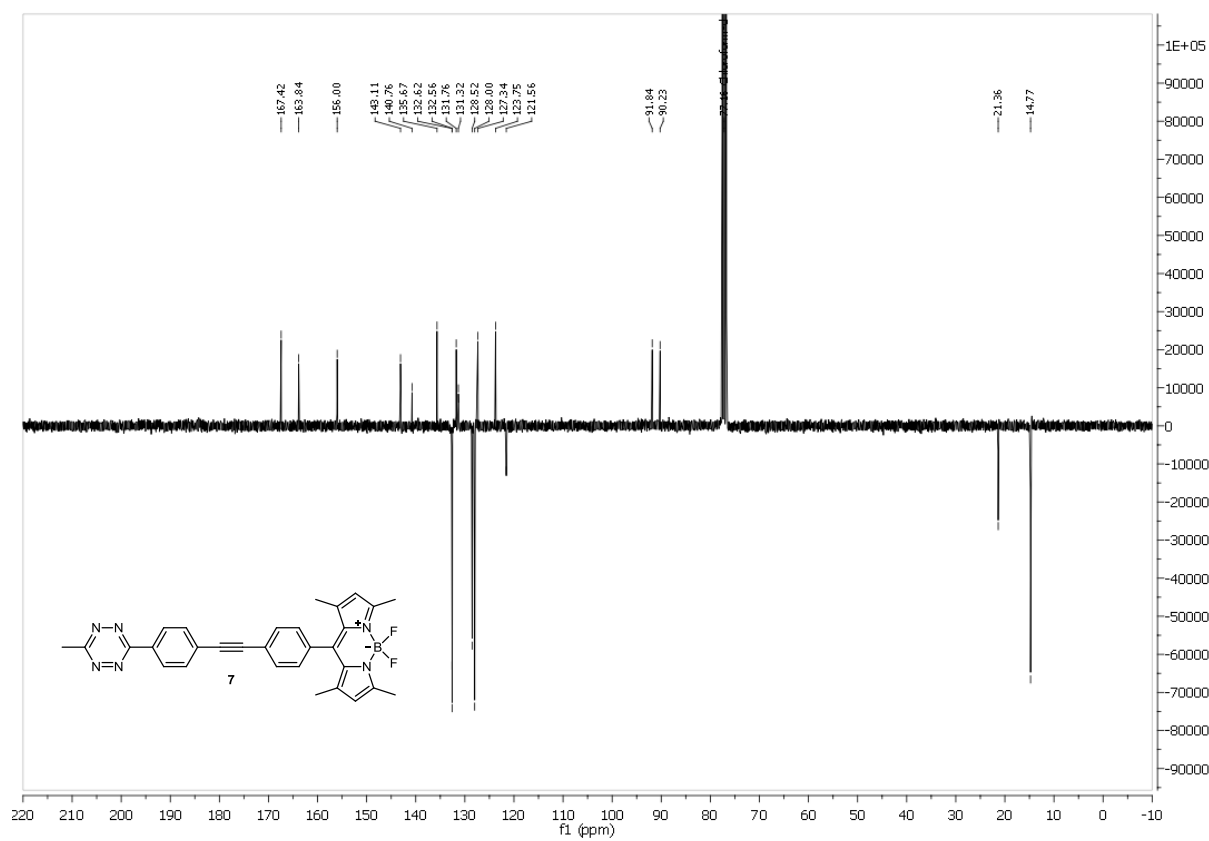¹H-NMR spectrum of compound **6** (d⁶-DMSO)

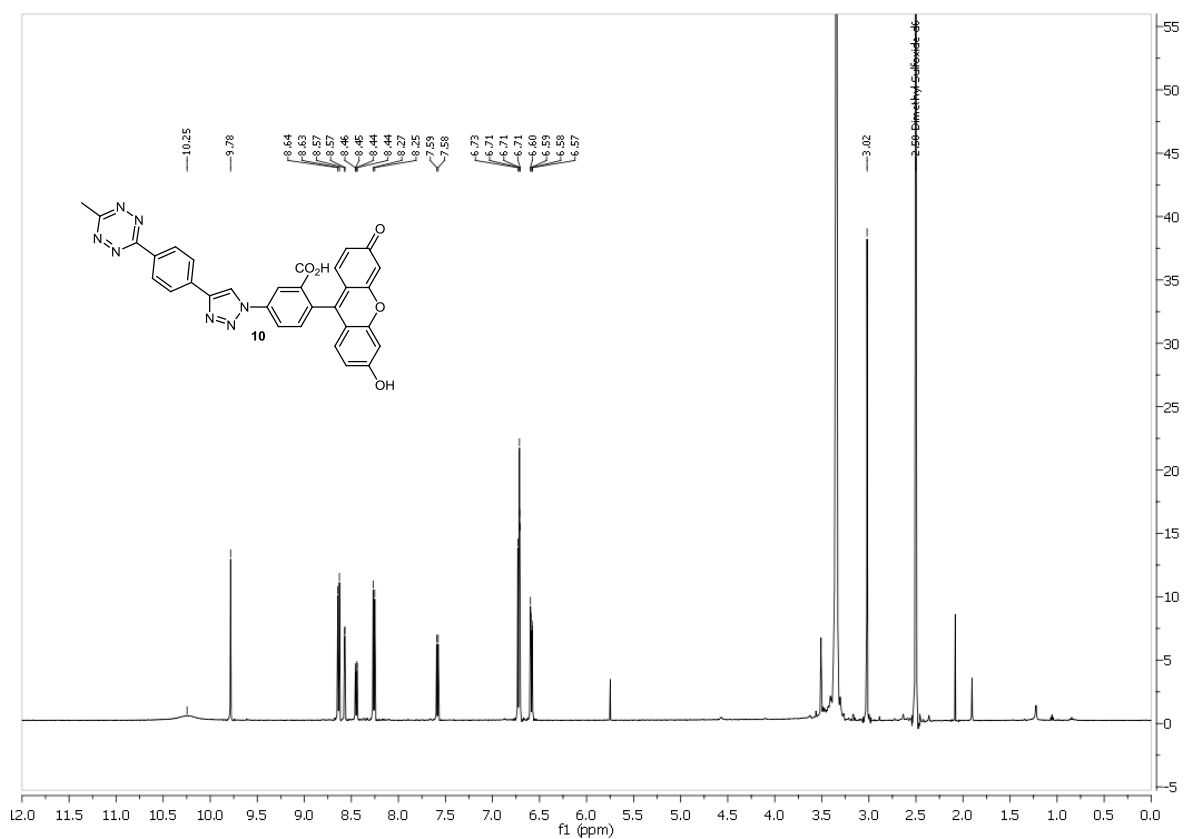¹³C-NMR spectrum of compound **6** (d⁶-DMSO)

^{1}H-NMR spectrum of compound **7** (CDCl$_3$)
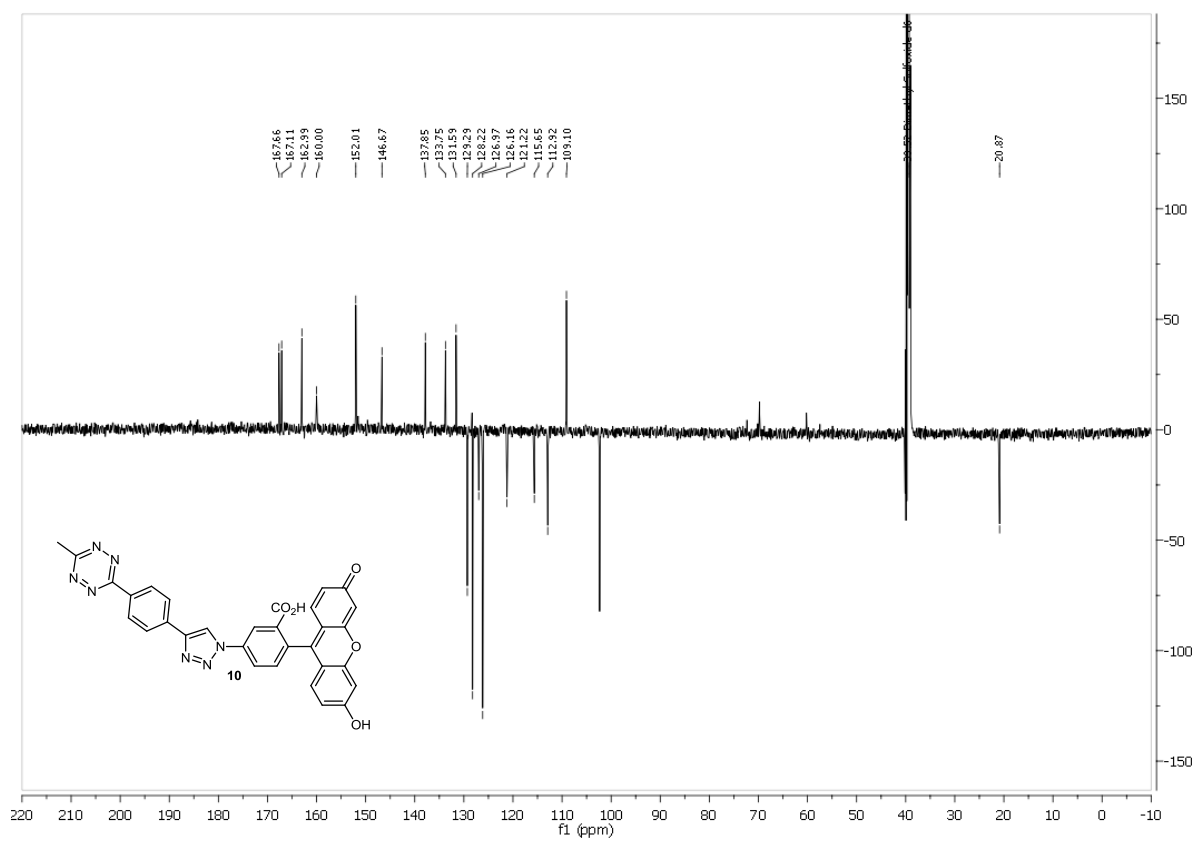

^{13}C-NMR spectrum of compound **7** (CDCl$_3$)
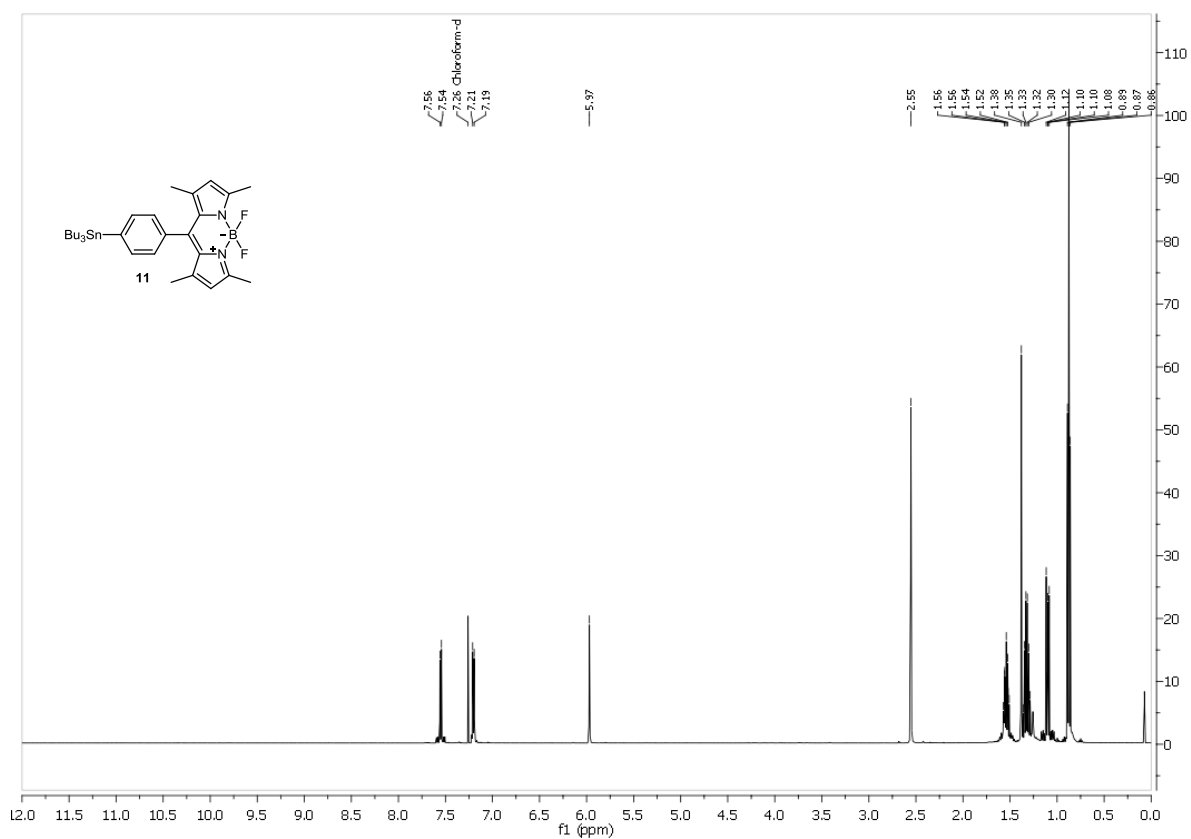
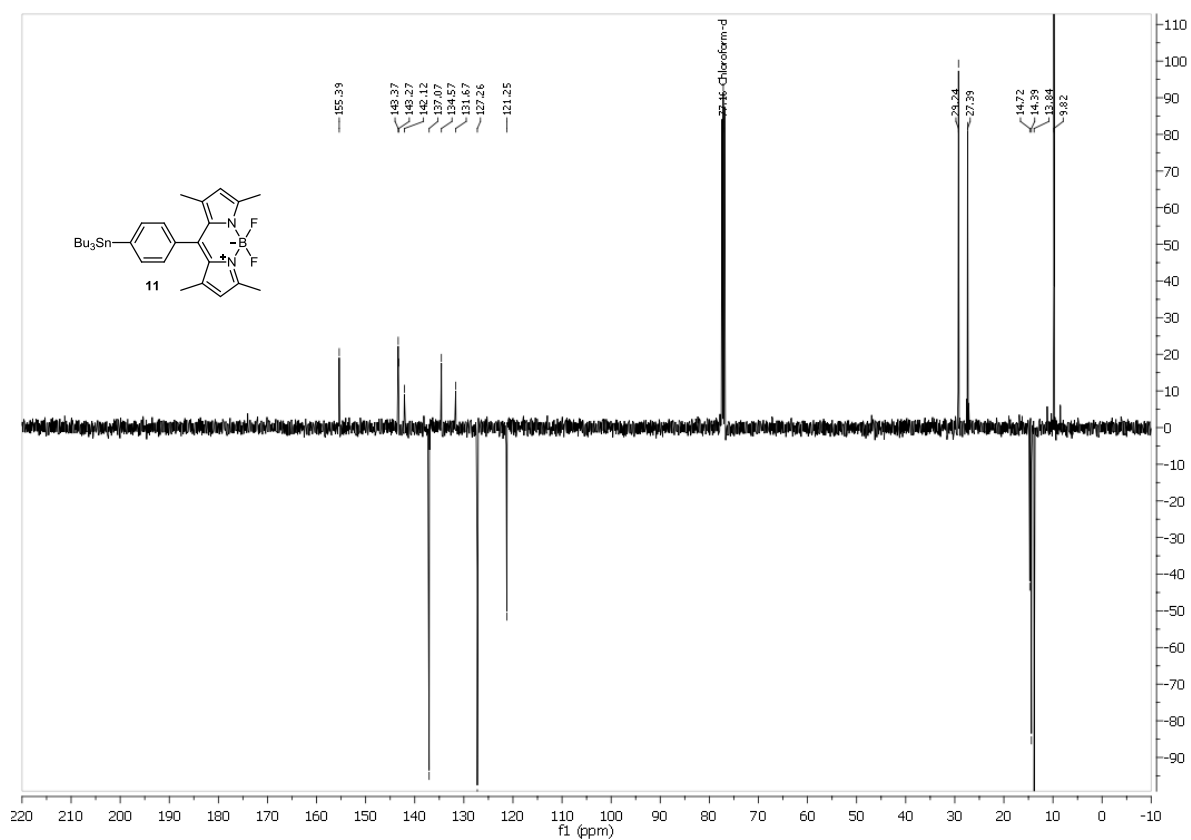¹H-NMR spectrum of compound **10** (d⁶-DMSO)

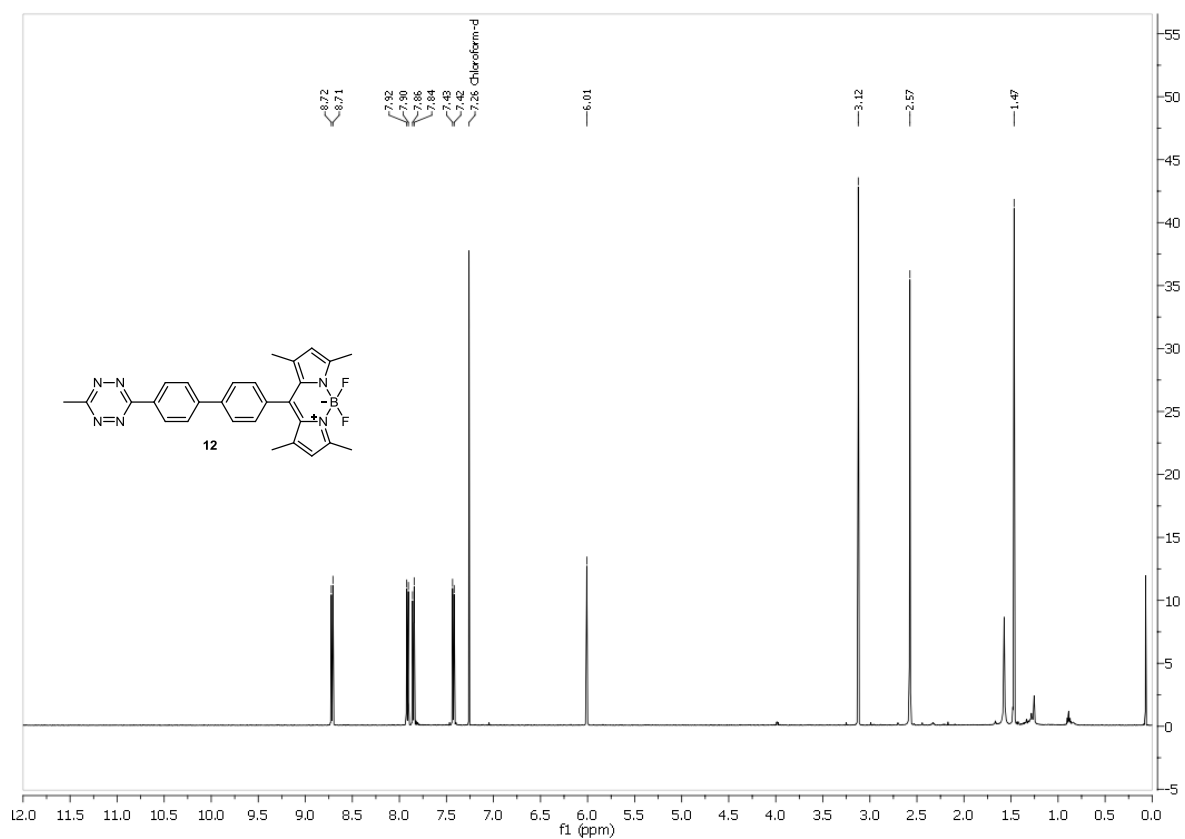¹³C-NMR spectrum of compound **10** (d⁶-DMSO)

¹H-NMR spectrum of compound 11 (CDCl₃)
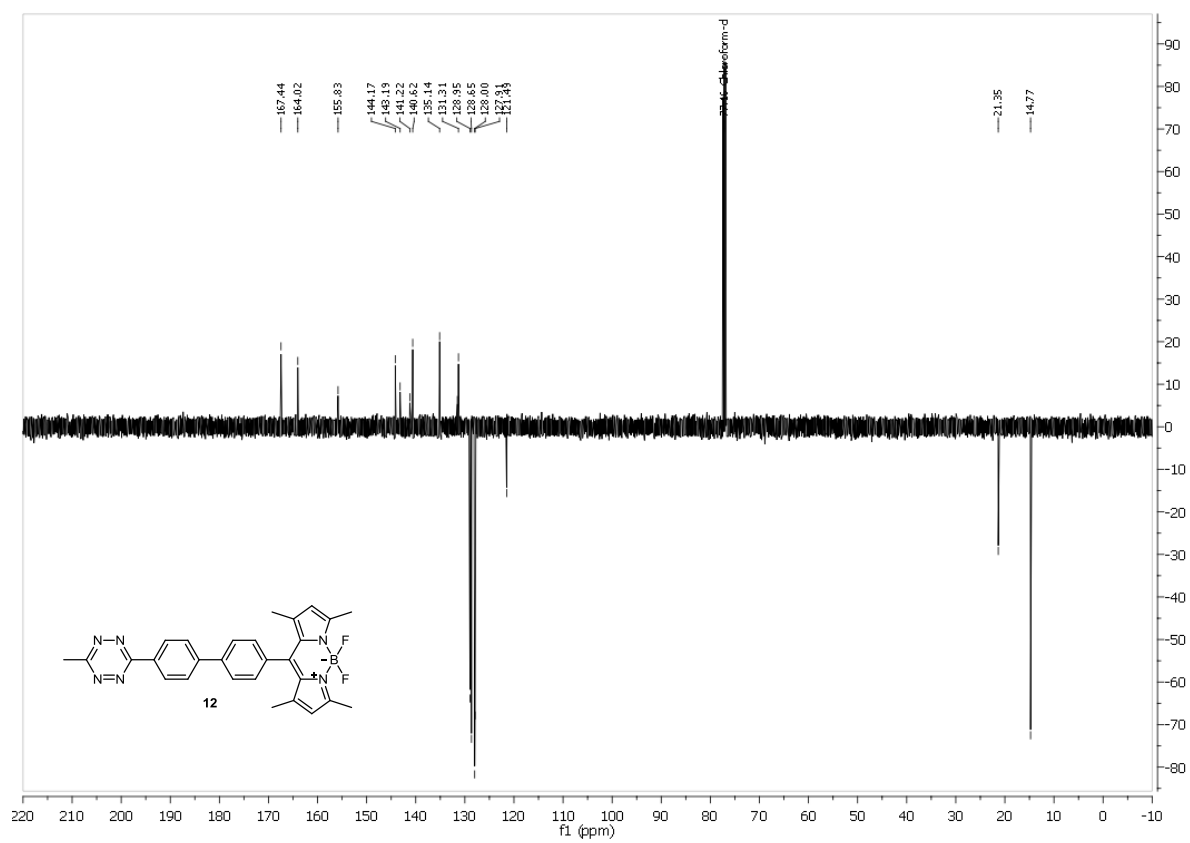
^{1}H-NMR spectrum of compound **11** (CDCl$_3$)

^{13}C-NMR spectrum of compound **11** (CDCl$_3$)

^{1}H-NMR spectrum of compound **12** (CDCl$_3$)

^{13}C-NMR spectrum of compound **12** (CDCl$_3$)

10 References

1.   J. R. Lakowicz, *Principles of fluorescence spectroscopy*. 3rd ed.; Springer: New York, 2006.
2.   F. Al-Saffar, S. Berlin, T. Musil and S. Sivadasan, *Process for preparing protected amidines*. WO/2006/090153, Aug. 31, 2006.
3.   J. Yang, M. R. Karver, W. Li, S. Sahu and N. K. Devaraj, *Angew. Chem. Int. Ed.*, 2012, **51** (21), 5222-5225.
4.   D. S. Liu, A. Tangpeerachaikul, R. Selvaraj, M. T. Taylor, J. M. Fox and A. Y. Ting, *J. Am. Chem. Soc.*, 2012, **134** (2), 792-5.