Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Online Monitoring the Isomerization of an Azobenzene-Based Dendritic Bolaamphiphile Using Ion Mobility-Mass Spectrometry

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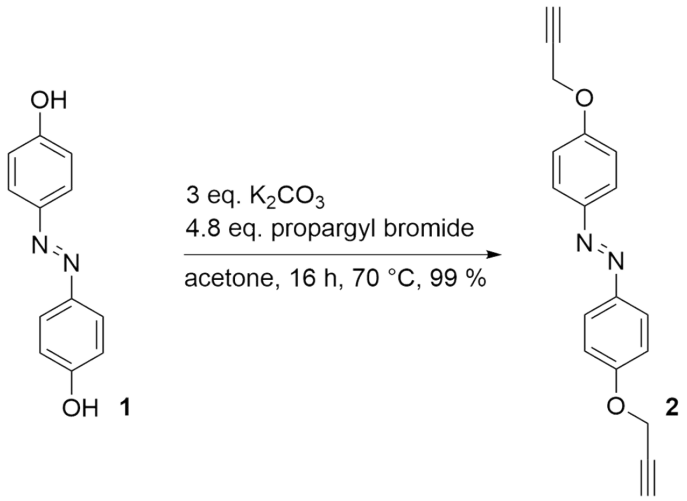
1. Synthesis

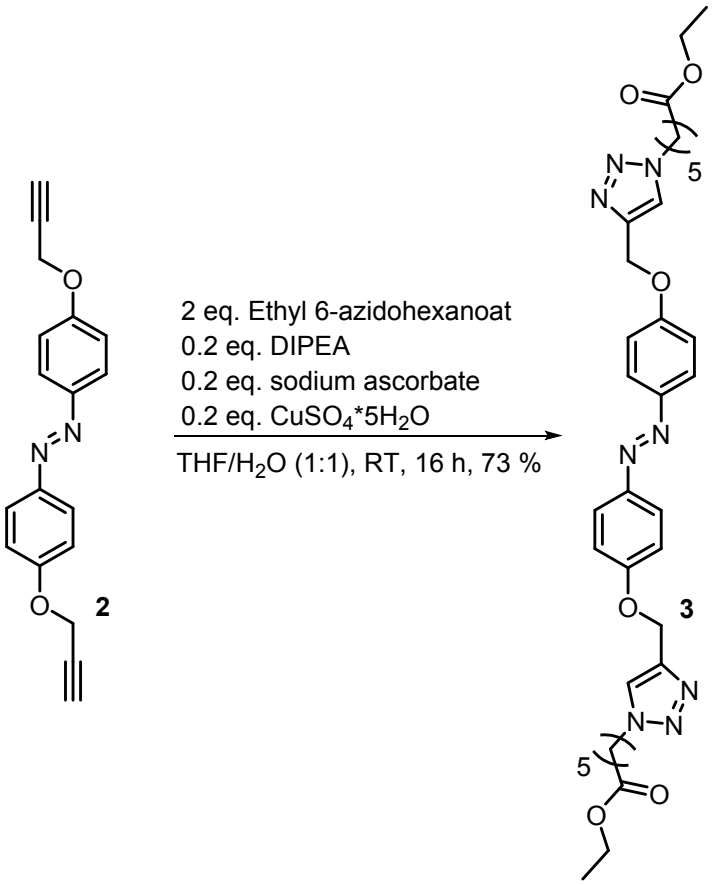
All solvents and reagents were used as supplied. The synthesis of the acetal protected triglycerol amine (protG$_1$-NH$_2$) and compound **1** were carried out via previously reported procedures.[1-3] The NMR spectra were detected by Bruker DPX400 (^{1}H NMR: 400 MHZ, ^{13}C NMR: 133 MHz) and Bruker AVANCEIII 500 (^{1}H NMR: 500 MHz, ^{13}C NMR: 166 MHz). ESI-MS were measured at Agilent 6210 ESI-TOF from Agilent Technologies. The flow rate was 4 µl/min.

1.1 Preparation of Compound 2



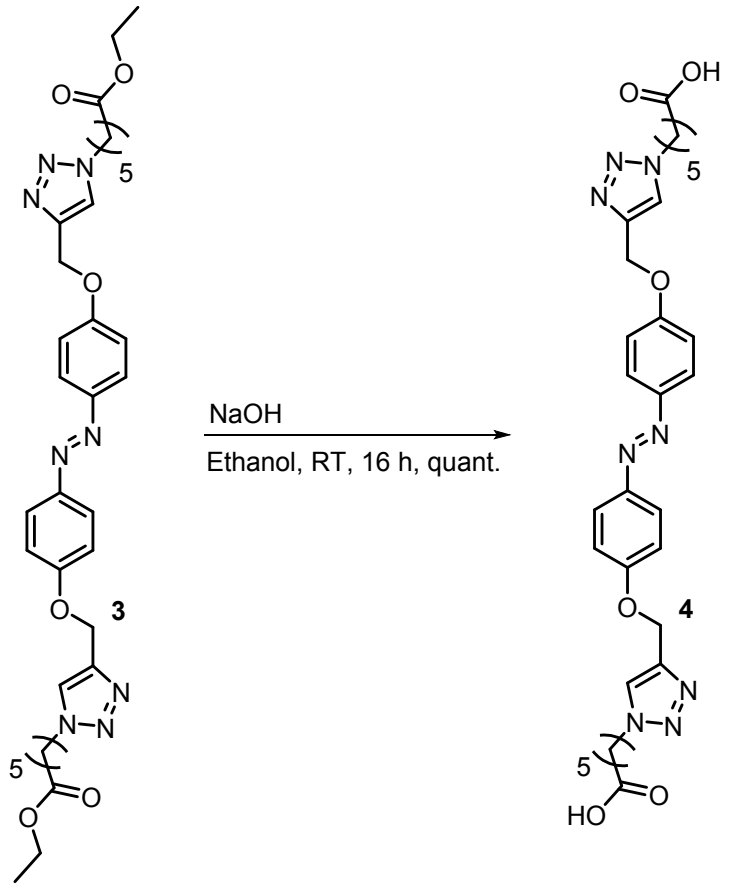
Compound **1** (500 mg, 2.33 mmol) and K_2CO_3 (774 mg, 5.60 mmol) were dissolved in dry acetone (25 mL). Propargyl bromide (1.24 mL, 11.232 mmol, 80 wt.% in toluene) was added and the mixture was stirred for 16 h under gentle reflux. Solids were filtered off and solvent was removed under reduced pressure. A saturated solution of NH_4Cl and water were added and the mixture was extracted with DCM. The organic layers were dried over Na_2SO_4. Solvent was removed under reduced pressure to yield the desired compound **2** (667 mg, 2.30 mmol, isolated yield: 99 %). δ_H (400 MHz, DMSO-d$_6$) 7.88 - 7.83 (m, 4H), 7.19 - 7.14 (m, 4H), 4.92 (d, 4H), 3.63 (s, 2H). δ_C (133 MHz, DMSO-d$_6$) 159.3, 146.6, 124.0, 115.4, 78.8 - 78.6, 55.8. *m/z* calcd for $C_{18}H_{15}N_2O_2^+$ $[M+H]^+$: 291.1128; found 291.1179.

1.2 Preparation of Compound 3



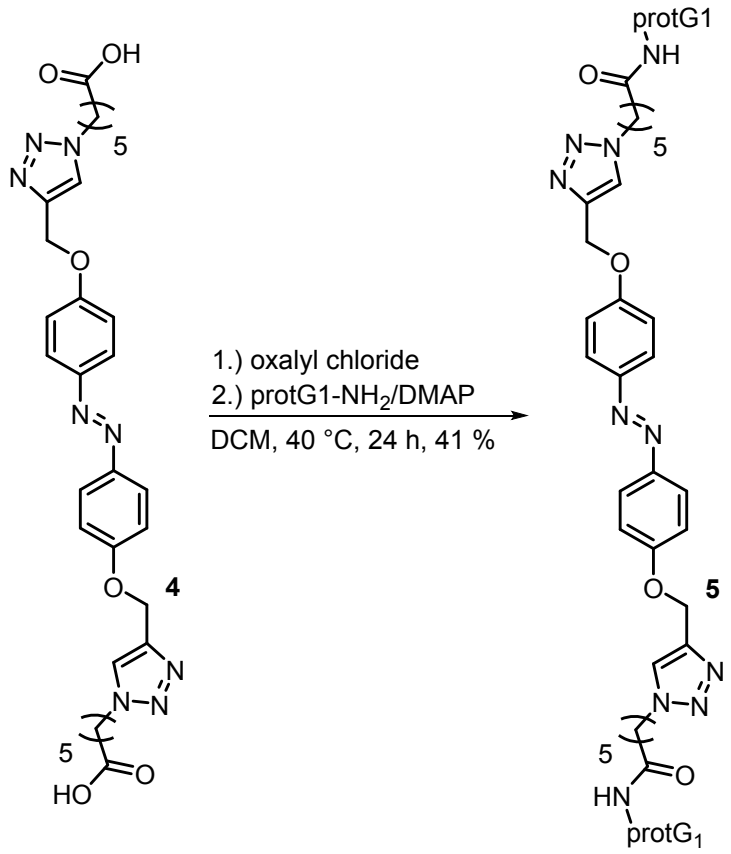
Compound **2** (199 mg, 0.69 mmol) and ethyl 6-azidohexanoat (254 mg, 1.37 mmol) were dissolved in THF (3 mL). Water (3 mL), DIPEA (45.3 µL, 274 µmol), sodium ascorbate (54.3 mg, 274 µmol) and $CuSO_4*5H_2O$ (68.4, 274 µmol) were added. The mixture was stirred for 16 h. Water (20 mL) and some drops of EDTA (sat.) were added and the mixture was extracted with DCM. The organic layer was dried over Na_2SO_4. Subsequent flash chromatography (SiO_2, DCM/EtOAc 2:1 → 1:1) gave the desired compound **3** (331 mg, 0.50 mmol, isolated yield: 73 %). δ_H (400 MHz, $CDCl_3$) 7.89 - 7.84 (m, 4H), 7.65 - 7.61 (s, 2H), 7.11 - 7.06 (m, 4H), 5.28 (s, 4H), 4.37 (t, 4H), 4.10 (q, 4H), 2.29 (t, 4H), 1.98-1.90 (m, 4H), 1.73 - 1.61 (4H, m), 1.41 - 1.31 (m, 4H), 1.23 (t, 6H). δ_C (133 MHz, $CDCl_3$) 173.4, 160.3, 147.4, 124.5, 115.1, 62.4, 60.4, 50.3, 34.0, 30.0, 26.0, 24.3, 14.3. *m/z* calcd for $C_{34}H_{44}N_8Na_1O_6^+$ $[M+Na]^+$: 683.3276; found 683.3297.

1.3 Preparation of Compound 4



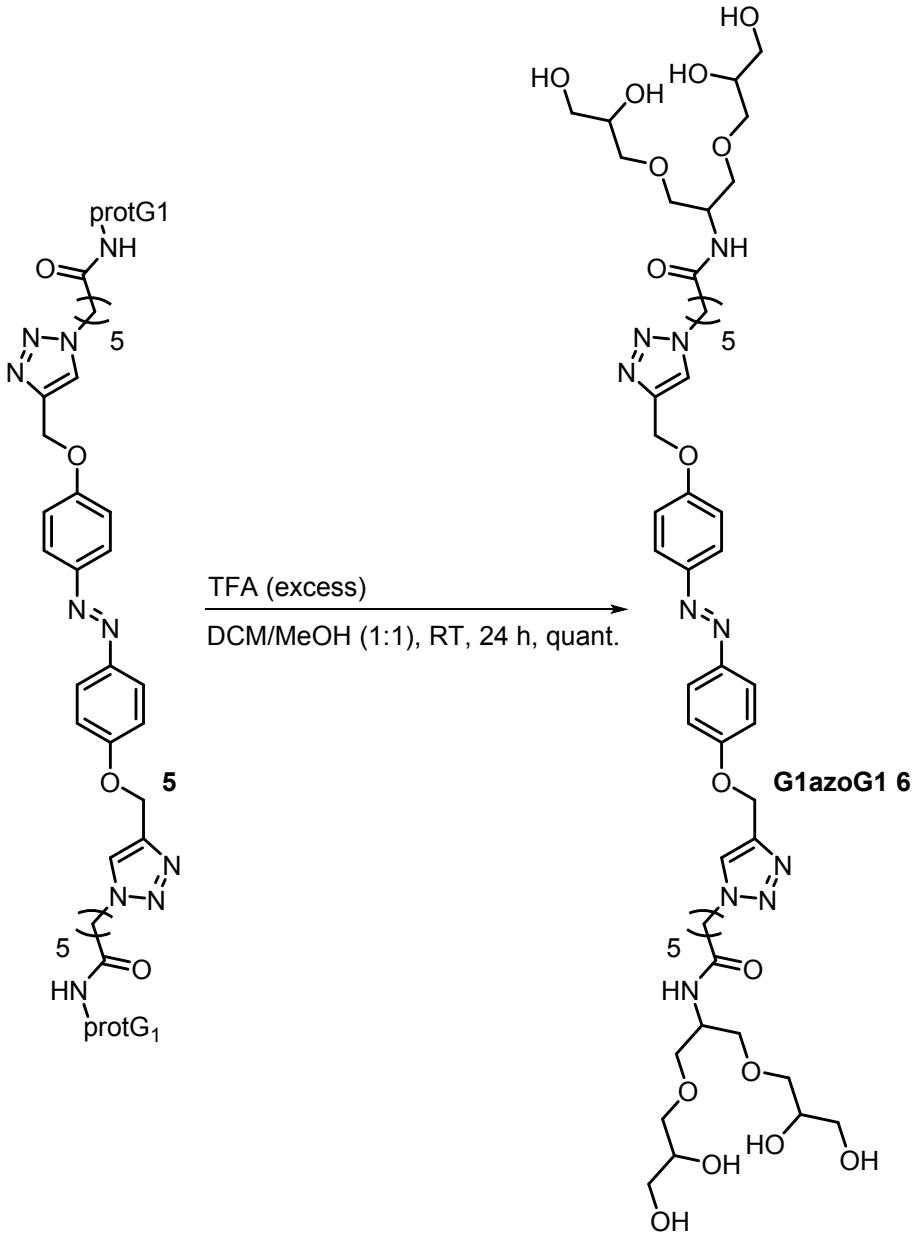
A mixture of diester **3** (150 mg, 230 μmol) and ethanol was treated with NaOH (1 mL, 1 M) and stirred for 16 h at RT. The suspension was acidified with HCl (1M) and extracted with DCM. The precipitate was filtered off and washed with water. Solvent was removed under reduced pressure to give the desired diacid **4** (137 mg, 230 μmol, quant). δ_H (400 MHz, DMSO-d_6) 12.03 (s, 2H), 8.28 (s, 2H), 7.86 - 7.84 (m, 4H), 7.27 - 7.18 (m, 4H), 5.25 (s, 4H), 4.37 (t, 4H), 2.19 (t, 4H), 1.82 (dt, 4H), 1.52 (dt, 4H), 1.29 - 1.18 (m, 4H). δ_C (133 MHz, DMSO-d_6) 174.4, 160.2, 146.3, 142.2, 124.6, 124.1, 115.3, 61.5, 49.2, 33.4, 29.4, 23.8. *m/z* calcd for $C_{30}H_{36}N_8Na_1O_6^+$ $[M+Na]^+$: 627.2650; found 627.2688.

1.4 Preparation of Compound 5



The diacid **4** (83.0 mg, 130 µmol), and oxalyl chloride (33.0 µL, 38.0 µmol), were dissolved in dry DCM and stirred for 2 h under gently reflux. The solvent was removed under vacuum by cryo distillation technique. The residue was dissolved in dry DCM. DMAP (100 mg, 820 µmol), and $protG_1$-NH_2 (105 mg, 320 µmol) were added and the mixture was stirred for 16 h under gentle reflux. Solvent was removed under reduced pressure and the raw product was purified via column chromatography (SiO_2, DCM/MeOH 10:1 and DCM/MeOH/toluene 10:1:5) to give the desired compound **5** (65.0 mg, 50.0 µmol, isolated yield: 41 %). δ_H (500 MHz, $CDCl_3$) 7.89 - 7.83 (m, 4H), 7.64 (s, 2H), 7.11 - 7.06 (m, 4H), 5.97 - 5.91 (m, 2H), 5.27 (s, 4H), 4.36 (t, 4H), 4.27 - 4.15 (m, 6H), 4.05 - 3.99 (m, 4H), 3.73 - 3.44 (m, 20H), 2.15 (t, 4H), 1.98 - 1.88 (m, 4H), 1.72 - 1.61 (m, 4H), 1.41 - 1.31 (m, 28H). δ_C (166 MHz, methanol-d_4) 174.3, 160.5, 147.2, 143.3, 124.1, 114.9, 109.2, 74.8, 71.9 - 71.7, 69.9 - 69.8, 66.1, 61.4, 49.9, 48.8, 35.3, 29.6, 25.7, 24.9, 24.2. *m/z* calcd for $C_{60}H_{91}N_{10}O_{16}^+$ $[M+H]^+$: 1207.6610; found 1207.6552.

1.5 Preparation of G1azoG1 6



Compound **5** (40.0 mg, 0.03 mmol), was dissolved in methanol (4 mL) and dichloromethane (4 mL). TFA (excess) was added and the mixture was stirred for 16 h at room temperature. Solvent was removed under reduced pressure (36.0 mg, 0.03 mmol, quant.). δ_H (500 MHz, D_2O) 7.92 (s, 2H, triazol), 7.67 - 7.58 (m, 4H, CH-arom), 6.92 (d, 4H, CH-arom), 5.01 (s, 4H, methylene), 4.39 (t, 4H, methylene), 4.37 - 4.31 (m, 2H, methane), 4.05 - 3.62 (combined signals, 28 H), 2.40 - 2.28 (m, 4H, methylene), 1.93 - 1.87 (m, 4H, methylene), 1.74 - 1.61 (m, 4H, methylene), 1.40 - 1.29 (m, 4H, methylene). δ_C (166 MHz, D_2O) 176.1, 160.2, 147.0, 143.0, 124.8, 115.4, 72.4 - 62.9, 61.8, 50.9, 48.8, 35.7, 29.3, 25.5, 24.8. *m/z* calcd for $C_{48}H_{75}N_{10}O_{16}^+$ $[M+H]^+$: 1047.5358; found 1047.5280.

2. UV/VIS Spectroscopy

UV/Vis measurements were performed on a PerkinElmer LAMBDA 950 UV/Vis/NIR spectrophotometer. Standard disposable PMMA UV/Vis cuvettes with a path length of 1 cm from PLASTIBRAND were used.

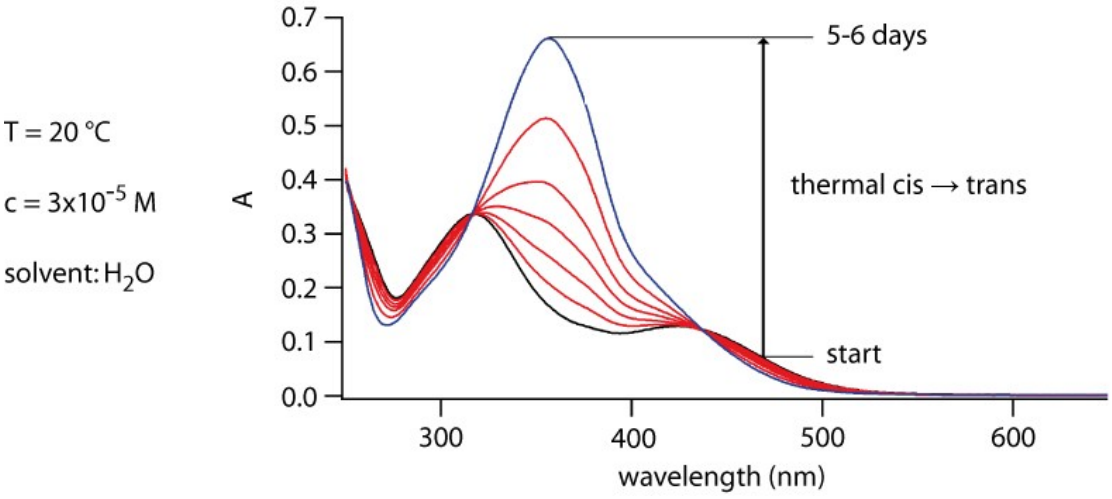


Fig. ES1: Transition of UV/VIS absorption spectra from *cis* to *trans* G1azoG1 **6**. The thermal reconversion is completed after 5-6 days.

3. DOSY Experiments

DOSY experiments were performed on a Bruker AVANCEIII 500 NMR spectrometer at a temperature of 300 K (10 mg of G1azoG1 **6** + 0.6 mL D_2O). Slight shifts in the ^1H NMR spectrum have been observed upon irradiation of the NMR tube at 366 nm for 30 min (Fig. ES2). However, both isomers do not differ with respect to their diffusion coefficients.

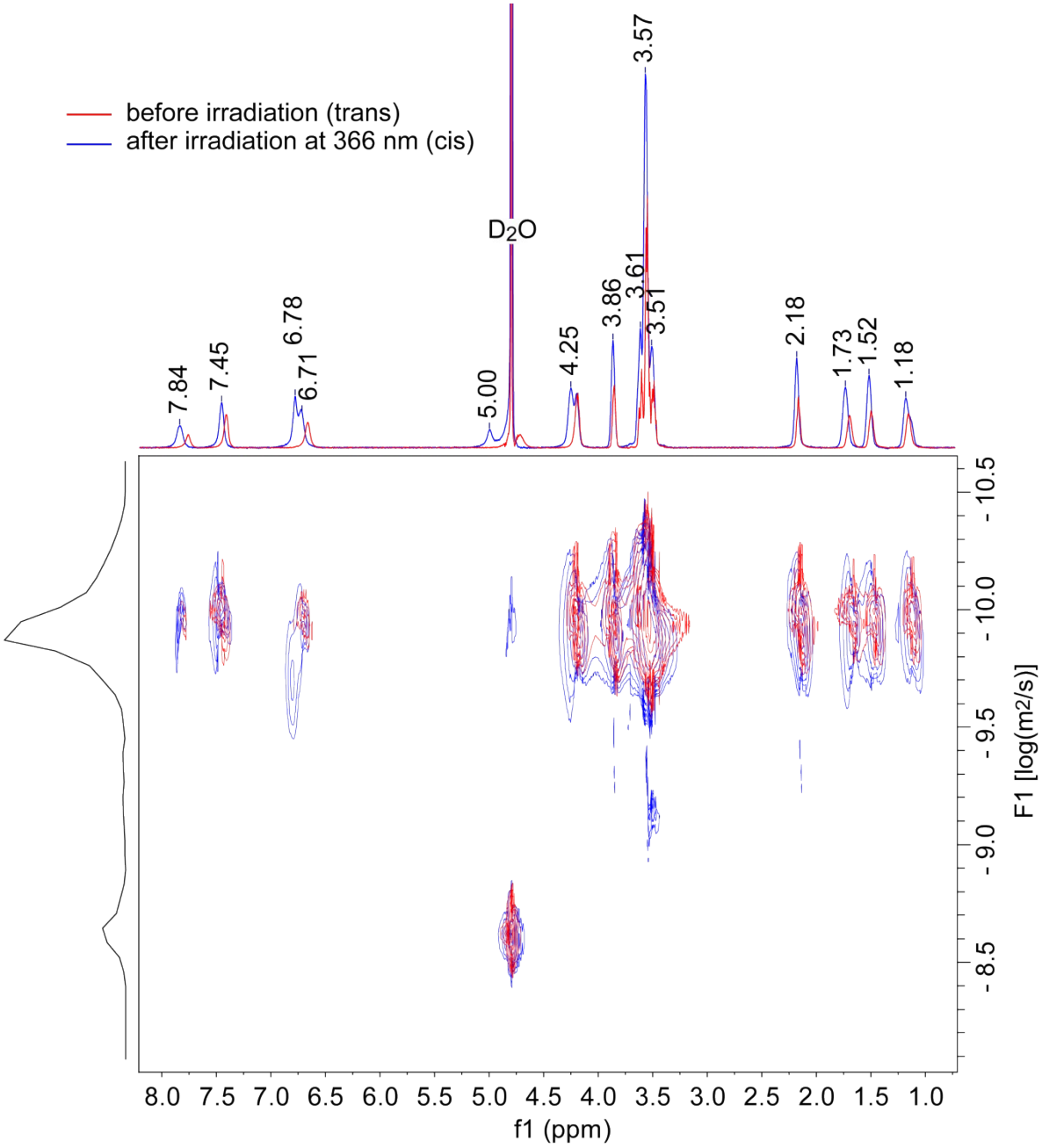


Fig. ES2: ^1H NMR spectra of G1azoG1 **6** before and after irradiation (f1, x-axis) against the observed diffusion coefficients on a logarithmic scale (F1, y-axis).

4. Ion Mobility-Mass Spectrometry

The mass spectrum of G1azoG1 **6** ($0.5 \cdot 10^{-5}$ M in MeOH/H2O 1:1 (v:v)) shows singly charged $[M+H]^+$ and $[M+Na]^+$ ions as well as a multitude of doubly charged species such as $[M+2H]^{2+}$, $[M+2Na]^{2+}$, $[M+ HNa]^{2+}$, $[M+HK]^{2+}$, $[M+2K]^{2+}$, and $[M+NaK]^{2+}$ (Fig. ES3).

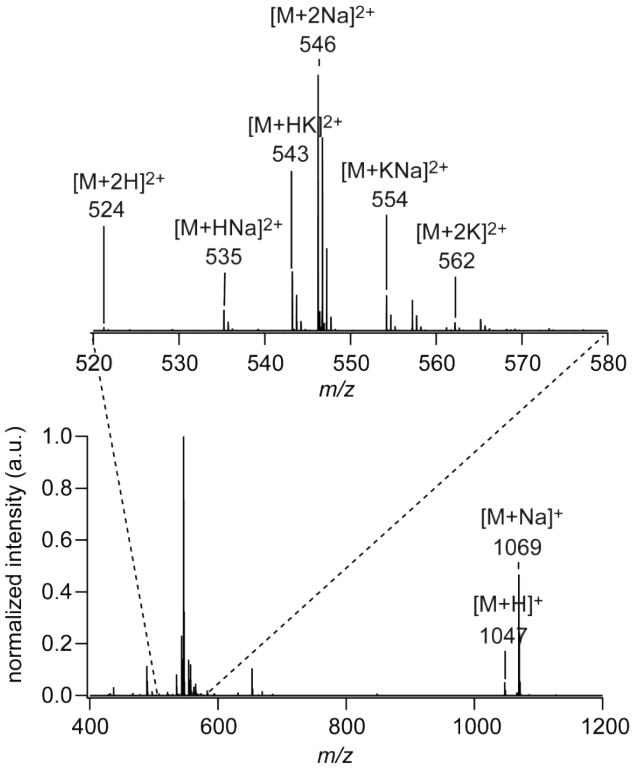


Fig. ES3: Mass spectrum of G1azoG1 **6** ($0.5 \cdot 10^{-5}$ M in MeOH/H2O 1:1 (v:v)).

Ion mobility analysis was carried out using a home-built drift tube IM-MS instrument (Fig. ES4). A similar instrument was described previously.[4, 5]

Ions are generated in a nano electrospray ionization (nESI) source and transferred into the vacuum (Fig. ES4). An electrodynamic ion funnel collects and pulses ions into a helium-filled drift tube (~5 mbar), which they traverse under the influence of a weak electric field (~10 V/cm). The drift velocity of the ions is dependent on their mobility, which in turn is governed by the molecules' overall shape and charge. Compact molecules traverse the drift tube faster than more elongated species of the same mass-to-charge ratio (*m*/*z*), before a second electrodynamic ion funnel guides them into high vacuum where mass analysis occurs by means of a quadrupole mass analyzer. So-called arrival time distributions (ATDs) are recorded by measuring the time that ions of a particular *m/z* need to traverse the drift region.

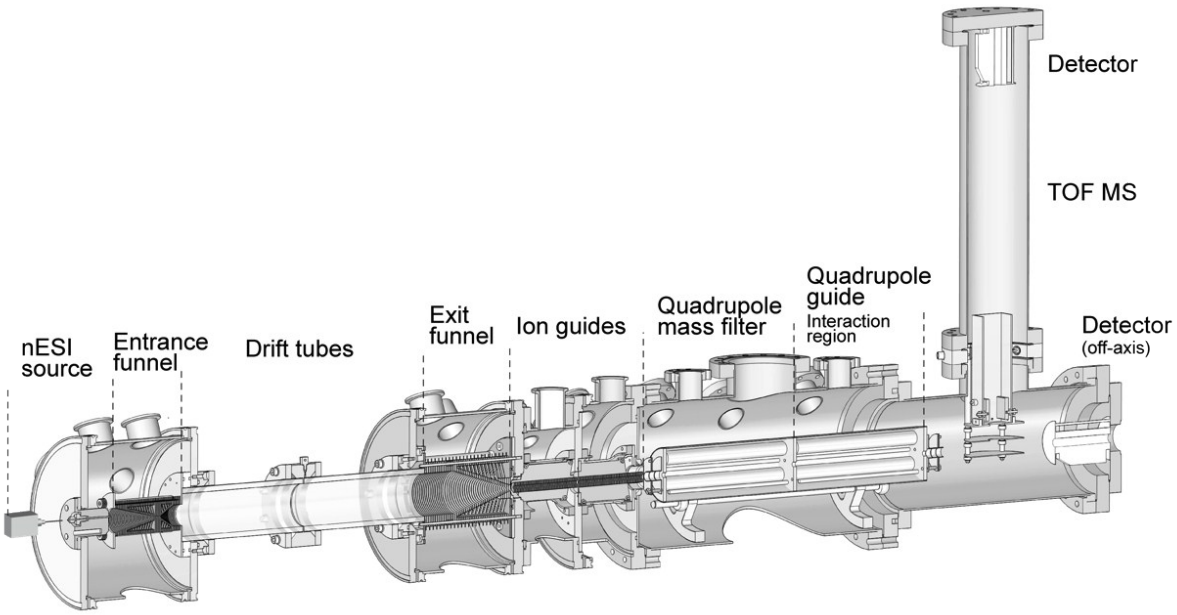


Fig.ES4: Instrumental setup of the DT instrument.[5]

The entrance of the drift tube instrument was further equipped with two lamps as shown in the manuscript (Fig. 2a) in order to irradiate the nESI interface:

- UV lamp from CAMAG (40 W) for irradiation at 366 nm
- VIS lamp from Schott, KL 1500 LCD (150 W), for irradiation with visible light (T = 3300 K, $\lambda_{max} \approx 880$ nm).

The UV lamp is typically used for detection of UV active compounds on silica gel (thin-layer chromatography) and the VIS lamp is typically used to illuminate the source region of the DT instrument. Both light sources are of rather low intensity and further photo induced chemical reactions within the samples can therefore be regarded.

The ionization process was started by applying a high voltage to the nESI capillary (0.8 - 1.2 kV). Once a stable spray was achieved, the capillary was irradiated by the VIS lamp until a maximum and constant intensity for the *trans* isomers was observed. In order to monitor the *trans* to *cis* conversion, the VIS lamp was switched off and the UV lamp was switched on. The switching between both lamps was performed almost simultaneously. After turning on the UV lamp, ATDs have been recorded every 2.5 seconds until the observed change in intensity between the ATDs of both isomers was completed. For monitoring the *cis* to *trans* conversion, the UV lamp was switched off and the VIS lamp was switched on. ATDs have been recorded again every 2.5 seconds until the observed change in the ATDs was completed.

5. Sample Conditions UV/VIS and IM-MS

The fact that the observed transition between first order kinetics and equilibrium conditions during *trans* to *cis* isomerization occurs simultaneously in both methods is purely coincidental. It is important to mention that the isomerization rate is depending on different factors like the distance between the light source and the sample, the intensity of light, the material of the cuvette/nESI capillary, the concentration and the layer thickness of the sample (For comparison see Tab. ES1).

Tab. ES1: Sample conditions during UV irradiation from UV/VIS and IM-MS experiments.

	UV/VIS	**IM-MS**
layer thickness	1 cm	max 0.78 mm
cuvette/capillary material	PMMA	Pd/Pt coated borosilicate
distance light source ↔ sample	~ 10 cm	~ 20 cm
concentration	$3x10^{-5}$ M	$0.5x10^{-5}$ M
Volume	3.3 mL	~ 10 µL

6. Surface Tension Measurements

Surface tension was measured with an optical contact angle apparatus (OCA20 from Dataphysics). The critical aggregation concentration (cac) was measured by the concentration dependency of the surface tension of a pendant drop. For G1azoG1 **6**, the surface tension of ten different concentrations ranging from $0.5 \cdot 10^{-7}$ M to 10^{-3} M was determined. The samples were stored in the dark for 6 days to ensure maximum of molecules in the *trans* state. Subsequently, the samples were irradiated for 2 min at 366 nm to ensure maximum concentration of molecules in the *cis* state. The equilibration time of the droplet before the measurement ranged from 30 min to 90 min. Finally, the interfacial surface tension (IFT) values for the irradiated and non-irradiated samples were plotted against the logarithm of the corresponding concentration. Moreover, the IFT values were fitted to a sigmoid equation (Fig. ES5 a-b). The curvatures show three typically regions: a flat region at higher concentration, a steep linear decline at intermediate concentrations and a second flat region at lower concentration. The point at which the steep linear decline becomes the flat region at lower concentration is taken as the cac. This point can be approximated by intersection of two extrapolated lines (Fig.ES5 a-b).

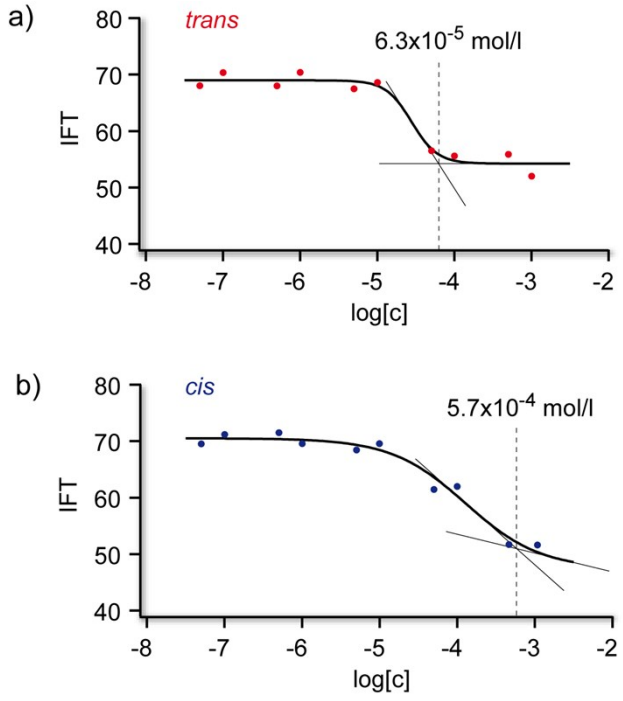


Fig. ES5: IFT values against logarithm of the concentration a) before and b) after irradiation at 366 nm.

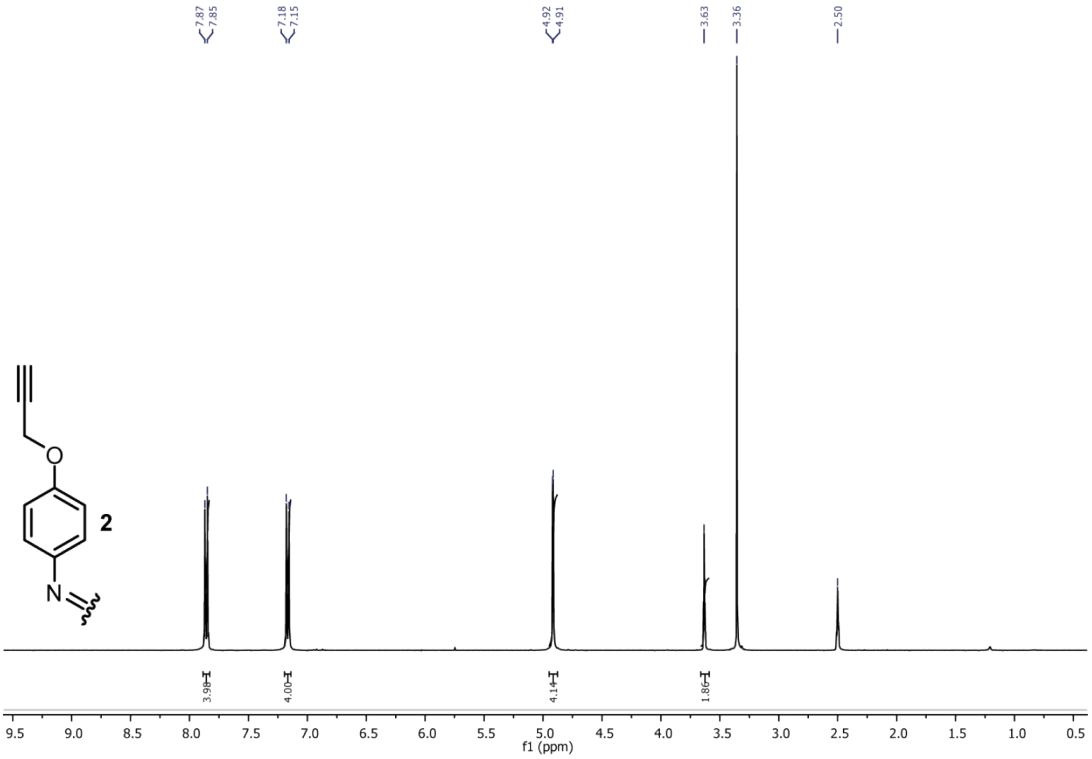
7. Transmission Electron Microscopy (TEM)

TEM measurements were carried out at room temperature using a Philips CM12 (FEI Company, Oregon, USA) instrument equipped with a LaB6 cathode operated at 100 kV accelerating voltage. Exposures were made at the low-dose mode (< 100 e/Å).

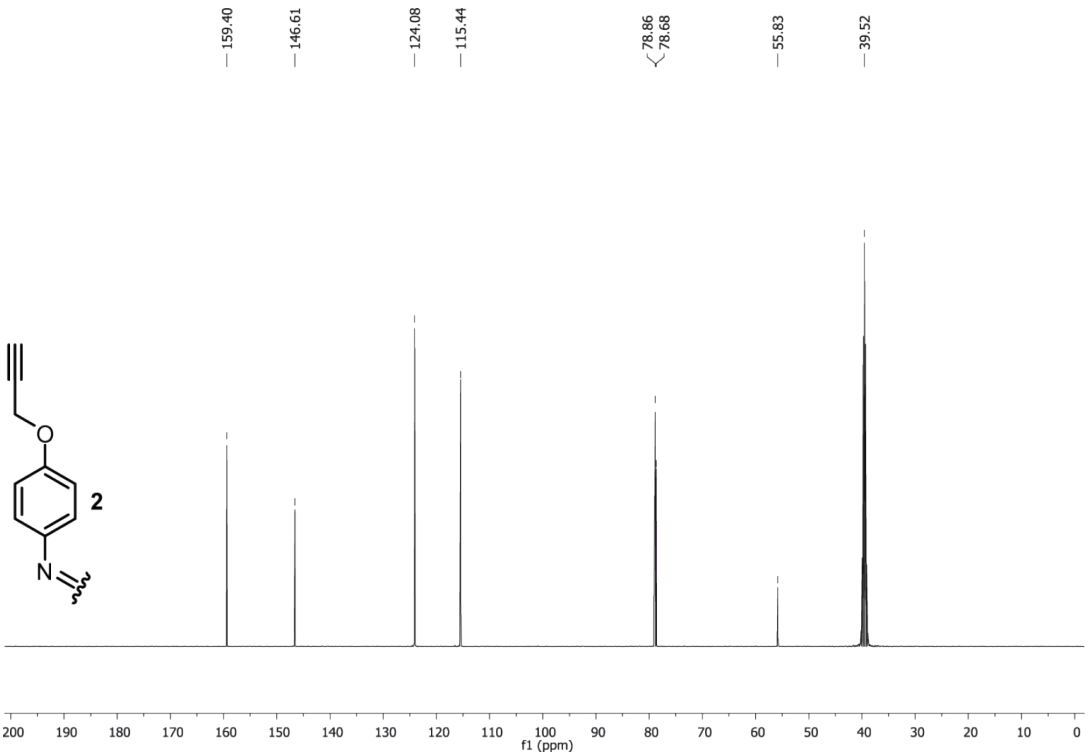
A droplet (5 µL) of sample solution was placed on a hydrophilized (60 s glow discharging at 8 W using a BALTEC MED 020 device) copper grid (400 mesh, Plano, Wetzlar) coated with 0.5 % collodium film evaporated with a carbon layer. Excess fluid was removed by blotting with a filter paper after 60 s. A droplet (5 µl) of 1% (w/v) phosphotungstic acid (pH 7.0) has been applied. Excess contrasting material was removed by means of filter paper and the sample was allowed to dry in the air.

The non-irradiated samples (*trans* form) were prepared at red light, allowed to dry under dark conditions and transfer into the microscope at red light. *Cis* G1azoG1 **6** (10^{-4} M) was generated by irradiation at a wavelength of 366 nm for 30 min and prepared for microscopy under similar conditions.

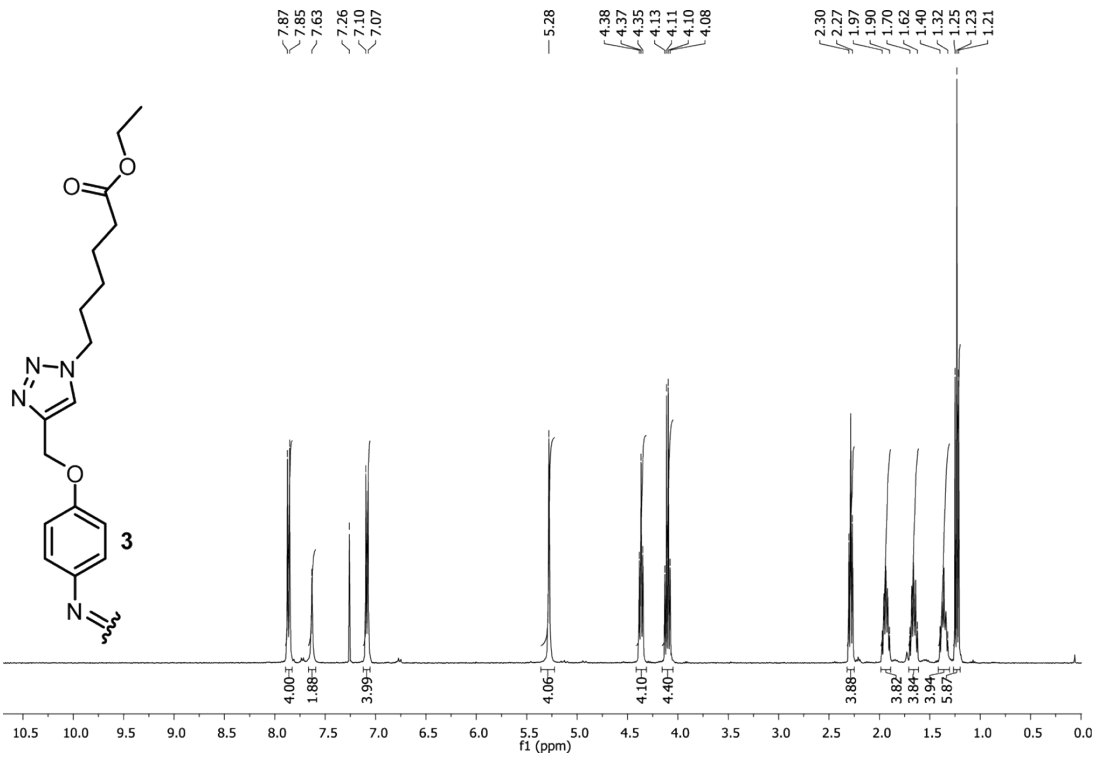
8. NMR



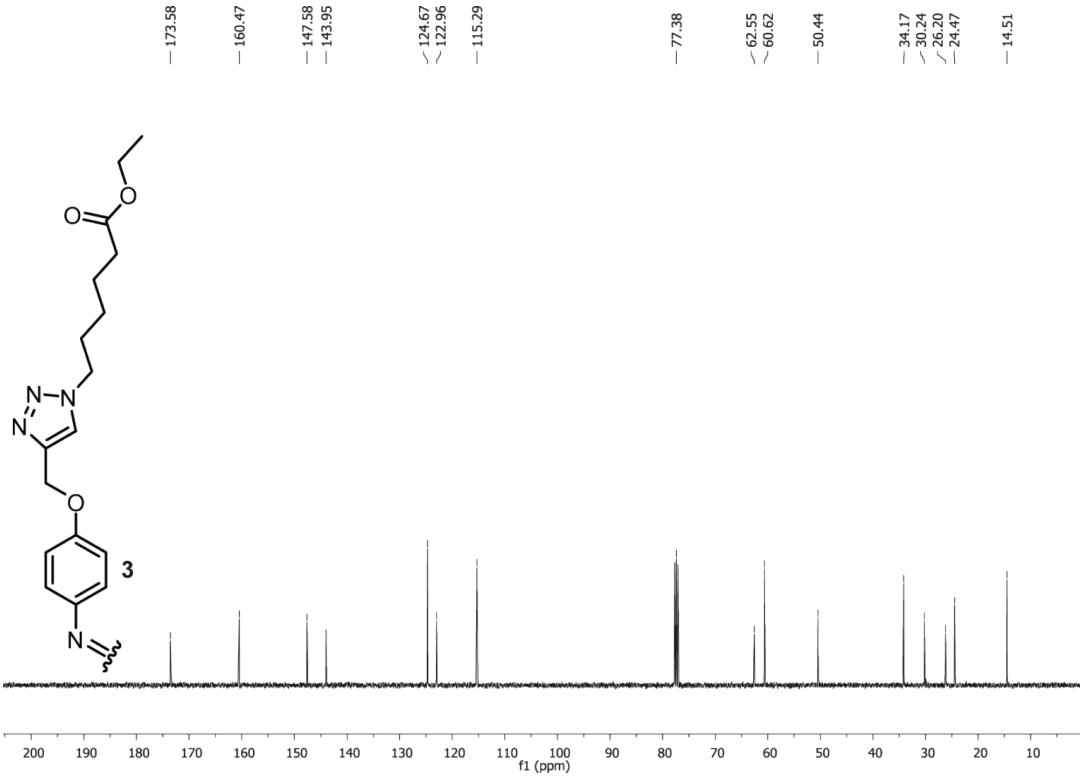
^{1}H-NMR(400 MHZ, DMSO-d_6)



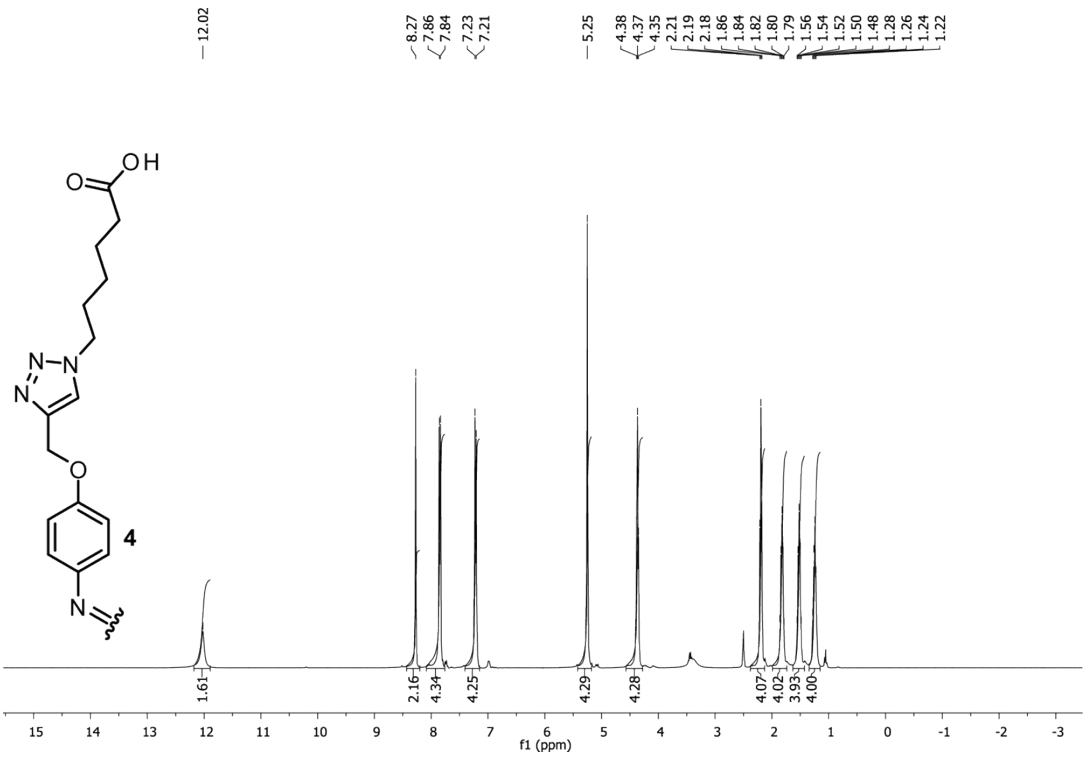
^{13}C-NMR(133 MHZ, DMSO-d_6)



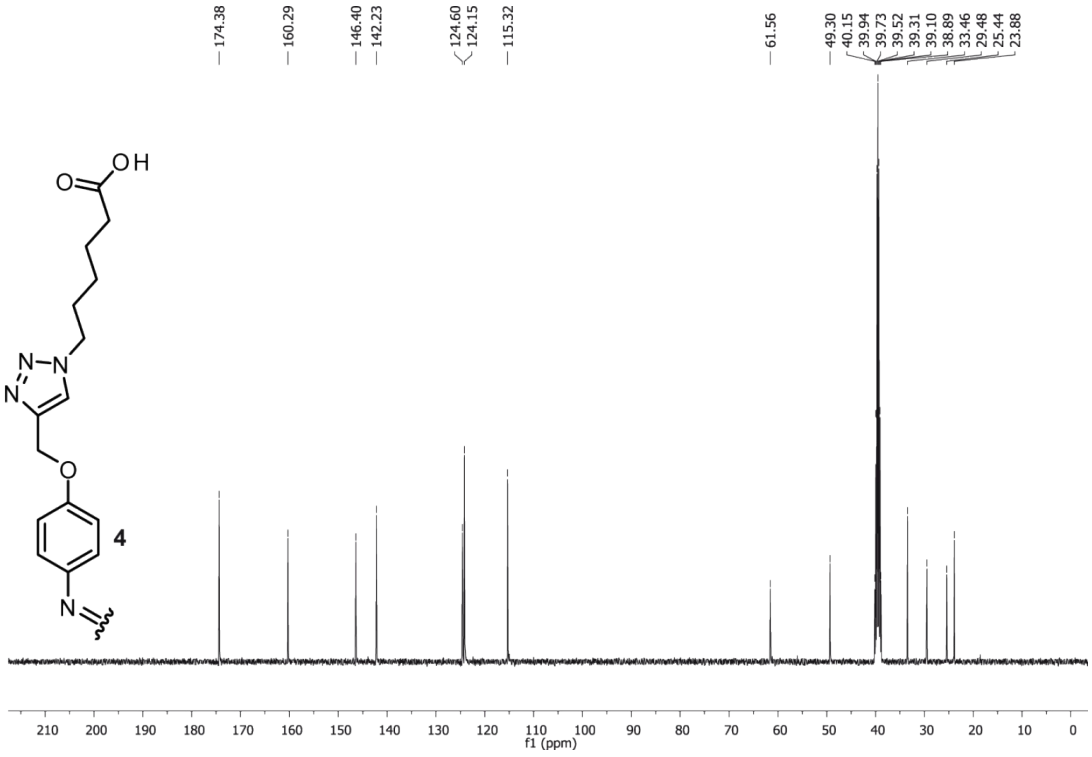
^{1}H-NMR (400 MHz, $CDCl_3$)



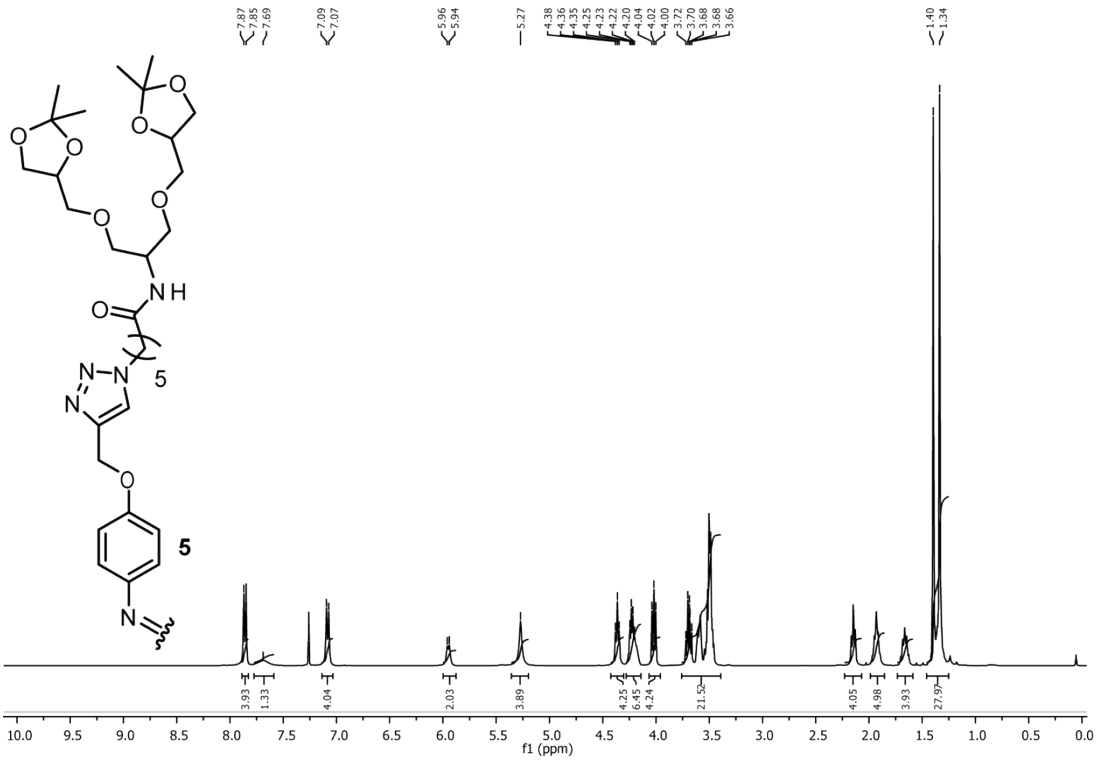
^{1}H-NMR (133 MHz, $CDCl_3$)



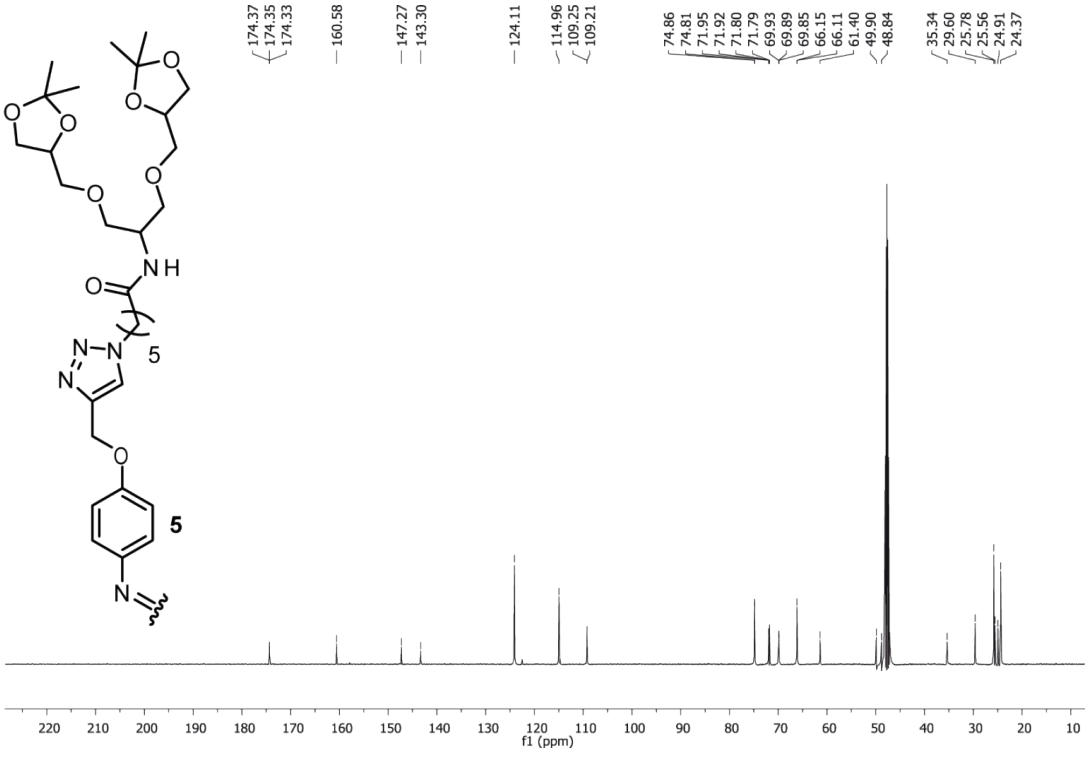
^{1}H-NMR(400 MHZ, DMSO-d_6)



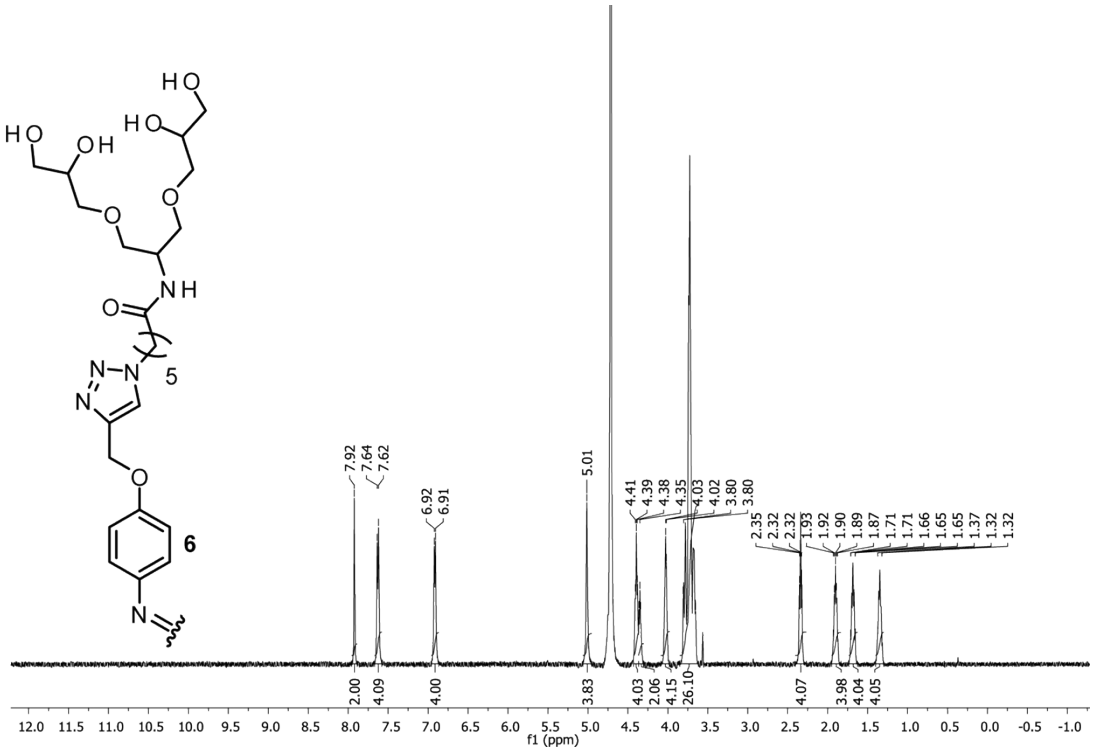
^{13}C-NMR(133 MHZ, DMSO-d_6)



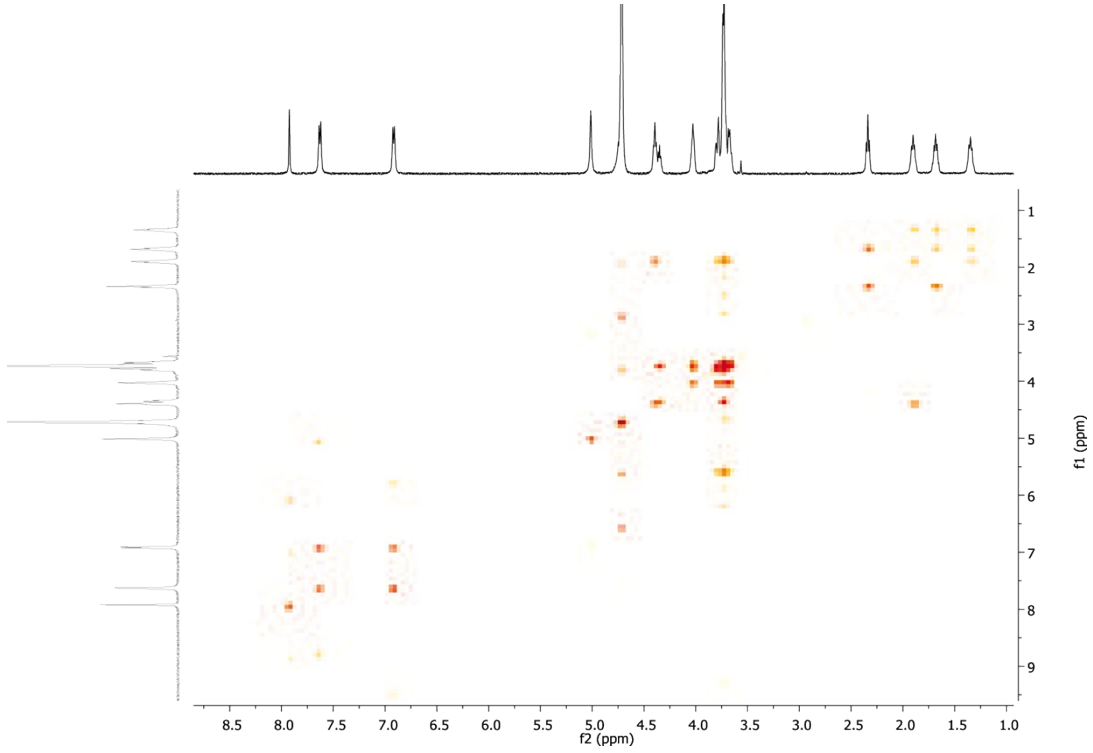
^{1}H-NMR(500 MHZ, $CDCl_3$)



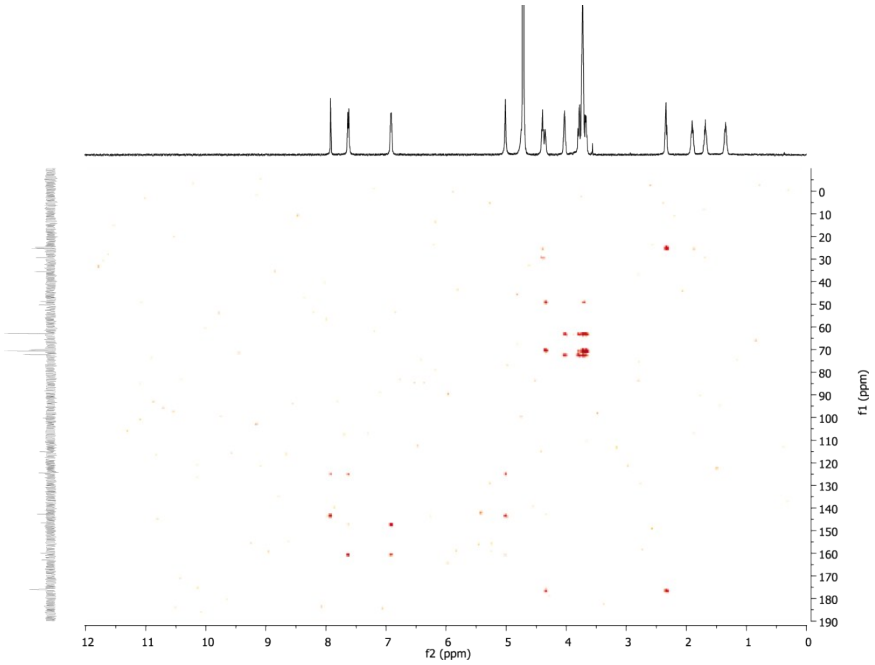
^{13}C-NMR(166 MHZ, methanol-d_4)



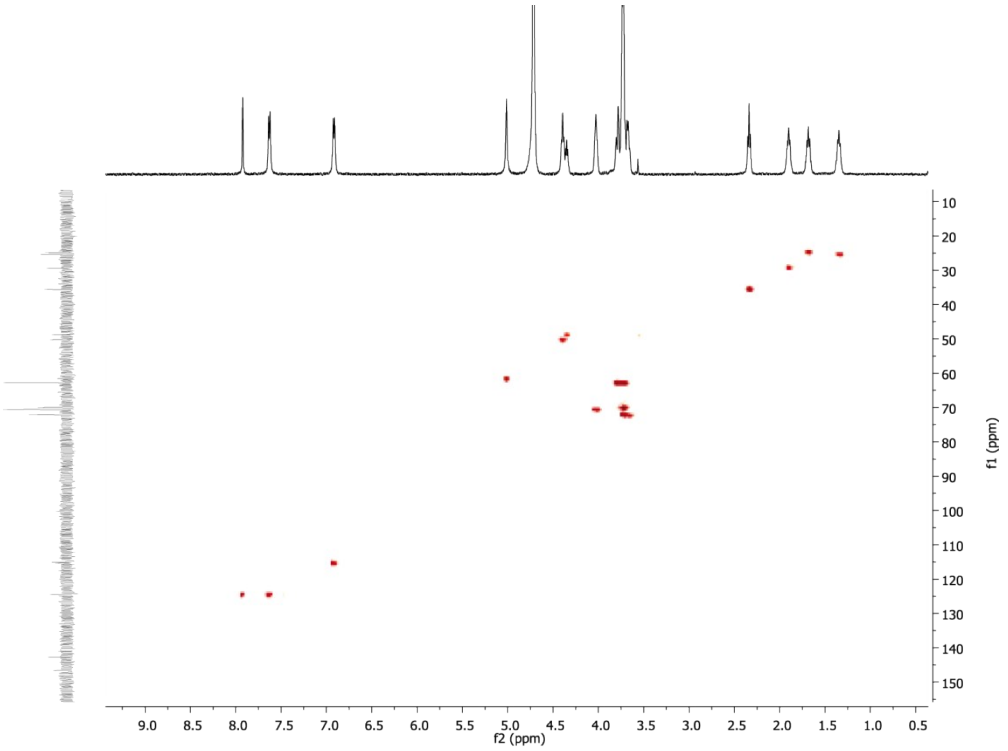
^{1}H-NMR(500 MHZ, D_2O) - G1azoG1 **6**



^{1}H-COSY(500 MHZ, D_2O) - G1azoG1 **6**



HMBC(500 MHZ, D_2O) - G1azoG1 **6**



HMQC(500 MHZ, D_2O) - G1azoG1 **6**

9. Literature

1. R. Haag, J.-F. Stumbé, A. Sunder, H. Frey and A. Hebel, *Macromolecules*, 2000, **33**, 8158-8166.
2. C. Kordel, C. S. Popeney and R. Haag, *Chem. Commun.*, 2011, **47**, 6584-6586.
3. M. Wyszogrodzka and R. Haag, *Chem. Eur. J.*, 2008, **14**, 9202-9214.
4. P. R. Kemper, N. F. Dupuis and M. T. Bowers, *Int. J. Mass. Spectrom.*, 2009, **287**, 46-57.
5. S. Warnke, C. Baldauf, M. T. Bowers, K. Pagel and G. von Helden, *J. Am. Chem. Soc.*, 2014, **136**, 10308-10314.

10. Abbreviations

$CuSO_4*5H_2O$	copper(II)sulfate pentahydrate
DCM	dichloromethane
DIPEA	N,N-diisopropylethylamine
EDTA	ethylenediaminetetraacetic acid
EtOAc	ethyl acetate
H2O	water
HCl	hydrochloric acid
K_2CO_3	potassium carbonate
Na_2SO_4	sodium sulfate
NaOH	sodium hydroxide
NH_4Cl	ammonium chloride
quant.	quantitatively
RT	room temperature
TFA	trifluoroacetic acid
THF	tetrahydrofurane