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Rhenium and technetium-complexed silicon rhodamines as near-infrared imaging probes for bimodal SPECT- and optical imaging

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1. General remarks

Unless otherwise stated reactions requiring exclusion of oxygen and moisture were carried out in heat-gun flasks dried under argon gas or nitrogen atmosphere using the Schlenk-technique.
All **chemicals** and **solvents** were taken from Sigma-Aldrich Laborchemikalien GmbH, abcr GmbH, Acros Organics and were used as received without further purification. Deuterated solvents were used from Deutero GmbH. Dry solvents as dimethylformamide, diethyl ether, methanol and tetrahydrofuran were purchased from Sigma-Aldrich Laborchemikalien GmbH in Sure/Seal™ bottles.

NMR spectra were recorded at room temperature on the following spectrometers: 400 MHz and 600 MHz: Bruker Avance III for ^{1}H-NMR spectra and 101 MHz and 151 MHz for ^{13}C-NMR spectra. Chemical shifts are reported in δ units relative to chloroform-d (δ_H = 7.26 ppm; δ_C = 77.2 ppm) or methanol-d_4 (δ_H = 3.31 ppm; δ_C = 49.0 ppm).[1] Analyses followed first order and the following abbreviations were used throughout: s = singlet, d = doublet, t = triplet, dd = doublet of doublet etc., m = multiplet. Coupling constants (*J*) are given in Hz and refer to H, H-couplings.

High Resolution Mass spectra (HR-MS) were determined in the Institute for Organic Chemistry of the University of Heidelberg under the direction of Dr. Jürgen Gross. The ionization method ESI was applied using spectrometer BrukerApexQe hybrid 9.4 T FT-ICR. High-resolution mass spectra (HR-MS) were recorded with a JEOL JMS-700 spectrometer. The molecule ions are reported as mass to charge (*m/z*) relation.

UV/VIS/NIR spectra were measured with the NanoDrop ^{1}C instrument (AZY1706045). Spectra were recorded from 300 to 800 nm in a quartz cuvette with 1 cm path length. All measurements were performed in phosphate buffered saline solution (PBS), methanol or deionized water containing ethanol (5%). The relative quantum yields were determined with $[Ru(bipy)_3]Cl_2$ in water as a calibration standard by comparing the area under the curve of the emission spectra of four different concentrations of the sample with a standard sample. Fluorescence properties were determined using Hitachi F-7100 FL spectrophotometer at room temperature. The excitation wavelength for the samples is account for 650 nm. The data were analysed with OriginPro 2018b (64-bit)

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[1] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg, *Organometallics* **2010**, *29*, 2176–2179.

Photostability experiments were investigated on a Perkin Elmer LS-55 fluorescence spectrometer by irradiation of the samples (c~0.8 µM for dye **6** and **7** and c~1 µM for dye **9** and **10**) in a quartz cuvette (1 cm path length) with a pulsed laser of the wavelength of 650 nm (20 kW, pulse width at half peak height < 10 µs) for two hours. During this time at several time points (10 min, 30 min, 1 h and 2 absorption spectra were measured on a spectrophotometer Specord 50 (Analytik Jena) to determine the photostability and were additionally normalized in reference before irradiation.

Infrared spectra were carried out with a FT-IR-spectrometer VERTEX 80/80v from Bruker. The samples were pressed with potassium bromide and were measured as pellets. The spectra were measured in the range from 500 cm^{-1} to 4000 cm^{-1} and the relevant type of signals were indicated as very strong (vs), s (strong), w (weak), and br (broad).

Analytical Thin Layer Chromatography (TLC) was carried out on polygram-TLC-plates produced by Machery-Nagel (40 x 80 mm, SIL G/UV254, 0.2 mm layer thickness). Detection was carried out using UV-light (254 nm or 366 nm).

Flash column chromatography was carried out on silica gel (0.032–0.062 mm, produced by Macherey-Nagel) using manual techniques. As mobile phase mixtures of solvents (hexanes/ethyl acetate, dichloromethane/methanol and chloroform/methanol) were used.

High-Performance Liquid Chromatography (HPLC) system was used for purification: LaPrep P314 (VWR International, Radnor, USA) equipped with a DAD and a Nucleodur Sphinx® RP 5 µm preparative column. Radio HPLC analysis and purification of the technetium-99m radiolabelled Si-rhodamine was measured and purified by using a Shimadzu HPLC20AR equipped with a binary gradient pump, UV/VIS detector, auto injector and Laura™ radio detector on a Gemini-NX C18 column (100 mm × 3 mm, 110 Å, AXIA packed). For analytical purity purposes the HPLC from Agilent 1100 series with a Chromolith® Performance (RP-18e, 4.6–100 mm, Merck KGaA) was used.

Serum stability tests were performed in human serum and PBS/EtOH (5%) in Eppendorf Protein Lowbind tubes at room temperature and 37 °C. The TLC plates were analyzed by radio-TLC (Rita – Elysia-raytest) after several time points up to 24 hours. Radio-TLC was performed using reverse-phase modified C18 TLC plates from Merck KGaA.

Partition coefficient of complex **11** was determined by using the shake flask essay in PBS (pH= 7.4) and *n*-octanol (n=10). For this purposes a Gammacounter from Hidex AMG Automatic Gamma Counter was used.

IVIS Spectrum in Vivo Imaging System was performed with Lumina Series II from Caliper LifeSciences small 3 animal imager for the samples to determine the suitable amount of the rhenium-complex **11** for *in vivo* experiments. The image was recorded with blocked excitation filter at 640 nm and emission record at 710 nm. Images were analyzed with Living Image software version 4.3.1.0.16427.

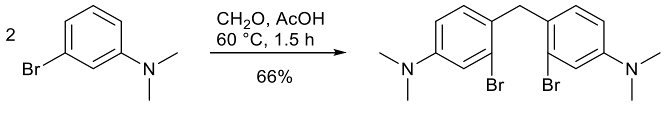
Melting points were measured with Gallenkamp Melting Point Apparatus – Gemini BV.

Chemical formulas were drawn with ChemDraw Professional 16.0.

NMR data were analyzed with MestReNova 10.0.1-14719.

2. Synthesis and Characterization

2.1 Synthesis of 4,4‘-methylenebis(3-bromo-*N,N*-dimethylaniline) (2)



1 **2**

3-Bromo-*N*,*N*-dimethylaniline (**1**) (10.0 g, 50.0 mmol, 1.0 eq.) was dissolved in concentrated acetic acid (80 mL). Formaldehyde (37% in water, containing 10–15% methanol, 10.0 mL, 134 mmol, 2.7 eq.) was added to the colorless solution. The reaction mixture was heated to 60 °C and stirred for 1.5 hours. After complete conversion (monitored by TLC) the solution was allowed to cool down. The excess of acetic acid was evaporated under reduced pressure. A saturated solution of sodium bicarbonate (20 mL) was added carefully to the reaction mixture. The suspension was extracted with ethyl acetate (4 x 50 mL) then the combined organic phases were dried with sodium sulfate and filtered off. The solvent was removed by evaporation in vacuum. The crude product was purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate 95:5) to afford pale 4,4‘-methylenebis(3-bromo-*N*,*N*-dimethylaniline) (**2**) (6.81 g, 16.5 mmol, 66%) as a colorless solid.

^{1}H-NMR (400 MHz, $CDCl_3$, 300 K):

δ (ppm) = 6.97 (s, 2 H, H_{arom}), 6.86 (d, J = 8.5 Hz, 2 H, H_{arom}), 6.62 (d, J = 8.5 Hz, 2 H, H_{arom}), 4.01 (s, 2 H, CH_2), 2.92 (s, 12 H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$, 300 K):

δ (ppm) = 150.1, 130.9, 127.2, 125.7, 116.4, 112.0, 40.7, 40.0.

IR (KBr):

υ [cm^{-1}] = 1669 (s), 1652 (s).

R$_f$ value (*n*-hexane/ethyl acetate: 95:5) = 0.30.

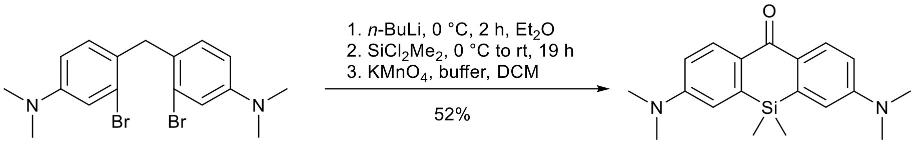
Melting point: T = 101–103 °C.

HR-ESI-MS:

$C_{17}H_{20}{}^{79}Br^{81}BrN_2$	calculated m/z	found m/z
$[M+H]^+$	413.0046	413.0050

The analytical data are in accordance with the data in the literature.[2, 3]

2.2 Synthesis of Si-xanthone 3[4]



2 **3**

Under an argon atmosphere **2** (1.50 g, 3.64 mmol, 1.0 eq.) was dissolved in dry diethyl ether (80 mL) in a heat-dried round-bottom flask. The colorless solution was cooled down to 0 °C and *n*-BuLi (4.0 mL, 2.5 M in *n*-hexane, 10.2 mmol, 2.8 eq.) was added dropwise. The bright yellow solution was stirred for two hours at 0 °C. Subsequently dichlorodimethylsilane (470 mg, 3.64 mmol, 1.0 eq.) was added dropwise *via* a syringe to the reaction mixture at 0 °C. A colorless precipitation was observed within few minutes. The solution was allowed to warm up to room temperature and was stirred for further 19 h. After complete conversion (monitored by TLC) the

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[2] G. Lukinavičius, K. Umezawa, N. Olivier, A. Honigmann, G. Yang, T. Plass, V. Mueller, L. Reymond, I. R. Corrêa, Z. G. Luo, C. Schultz, E. A. Lemke, P. Heppenstall, C. Eggeling, S. Manley, K. Johnsson, *Nat. Chem.* **2013**, *5*, 132–139.

[3] J. H. Gorvin, *J. Chem. Soc.* **1953**, 1237–1241.

pale yellow mixture was quenched with water and extracted with diethyl ether (3 x 150 mL). The combined organic phases were washed with brine and were dried with sodium sulfate. After filtration the solvent was removed by evaporating. The subsequent benzylic oxidation was conducted using a procedure published by Bertozzi et al.[4] The residual brown solid was dissolved in DCM (40 mL) and cooled down to 0 °C. Then a mixture of potassium permanganate (1.73 g, 10.9 mmol, 3.0 eq.) in water (30 mL), 1 M potassium hydroxide solution (7.5 mL) and tetrabutylammonium bisulfate (510 mg, 1.50 mmol, 0.4 eq.) was freshly prepared in a separate flask. The dark violet solution was added carefully to the blue DCM solution at 0 °C. The reaction mixture was stirred for 30 minutes at room temperature. The solution was quenched by addition of acetic acid (7.5 mL). Then sodium sulfite (2.85 g, 22.7 mmol, 6.0 eq.) was added in one portion. The color of the solution changed from brown to dark green immediately. The reaction mixture was diluted with water and the organic phase was separated. The aqueous phase was extracted with ethyl acetate (2 x 100 mL) and with DCM (3 x 100 mL) afterwards. The combined organic phases were washed with brine and dried over sodium sulfate. After filtration the solvent was removed under vacuum. The crude product was purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate 95:5 to 60:40) to afford Si-xanthone **3** (614 mg, 1.89 mmol, 52%) as a yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$, 300 K):
δ (ppm) = 8.40 (d, *J* = 9.0 Hz, 2 H, H_{arom}), 6.85 (d, *J* = 2.1 Hz, 2 H, H_{arom}), 6.80 (dd, *J* = 8.7 Hz, 2.6 Hz, 2 H, H_{arom}), 3.10 (s, 12 H, N-CH_3), 0.47 (s, 6 H, Si-CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$, 300 K):
δ (ppm) = 185.4, 165.0, 151.5, 140.6, 131.8, 114.4, 113.3, 40.2, –0.9.

IR (KBr):
υ [cm^{-1}] = 2948 (br), 2890 (br), 2805 (br), 1581 (CO; vs).

R$_f$ value (*n*-hexane/ethyl acetate: 8:2) = 0.18.

Retention time (analytical HPLC, 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1%; 95% MeCN in 16 min) = 5.58 min.

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[4] C. Bertozzi, P. Shieh, (2015). Alkyne-activated fluorogenic azide compounds and methods of use thereof. US9410958B2.

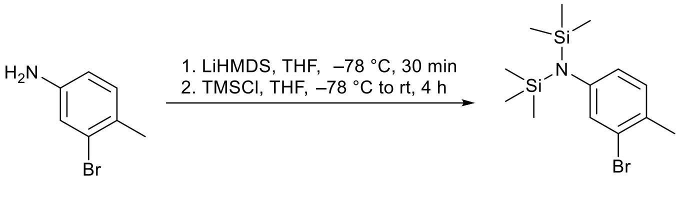
Melting point: T = 221–223 °C.

HR-ESI-MS:

$C_{19}H_{24}N_2OSi$	calculated m/z	found m/z
$[2M+Na]^+$	671.3208	671.3218

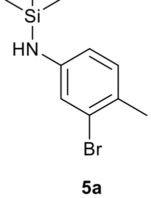
The analytical data are in accordance with the data in the literature.[5]

2.3 Synthesis of 3-bromo-4-methyl-*N*,*N*-bis(trimethylsilyl)aniline (5)[6]

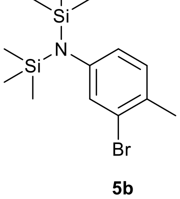


4 **5b**

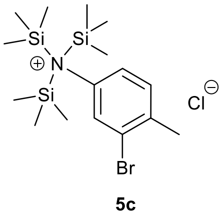
Under an argon atmosphere aniline **4** (390 mg, 2.09 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (80 mL) in a heat-dried round-bottom flask. The colorless solution was cooled down to –78 °C and lithiumbis(trimethylsilyl)amide (1 M in THF, 4.40 mL, 4.39 mmol, 2.1 eq.) was added dropwise to the solution. After full addition of the base the dark brown solution was stirred for 10 minutes at –78 °C and afterwards the cooling bath was removed for five minutes to ensure full deprotonation, before cooling down again to –78 °C. Subsequently trimethylsilyl chloride (477 mg, 4.39 mmol, 2.1 eq.) was added dropwise *via* a syringe to the dark yellow solution. After complete addition the reaction mixture was warmed up to room temperature and was stirred for further four hours under an argon atmosphere. Then the solvent was removed under reduced pressure. The brown oil was dissolved in *n*-hexane (50 mL) and then the solution was filtrated. Hereafter the solvent was removed under reduced pressure and the brown oil **5** was used directly without further purification in the next reaction step. The oil was received as a mixture of mono-**5a** (151 mg, 585 µmol, 28%), di-**5b** (387 mg, 1.17 mmol, 56%) and tris-**5c** (147 mg, 334 µmol, 16%) TMS-substituted anilines **5a**/**5b**/**5c** in a ratio of 7:14:4 (analyzed by ^{1}H-NMR).



5a



5b



5c

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[5] J. L. Bachman, P. R. Escamilla, A. J. Boley, C. I. Pavlich, E. V. Anslyn, *Org. Lett.* **2019**, *21*, 206–209.
[6] P. Shieh, M. S. Siegrist, A. J. Cullen, C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 5456–5461.

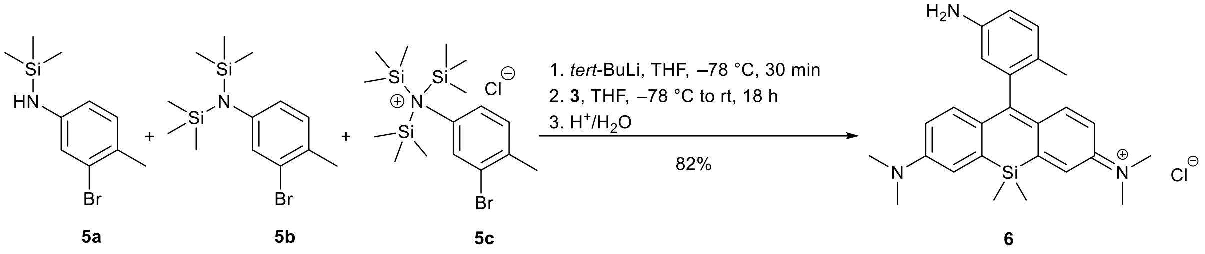
^{1}H-NMR (400 MHz, $CDCl_3$, 300 K):

δ (ppm) = 7.09 (d, 2 H, J = 2.2 Hz), 7.05 (d, 2 H, J = 8.0 Hz), 6.97 (d, 1 H, J = 8.0 Hz), 6.86 (d, 1 H, J = 2.5 Hz), 6.73 (dd, 2 H, J = 8.0, 2.2 Hz), 6.52 (dd, 1 H, J = 8.2, 2.5 Hz), 2.35 (s, 6 H), 2.27 (s, 3H), 0.27 (s, 9 H, Si-CH_3), 0.07 (s, 36 H, Si-CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$, 300 K):

δ (ppm) = 147.1, 146.6, 133.6, 132.9, 131.1, 130.4, 129.2, 126.3, 125.4, 124.2, 119.8, 115.3, 22.3, 21.8, 2.2

2.4 Synthesis of Si-rhodamine 6[6]



Under an argon atmosphere the mixture of TMS-protected anilines **5** (776 mg, 2.44 mmol, 7.3 eq.) were dissolved in anhydrous tetrahydrofuran (2 mL) in a heat-dried round-bottom flask. The brown solution was cooled down to –78 °C and was stirred for 10 minutes at this temperature. At –78 °C *tert*-butyllithium (1.7 M in pentane, 2.80 mL, 4.76 mmol, 14.2 eq.) was added dropwise to the solution. *CAUTION*: *solutions* of *tert-butyllithium react explosively* with *water and may ignite in moist air*. The orange solution was stirred for 30 minutes at –78 °C. Subsequently the Si-xanthone **3** (100 mg, 336 µmol, 1.0 eq.) dissolved in dry tetrahydrofuran (10 mL) was added *via* a syringe to the reaction mixture at –78 °C. The color of the solution turned to a bright orange. After full addition the reaction mixture was allowed to warm up to room temperature and the solution was stirred overnight. After full conversion (monitored by TLC) the solution was treated with hydrochloric acid (1 N, 5 mL). The color of the solution changed from orange over a green to finally a dark blue within minutes. The solution was diluted with dichloromethane (100 mL) and the organic phase was separated. The aqueous phase was extracted with dichloromethane (5 x 50 mL). The combined organic phases were washed with brine and dried over sodium sulfate. After filtration the solvent was removed under vacuum. The crude product was purified by flash column chromatography (silica gel, dichloromethane/methanol 99:1 to 88:12). The concentrated and combined fractions from the column were filtrated over cotton wool and Si-rhodamine **6** (124 mg, 276 µmol, 82%) was afforded as a gleaming dark blue solid.

^{1}**H-NMR** (400 MHz, MeOD-d_4, 300 K):

δ (ppm) = 7.34 (d, J = 2.8 Hz, 2 H, H_{arom}), 7.25 (d, J = 9.6 Hz, 2 H, H_{arom}), 7.10 (d, J = 8.2 Hz, 1 H, H_{arom}), 6.83–6.77 (m, 3 H, H_{arom}), 6.51 (d, J = 2.4 Hz, 1 H, H_{arom}), 3.35 (s, 12 H, N-CH_3), 1.87 (s, 3 H, CH_3), 0.60 (s, 3 H, Si-CH_3), 0.59 (s, 3 H, Si-CH_3).

^{13}**C-NMR** (101 MHz, MeOD-d_4, 300 K):

δ (ppm) = 172.1, 155.8, 149.5, 146.7, 142.7, 140.5, 131.9, 128.5, 125.6, 121.9, 117.0, 117.0, 115.0, 40.9, 18.4, –1.1, –1.3.

IR (KBr):

υ [cm^{-1}] = 3430 (br), 2937 (br), 2861 (br), 2810 (br), 1734 (w), 1606 (s), 1578 (w).

Optical properties:

Solvent	$\lambda_{abs, max}$	λ_{em}	ε_{max}	Φ_F
MeOH	653 nm	-	91900 $M^{-1}cm^{-1}$	<0.01
PBS (pH = 7.4)	651 nm	-	77300 $M^{-1}cm^{-1}$	<0.01

R$_f$ value (dichloromethane/methanol 9:1) = 0.22.

Retention time (analytical HPLC, 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1%; 95% MeCN in 16 min) = 4.00 min.

Melting point: T = 74 °C.

HR-ESI-MS:

$C_{26}H_{32}N_3Si^+$	calculated m/z	found m/z
$[M]^+$	414.2360	414.2358

The ^{1}H-NMR data are in accordance with the data in the literature.[7]

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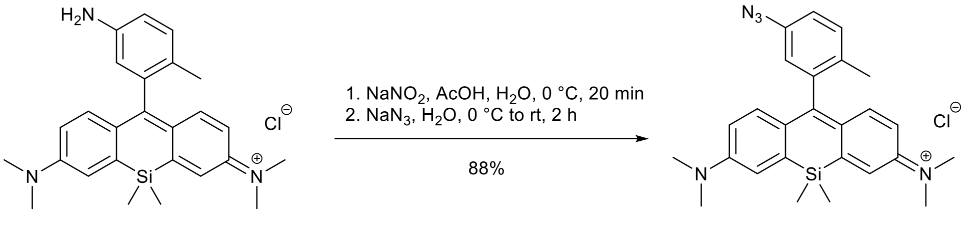
[7] Y. Koide, Y. Urano, K. Hanaoka, T. Terai, T. Nagano, *ACS Chem. Biol.* **2011**, *6*, 600–608.

2.5 Synthesis of Si-rhodamine 7[6]



6 **7**

The amine-functionalized Si-rhodamine **6** (114 mg, 253 µmol, 1.0 eq.) was dissolved in a 1:1 mixture of acetic acid (5 mL) and deionized water (5 mL). The dark blue solution was cooled down to 0 °C and sodium nitrite (26.2 mg, 380 µmol, 1.5 eq.) dissolved in deionized water (500 µL) was added. The color changed from dark blue to a slightly green-blue solution. After 20 minutes sodium azide (32.8 mg, 506 µmol, 2.0 eq.) in deionized water (500 µL) was added at 0 °C. A color change back to a dark blue was observed. The reaction mixture was stirred for two hours at 0 °C. Afterwards the solvents were carefully removed under reduced pressure. The reaction mixture was diluted with water and dichloromethane. *CAUTION: Generally working with azides should always be done cautiously. In the case of reactions with azide sources in dichloromethane, formation of explosive diazidomethane has been reported.[8] Anyway we never observed any incidents with this low scales.* Subsequently the organic phase was separated. The aqueous phase was extracted with dichloromethane (3 x 100 mL). The combined organic phases were washed with brine and dried over sodium sulfate. After filtration the solvent was removed under vacuum. The crude product was purified by flash column chromatography (silica gel, dichloromethane/methanol 99:1 to 90:10) to receive Si-rhodamine **7** (106 mg, 223 mmol, 88%) as a dark blue solid.

^{1}H-NMR (600 MHz, MeOD-*d*$_4$, 300 K):

δ (ppm) = 7.44 (d, J = 8.3 Hz, 1 H, H_{arom}), 7.37 (d, J = 2.8 Hz, 2 H, H_{arom}), 7.20 (dd, J = 8.3 Hz, 2.3 Hz, 1 H, H_{arom}), 7.09 (d, J = 9.6 Hz, 2 H, H_{arom}), 6.84 (d, J = 2.3 Hz, 1 H, H_{arom}), 6.80 (dd, J = 9.6 Hz, 2.8 Hz, 2 H, H_{arom}), 3.36 (s, 12 H, N-CH_3), 2.01 (s, 3 H, CH_3), 0.62 (s, 3 H, Si-CH_3), 0.60 (s, 3 H, Si-CH_3).

^{13}C-NMR (101 MHz, MeOD-*d*$_4$, 300 K):

δ (ppm) = 168.9, 155.8, 149.5, 142.1, 141.7, 139.5, 134.0, 132.9, 128.2, 122.3, 120.7, 120.5, 115.4, 41.0, 18.8, –1.1, –1.3.

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[8] A. Hassner, M. Stern, *Angew. Chem. Int. Ed.* **1986**, *25*, 478–479.

IR (KBr):

υ [cm^{-1}] = 3430 (br), 2979 (w), 2883 (w), 2383 (w), 2358 (w), 2342 (w), 2325 (w), 2300 (w), 2108 (w), 1608 (w), 1568 (w), 1492 (w).

Optical properties:

Solvent	$\lambda_{abs, max}$	λ_{em}	ε_{max}	Φ_F
MeOH	651 nm	670 nm	156500 M^{-1}cm^{-1}	0.18
H_2O/EtOH (5%)	651 nm	670 nm	123700 M^{-1}cm^{-1}	0.10
PBS (pH = 7.4)	651 nm	671 nm	99000 M^{-1}cm^{-1}	0.12

R$_f$ value (dichloromethane/methanol 9:1) = 0.45.

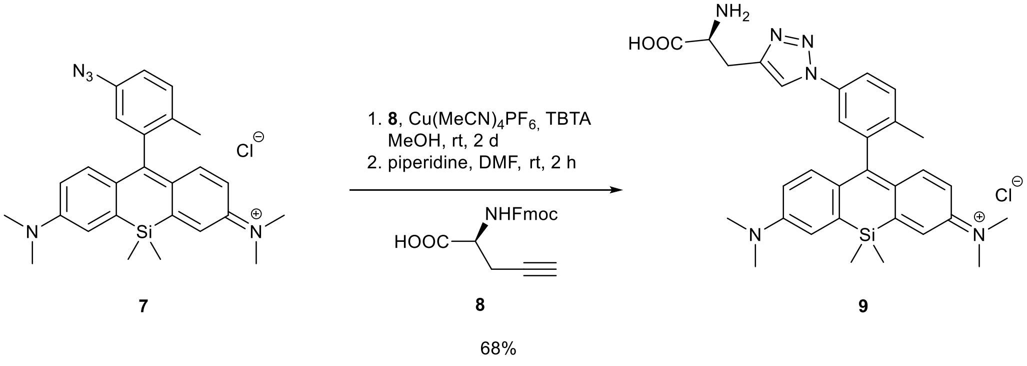
Retention time (analytical HPLC, 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1%; 95% MeCN in 16 min) = 6.03 min.

Melting point: decomposition >189 °C.

HR-ESI-MS:

$C_{26}H_{30}N_5Si^+$	calculated m/z	found m/z
$[M]^+$	440.2265	440.2271

2.6 Synthesis of Si-rhodamine 9



The azide-functionalized Si-rhodamine **7** (100 mg, 210 µmol, 1.0 eq.) was dissolved in dry methanol (5 mL) under an argon atmosphere. The solids Fmoc-*L*-propargylglycine **8** (141 mg, 420 µmol, 2.0 eq.), tetrakis(acetonitrile)copper(I) hexafluorophosphate (7.80 mg, 21.0 µmol, 0.1 eq.) and tris(benzyltriazolylmethyl)amine (11.1 mg, 21.0 µmol, 0.1 eq.) were added to the blue solution under an argon atmosphere. The reaction was stirred for two days. Subsequently the solvent was removed under reduced pressure. Afterwards the residue was dissolved in dichloromethane (100 mL) and deionized water was added. The organic phase was separated. The aqueous phase was extracted with dichloromethane (3 x 70 mL). The combined organic phases were washed with brine and dried over sodium sulfate. After filtration the solvent was removed under vacuum. The crude product was purified by flash column chromatography (silica gel, dichloromethane/methanol 96:4 to 80:20). The concentrated and combined fractions from the column were filtrated over cotton wool and 1,2,3-triazole-functionalized Si-rhodamine **9** (142 mg, 183 µmol, 87%) was received as a dark blue solid. Afterwards the Si-rhodamine was dissolved under argon atmosphere in dry dimethylformamide (2 mL) to cleave the Fmoc protection group. Piperidine (2 mL) was added and the reaction was stirred for further two hours at room temperature under an inert atmosphere. Then the solvent was removed under reduced pressure. The crude product was purified with HPLC to afford Si-rhodamine **9** (84.1 mg, 143 µmol, 68%) as blue solid.

^{1}H-NMR (500 MHz, MeOD-*d*$_4$, 300 K):

δ (ppm) = 8.49 (s, 1 H, H$_{arom}$), 7.98 (dd, J = 8.4 Hz, 2.3 Hz, 1 H, H$_{arom}$), 7.69 (d, J = 2.3 Hz, 1 H, H$_{arom}$), 7.64 (d, J = 8.4 Hz, 1 H, H$_{arom}$), 7.39 (d, J = 2.8 Hz, 2 H, H$_{arom}$), 7.13 (d, J = 9.7 Hz, 2 H, H$_{arom}$), 6.80 (dd, J = 9.7 Hz, 2.7 Hz, 2 H, H$_{arom}$), 4.41 (dd, J = 7.3 Hz, 5.0 Hz, 1 H, C$_\alpha$H), 3.50 (dd, J = 15.8 Hz, 5.0 Hz, 1 H, CH), 3.41 (dd, J = 15.8 Hz, 7.3 Hz, 1 H, CH), 3.36 (s, 12 H, N-CH$_3$), 2.13 (s, 3 H, CH$_3$), 0.63 (s, 3 H, Si-CH$_3$), 0.62 (s, 3 H, Si-CH$_3$).

^{13}C-NMR (151 MHz, MeOD-*d*$_4$, 300 K):

δ (ppm) = 170.7, 167.9, 155.8, 149.6, 142.0, 141.7, 138.4, 136.4, 133.0, 128.2, 123.2, 123.0, 122.5, 121.9, 121.7, 115.5, 53.6, 41.0, 27.2, 19.1, –1.2, –1.3.

IR (KBr):

υ [cm^{-1}] = 3426 (br), 2975 (w), 2886 (w), 1682 (w), 1609 (w), 1579 (s), 1497 (w).

Optical properties:

Solvent	$\lambda_{abs,\ max}$	λ_{em}	ε_{max}	Φ_F
MeOH	655 nm	672 nm	79900 M^{-1}cm^{-1}	0.13
H$_2$O/EtOH (5%)	653 nm	671 nm	73890 M^{-1}cm^{-1}	0.10
PBS (pH = 7.4)	655 nm	672 nm	79900 M^{-1}cm^{-1}	0.13

Retention time (analytical HPLC, 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1%; 95% MeCN in 16 min) = 4.27 min.

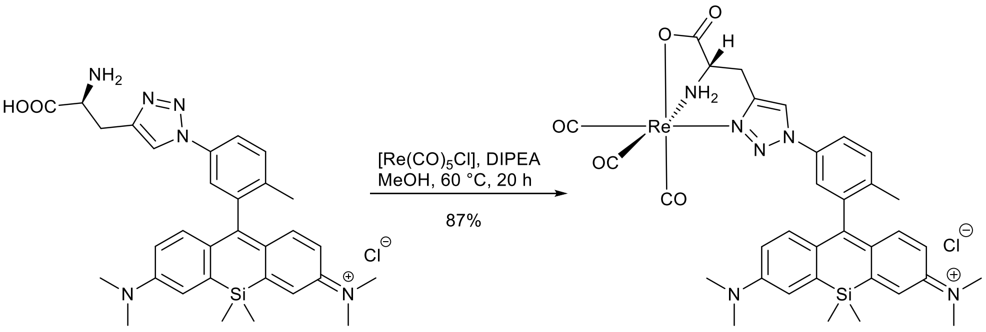
Retention time (analytical HPLC, 90% H_2O+0.1% TFA; 10% MeCN to 10% H_2O+0.1% TFA; 90% MeCN+0.1% TFA in 30 min [DAD detection at 650 nm]) = 15.3 min.

Melting point: T = 127 °C; decomposition >240 °C.

HR-ESI-MS:

$C_{31}H_{37}N_6O_2Si^+$	calculated m/z	found m/z
$[M]^+$	553.2742	553.2752
$[M\text{-}CH_3]^+$	539.2585	539.2596

2.7 Synthesis of the rhenium-complexed Si-rhodamine 10



9 **10**

Under an argon atmosphere Si-rhodamine **9** (15 mg, 25.5 µmol, 1.0 eq.) was dissolved in dry methanol (2 mL). Subsequently *N*,*N*-diisopropylethylamine (10 µL, 58.8 µmol, 2.3 eq.) was added to the blue solution. After 15 minutes rhenium(I) pentacarbonyl chloride (10.1 mg, 28.1 µmol, 1.1 eq.) was added in one portion. The dark blue solution was warmed up to 60 °C and the reaction was stirred for further 20 hours under an argon atmosphere. After full conversion (monitored by HPLC) the solutions were completely evaporated under reduced pressure. The crude product was purified with HPLC to afford the Re-complex **10** (19.0 mg, 22.2 µmol, 87%) as a blue solid.

^{1}H-NMR (500 MHz, MeOD-d_4, 300 K):

δ (ppm) = 8.63 (s, 1 H, H_{arom}), 8.03 (dd, J = 8.4 Hz, 2.2 Hz, 1 H, H_{arom}), 7.71–7.69 (m, 2 H, H_{arom}), 7.39 (d, J = 2.8 Hz, 2 H, H_{arom}), 7.12 (dd, J = 9.7 Hz, 3.3 Hz, 2 H, H_{arom}), 6.81 (dt, J = 9.6 Hz, 3.1 Hz, 2 H, H_{arom}), 5.97 (dd, J = 10.8 Hz, 5,5 Hz, 1 H, NH), 5.27 (d, J = 11.3 Hz, 1 H, NH), 4.14 (q, J = 3.7 Hz, 1 H, $C_\alpha H$), 3.52–3.40 (m, 2 H, CH_2), 3.36 (s, 12 H, N-CH_3), 2.15 (s, 3 H, CH_3), 0.63 (s, 3 H, Si-CH_3), 0.62 (s, 3 H, Si-CH_3).

^{13}C-NMR (101 MHz, MeOD-d_4, 300 K):

δ (ppm) = 167.3, 155.9, 149.5, 144.9, 142.0, 141.9, 139.8, 135.4, 133.3, 128.1, 125.0, 122.6, 122.2, 122.1, 115.6, 115.5, 52.9, 41.0, 27.5, 19.1, –1.1, –1.3.

IR (KBr):

υ [cm^{-1}] = 3431 (br), 2976 (w), 2885 (w), 2022 (CO; vs), 1889 (CO; vs), 1681 (w), 1638 (w), 1608 (CO; vs), 1579 (w).

Optical properties:

Solvent	$\lambda_{abs,\ max}$	λ_{em}	ε_{max}	Φ_F
MeOH	654 nm	672 nm	63900 $M^{-1}cm^{-1}$	0.14
H_2O/EtOH (5%)	654 nm	674 nm	22100 $M^{-1}cm^{-1}$	0.10
PBS (pH = 7.4)	654 nm	669 nm	39100 $M^{-1}cm^{-1}$	0.09

Retention time (analytical HPLC, 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1%; 95% MeCN in 16 min) = 5.11 min, 5.28 min.

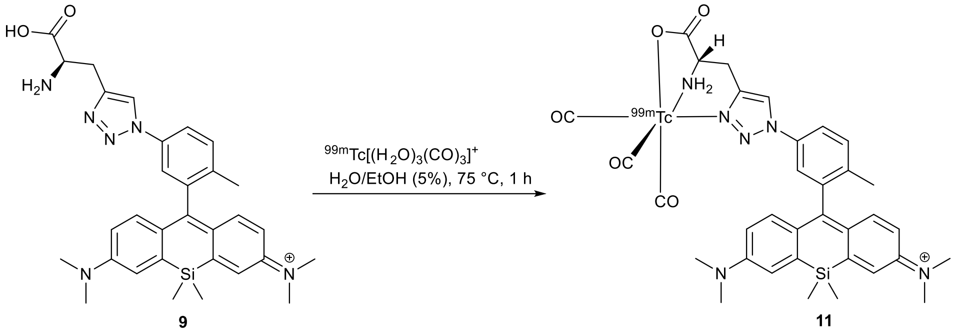
Retention time (analytical HPLC, 90% H_2O+0.1% TFA; 10% MeCN to 10% H_2O+0.1% TFA; 90% MeCN+0.1% TFA in 30 min [DAD detection at 650 nm]) = 15.4 min.

Melting point: T = decomposition >270 °C.

HR-ESI-MS:

$C_{34}H_{36}N_6O_5ReSi^+$	calculated m/z	found m/z
$[M]^+$	823.2068	823.2085

2.8 Synthesis of ^{99m}Tc-Si-rhodamine 11



Na[^{99m}TcO$_4$] was eluted using 1.0 mL of a 0.9% saline received from a ^{99}Mo/^{99m}Tc sterile generator from Triad Isotopes (Triad Isotopes – Canton, MA). The elute (2.2 mCi) was added to a sealed vial containing sodium-tartrate $Na_2C_4H_4O_6$ (7 mg), sodium tetraborate decahydrate $Na_2B_4O_7$*10 H_2O (7 mg) and sodium boranocarbonate $Na_2H_3BCO_2$ (4 mg). The sealed and with nitrogen flushed cocktail was heated for 40 minutes at 100 °C. Subsequently the solution was allowed to cool down to room temperature. The aqueous radioactive solution was treated with 1 M hydrochloride acid to adjust the pH to 7. Subsequently the aqueous technetium-99m tricarbonyl complex solution was added to a solution of Si-rhodamine **9** (86.9 nmol) in deionized water with ethanol (5%) (50 µL). The blue solution was warmed up to 70 °C and was held at this temperature for 1 h. Afterwards the reaction mixture was allowed to cool down. A small aliquot was taken and radio-HPLC was performed using deionized water (+ 0.1% trifluoroacetic acid) and acetonitrile (+ 0.1% trifluoroacetic acid). The purification was performed with deionized water (+ 0.1% TFA) and acetonitrile (+ 0.1% TFA) on the analytical radio-HPLC. Afterwards the aqueous solution was evaporated under reduced pressure and the residue was redissolved in PBS/EtOH (5%) to receive **11** with a radiochemical yield of 59%.[9, 10]

2.9 Stability tests of rhenium-complex 10 in PBS and challenging experiments with histidine

The rhenium-complexed Si-rhodamine **10** was dissolved in PBS/EtOH (5%) and the solution was injected after several time intervals into the RP-HPLC and stabilities were analyzed. In another experiment **10** was added to a solution of *L*-histidine (1 mM) and aliquots were withdrawn in the same time range and were analyzed in RP-HPLC at room temperature and at 37 °C.

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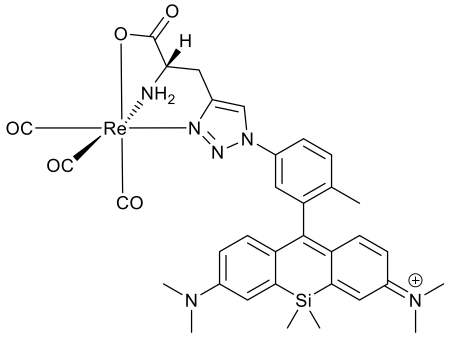
[9] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A. P. Schubiger, *J. Am. Chem. Soc.* **2001**, *123*, 3135–3136.
[10] C. C. Konkankit, B. A. Vaughn, S. N. MacMillan, E. Boros, J. J. Wilson, *Inorg. Chem.* **2019**, *58*, 3895–3909.



10

Table 1: Overview of the results from the HPLC stability tests of complex **10** in aqueous medium and challenging with histidine after several time points.

Time	PBS/EtOH (5%)	PBS/EtOH (5%) at 37 °C	PBS/EtOH (5%)+1 mM histidine at 37 °C
1 h	>99%	>99%	>99%
3 h	>99%	>99%	>99%
6 h	>99%	>99%	>99%
24 h	>99%	>99%	>99%

2.10 Stability tests of technetium complex 11 in human serum

The *in vitro* stability of the purified pure technetium-99m complex **11** was determined in human serum. The complex **11** (50 µL) dissolved in PBS/EtOH (5%) was added to human serum (500 µL) and at different time points up to 24 hours the stability was evaluated at room temperature and 37 °C. The stability was monitored by radio-TLC with the solvent system water/acetic acid (5%) for the RP-TLC plates. The degree of degradation was analyzed by comparison of increasing free pertechnetate on TLC and the results are shown in Table 2.

Table 2: Overview of the stability test results from the radio-TLC of complex **11** in human serum after several time points at room temperature and at 37 °C.

Time	Stability at room temperature	Stability at 37 °C
1 h	>99%	>99%
3 h	>98%	>99%
5 h	>97%	>93%
19 h	>96%	>93%
24 h	>96%	>92%

2.11 Partition coefficient determination of technetium-complex 11

The partition coefficient of the purified pure technetium-99m complex **11** was determined in PBS (pH=7.4) and *n*-octanol using the shake flask assay. For this purpose the complex **11** (20 µL/2 MBq) was added into an Eppendorf vial and PBS (500 µL) and *n*-octanol (500 µL) were added. The mixture was mixed for 30 minutes at room temperature. Afterwards the aqueous and organic phases were separated from each other and were centrifuged at 4000 rpm for 10 minutes. Subsequently two aliquots (100 µL) from each phase were taken and were counted at a gamma counter. The log D value at pH= 7.4 was calculated by using the following equation:

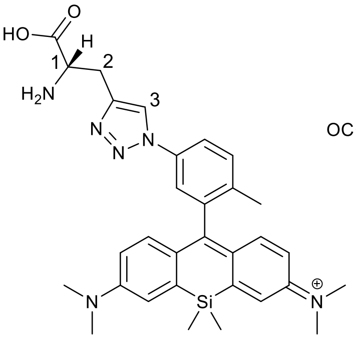
$$\log D_{ph=7.4} = \frac{\log A_{oct}}{\log A_{PBS}} \tag{1}$$

The A_{oct} stands for the decay corrected gamma counts per second from the *n*-octanol phase whereas the A_{PBS} stands for the decay corrected gamma counts per second from the aqueous phosphate-buffered saline (PBS) phase.

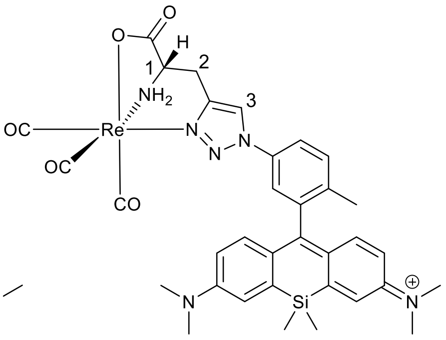
The reported value is the average of 10 measurements:

$$\log D_{ph=7.4} \text{ (technetium complex } \mathbf{11}\text{)} = 1.11.$$

2.12 Comparison of the NMR-data of 9 and 10 in methanol-*d₄*



9

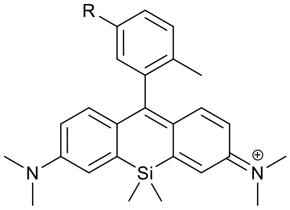


10

Table 3: Comparison of the NMR-data of **9** and **10** in methanol-d_4

protons	Ligand **9**		Re-complex **10**	
	δ (ppm)	*J* [Hz]	δ (ppm)	*J* [Hz]
1	4.41	7.3, 5.0	4.14	3.7
2	3.41	15.8, 5.0	3.46	-
	3.50	15.8, 7.3		
3	8.49	-	8.63	-
NH	-	-	5.97	10.8, 5.5
NH	-	-	5.27	11.3

2.13 Summary of the optical properties



12

Each dye was diluted in methanol to receive a general stock solution. Afterwards co-injection in PBS with pH 7.4 or water/ethanol (5%) outgoing from the stock solution was prepared. The extinction coefficient ε_{max} was calculated according to the Lambert-Beer equation (1) for the absorbance maximum whereas the absorption intensity was limited up to 0.1:

$$E = c \cdot \varepsilon \cdot d \qquad (2)$$

E means the extinction, c stands for the concentration, ε gives the molar absorption coefficient and d describes the thickness of the used cuvette (*d* = 1 cm).

For determination of the quantum yield the dyes were diluted to a dilution series considering an absorbance between 0.01 and 0.1 at the excitation wavelength of 650 nm. The quantum yield was calculated using the following equation:

$$QY_X = QY_S \cdot \frac{m}{m_{ref}} \cdot \frac{\eta}{\eta_{ref}} \qquad (3)$$

According to (2) QY_x is the unknown quantum yield, QY_s the quantum yield of the reference substance $[Ru(bpy)_3]Cl_2$ in water (QY_s = 0.042), m and m_{ref} are the slopes of the linear fit of the integrated emission and η and η_{ref} are belonging to the refractive index of the corresponding solvent.[11]

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[11] K. Nakamaru, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 1639–1640.

3. Analytical Data

3.1 NMR Spectra

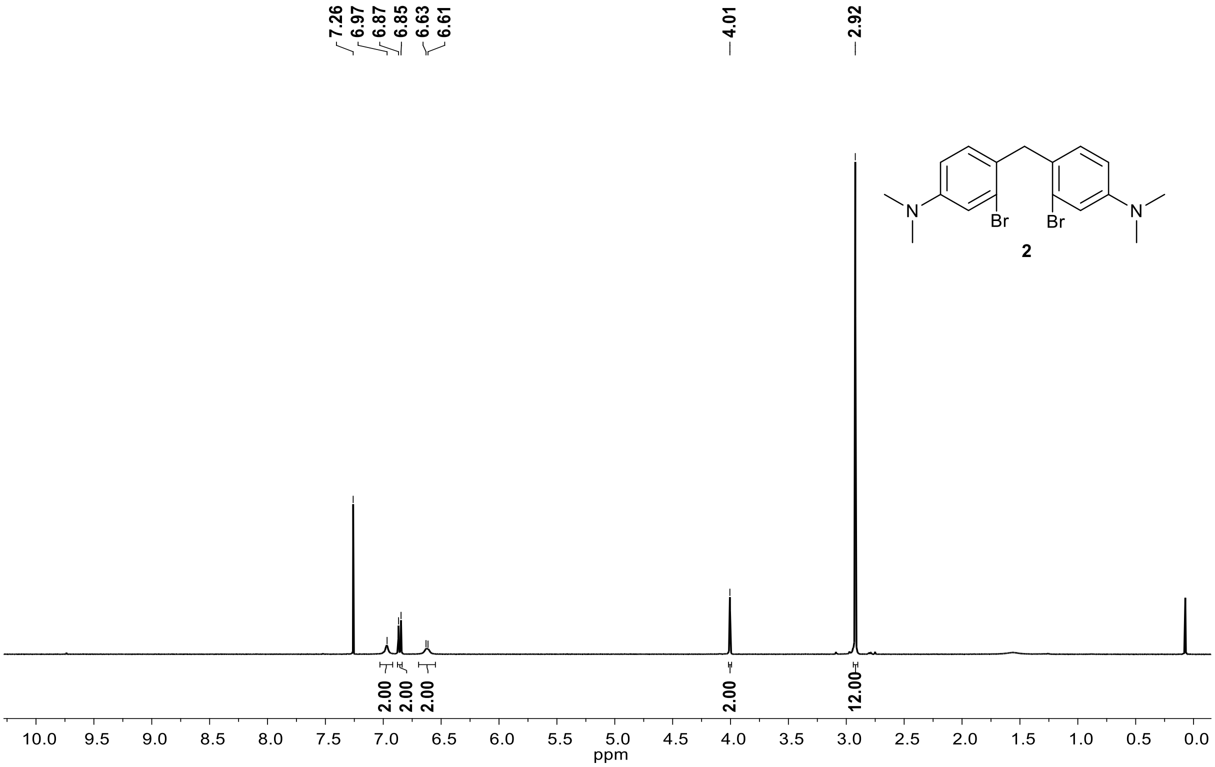


Figure 1: ^{1}H-NMR-spectrum of **2** in chloroform-*d* (400 MHz, 300 K).

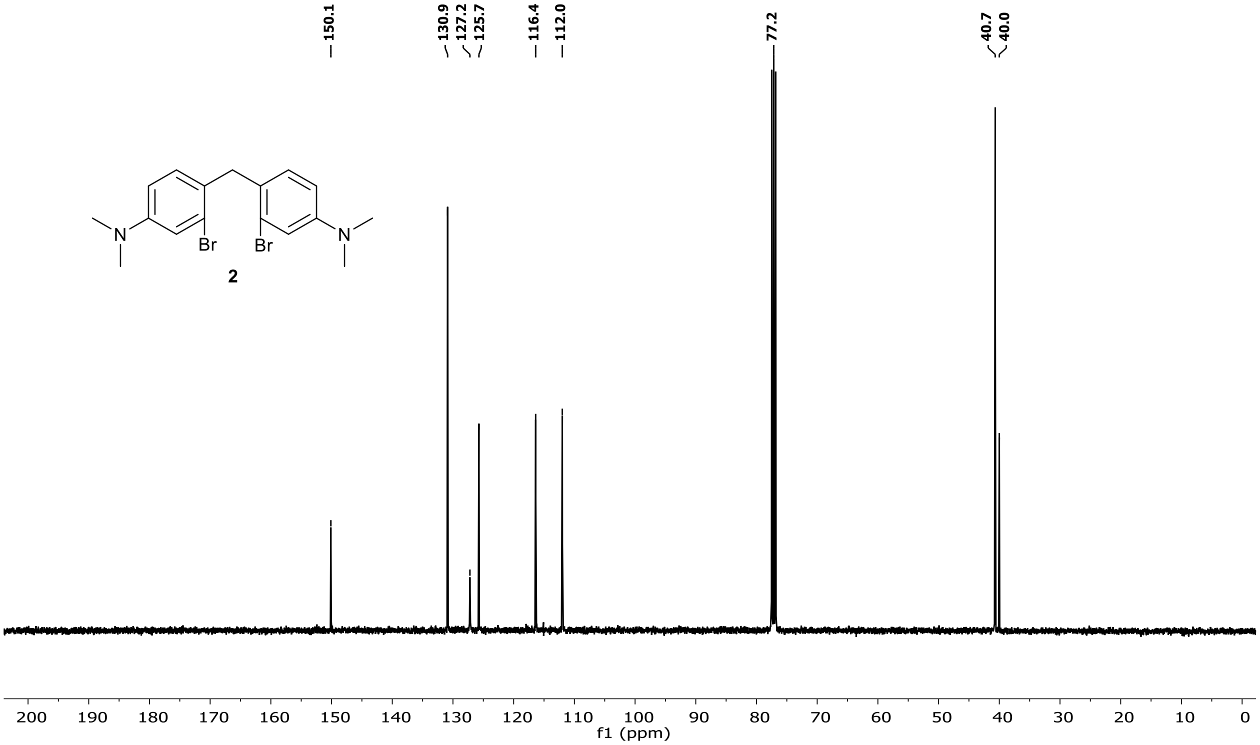


Figure 2: $^{13}C\{^1H\}$-NMR-spectrum of **2** in chloroform-*d* (101 MHz, 300 K).

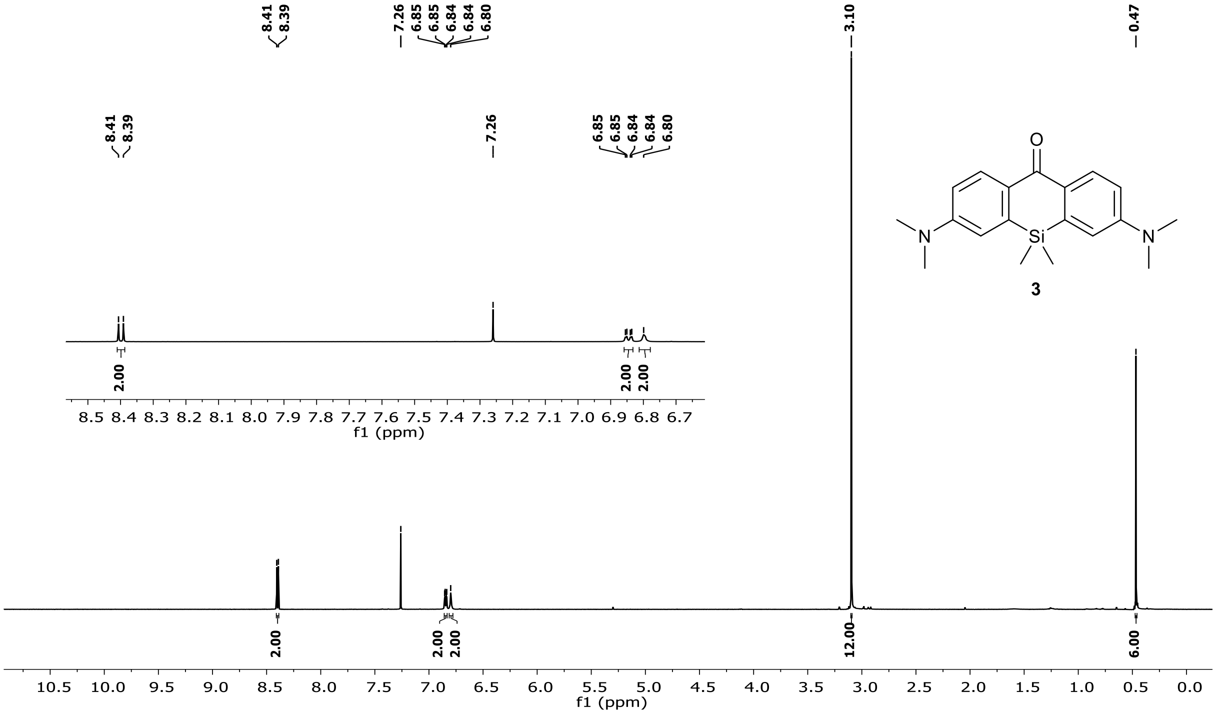


Figure 3: ^{1}H-NMR-spectrum of **3** in chloroform-*d* (600 MHz, 300 K).

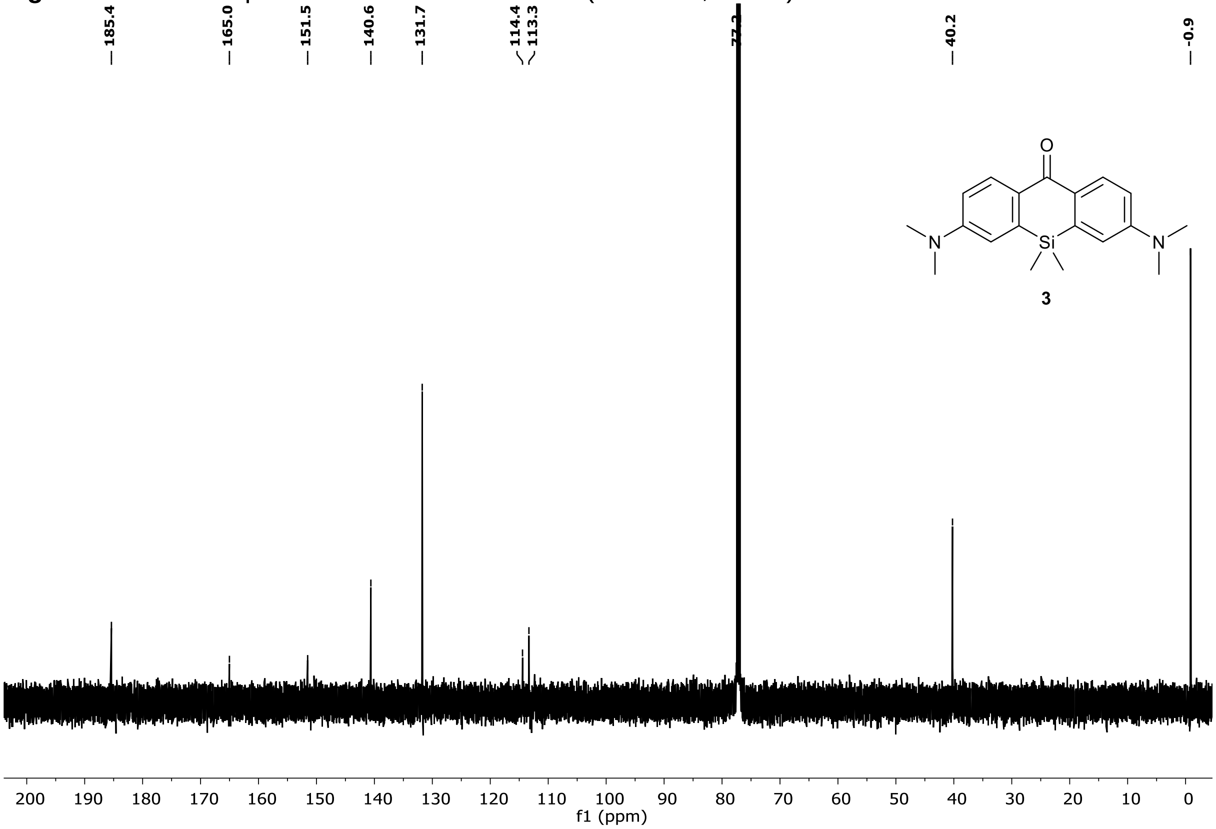


Figure 4: ^{13}C{^{1}H}-NMR-spectrum of **3** in chloroform-*d* (151 MHz, 300 K).

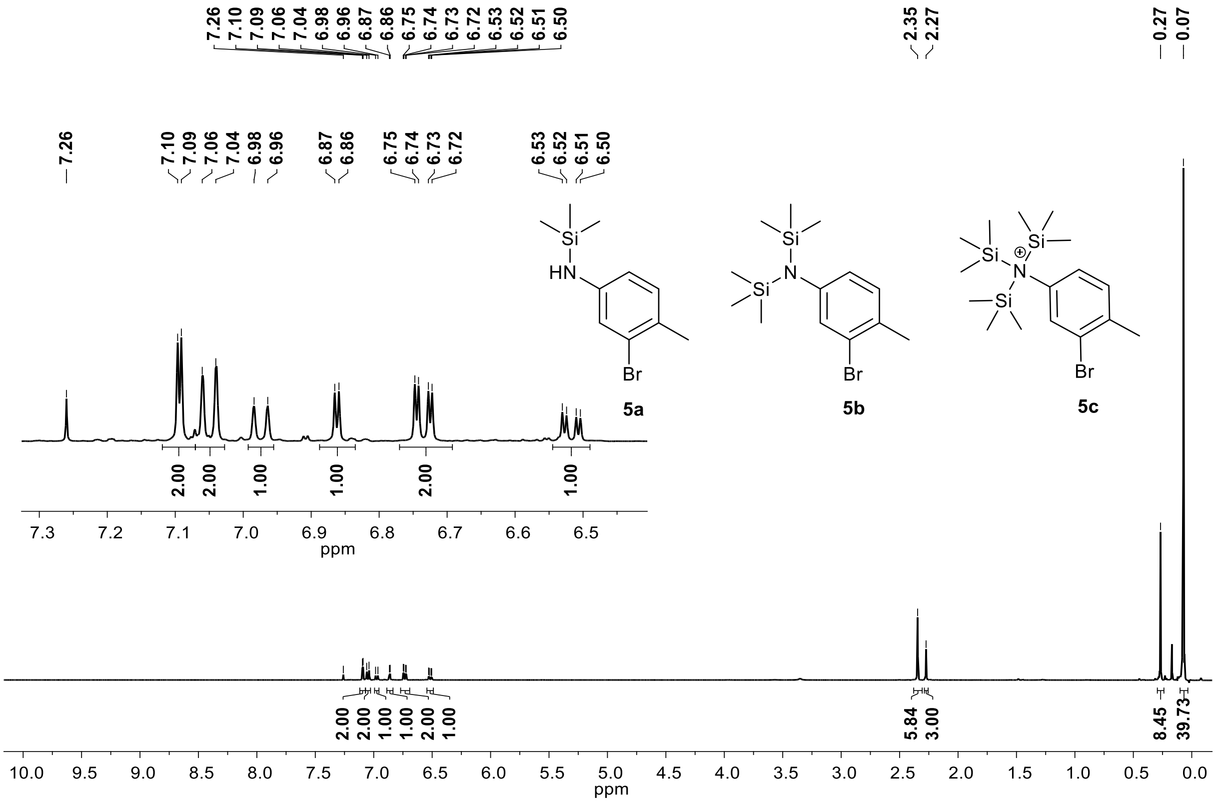


Figure 5: ^{1}H-NMR-spectrum of **5** in chloroform-*d* (400 MHz, 300 K).

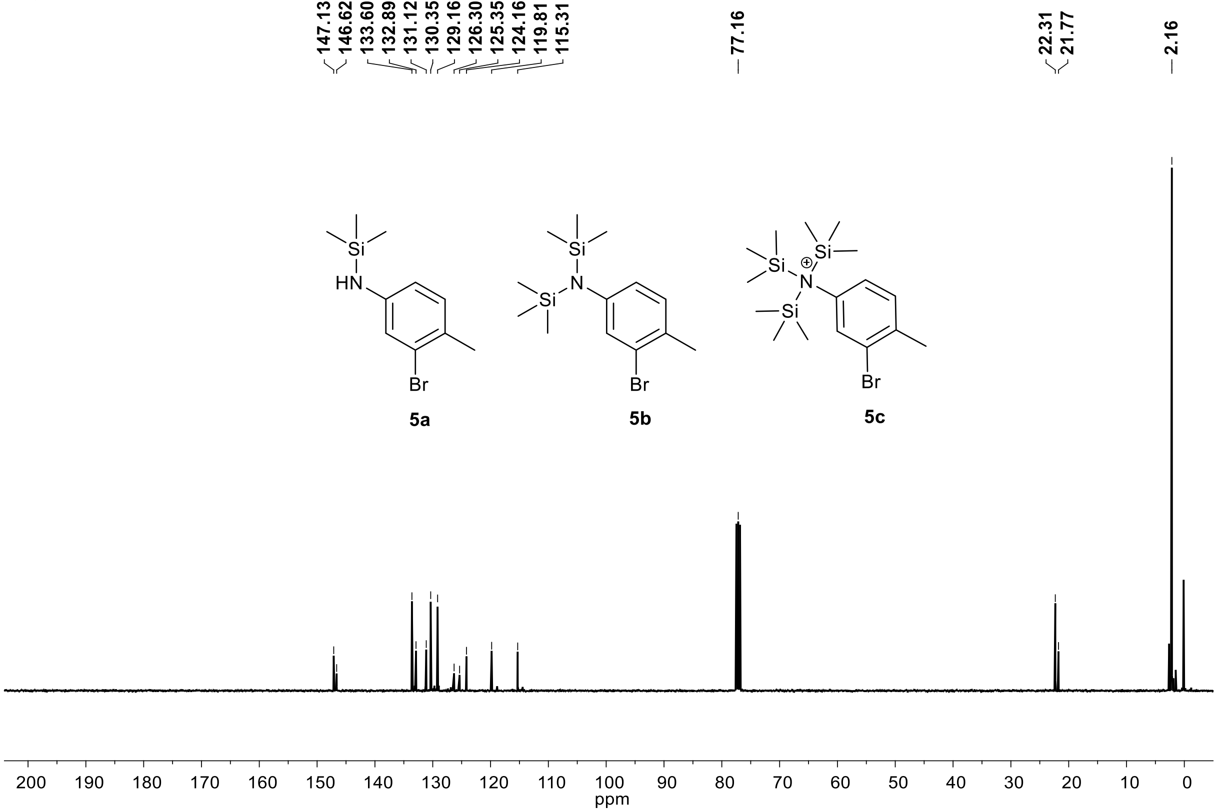


Figure 6: ^{13}C{^{1}H}-NMR-spectrum of **5** in chloroform-*d* (151 MHz, 300 K).

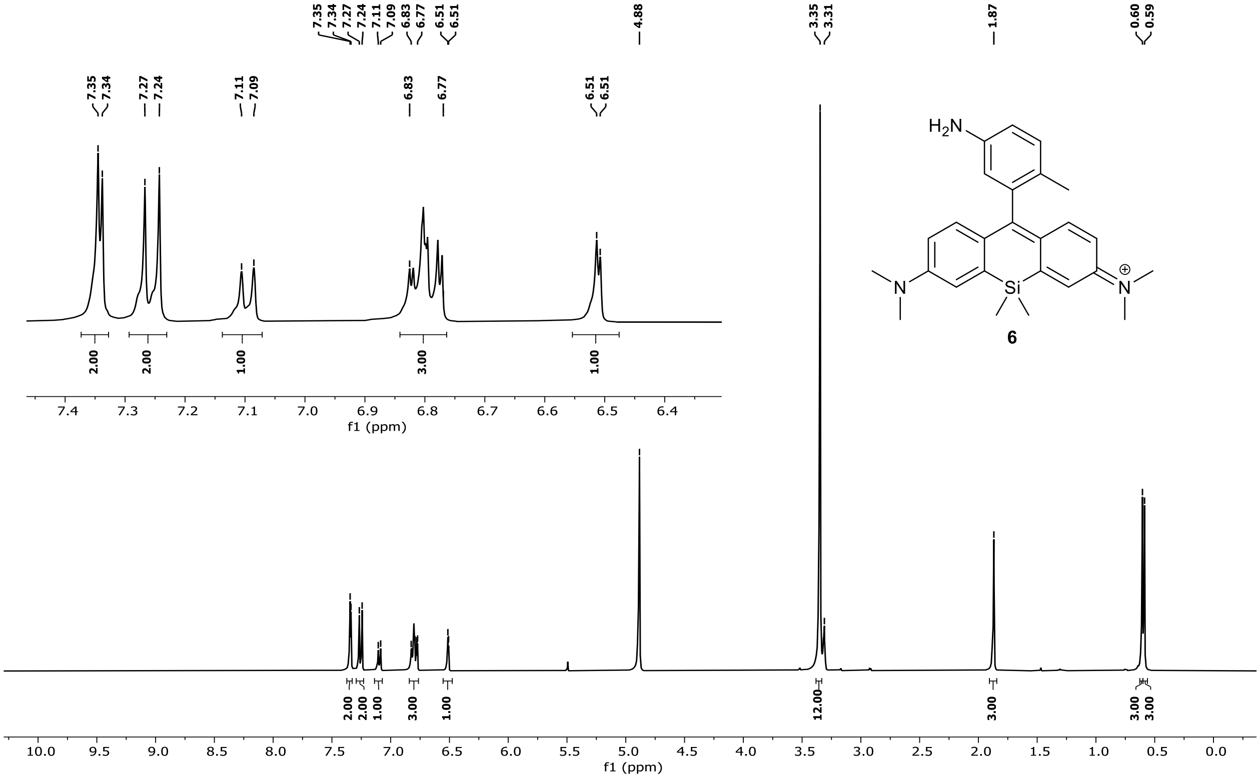


Figure 7: ^{1}H-NMR-spectrum of **6** in methanol-*d₄* (600 MHz, 300 K).

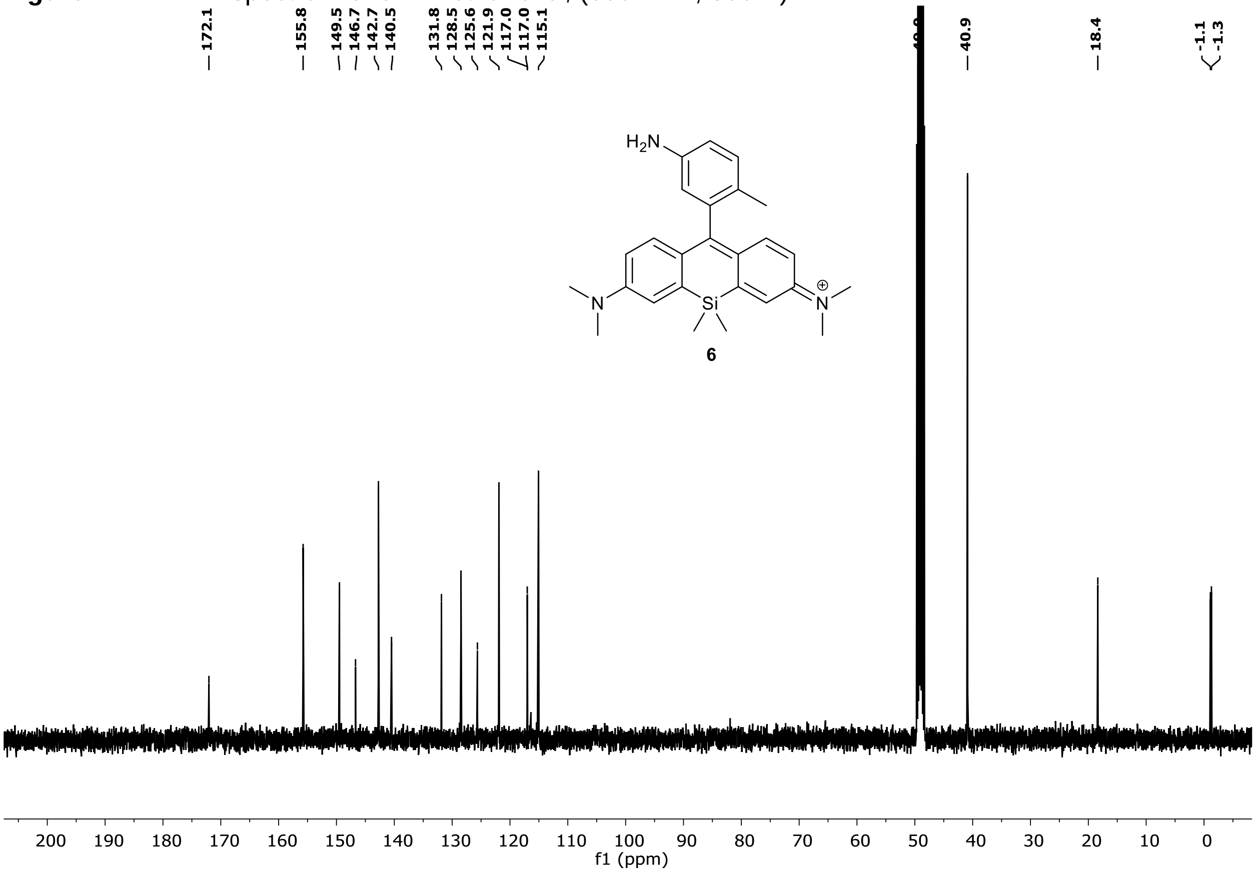


Figure 8: ^{13}C{^{1}H}-NMR-spectrum of **6** in methanol-*d₄* (101 MHz, 300 K).

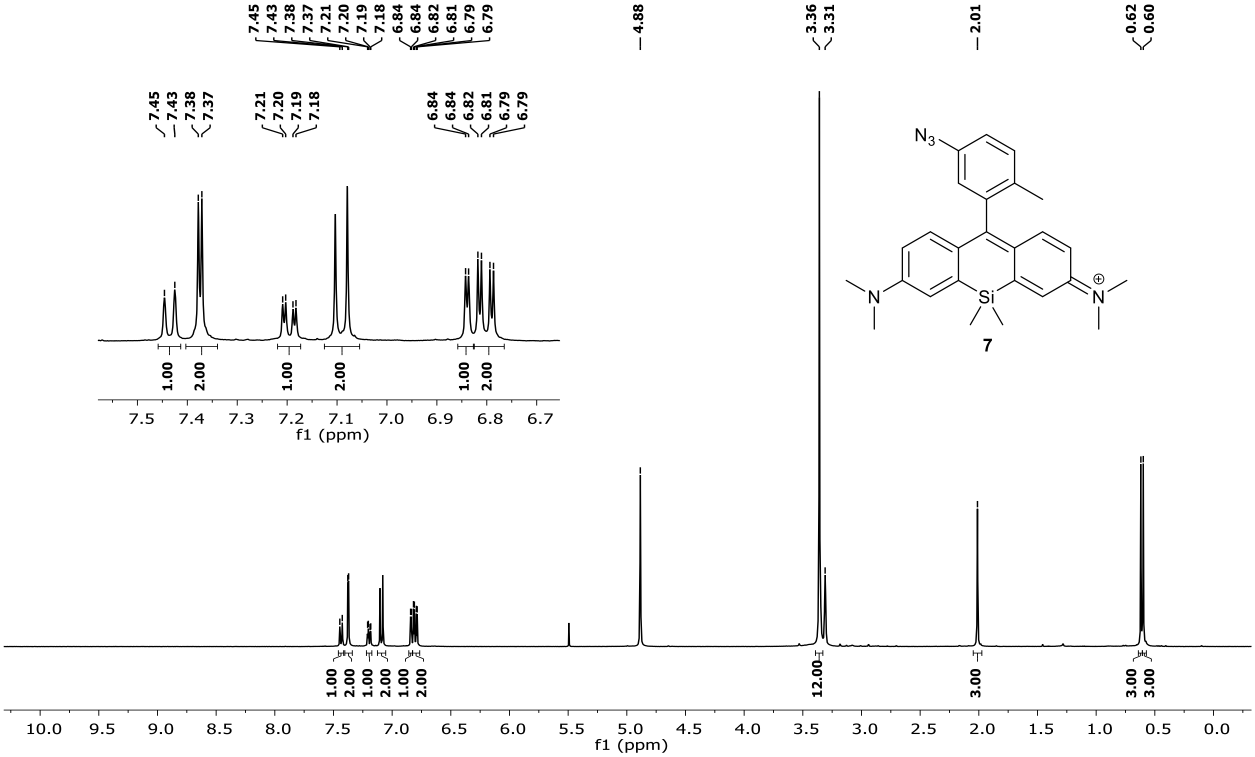


Figure 9: ^{1}H-NMR-spectrum of **7** in methanol-*d*$_4$ (600 MHz, 300 K).

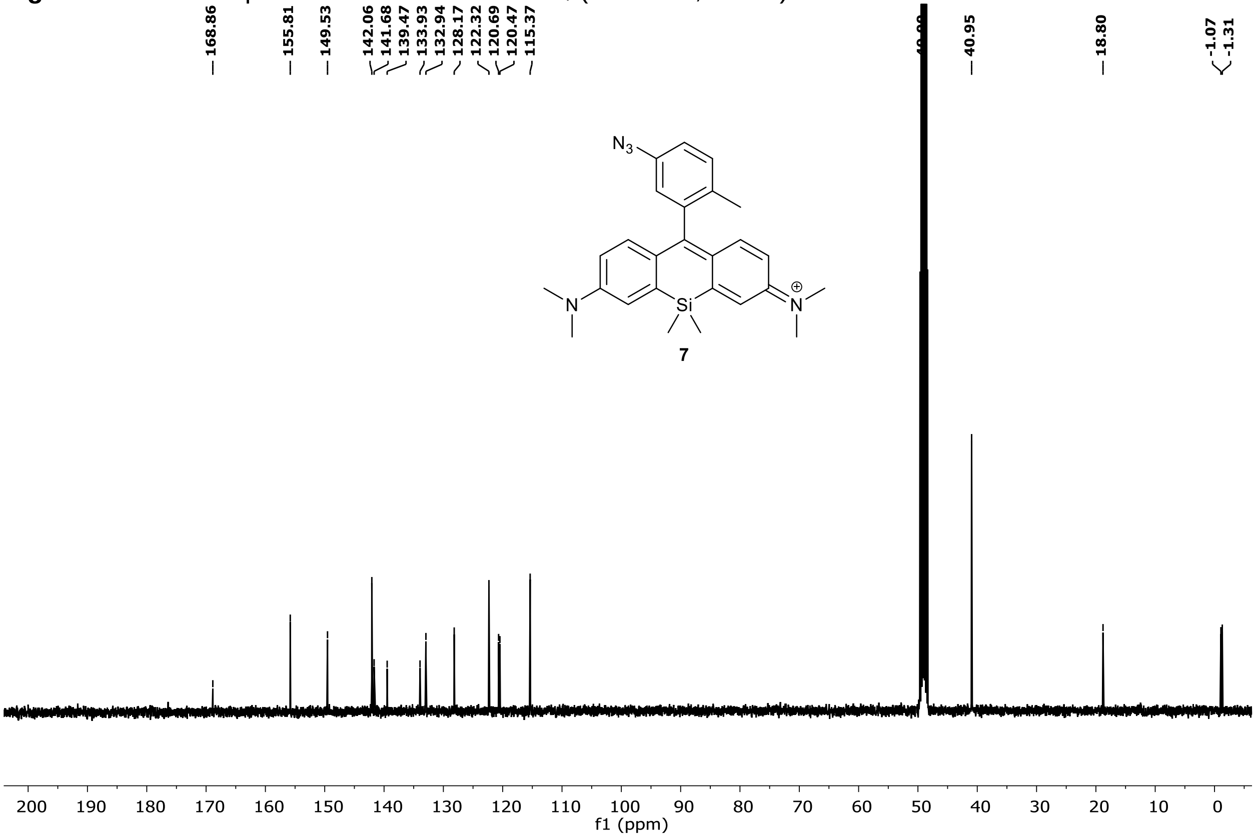


Figure 10: ^{13}C{^{1}H}-NMR-spectrum of **7** in methanol-*d*$_4$ (101 MHz, 300 K).

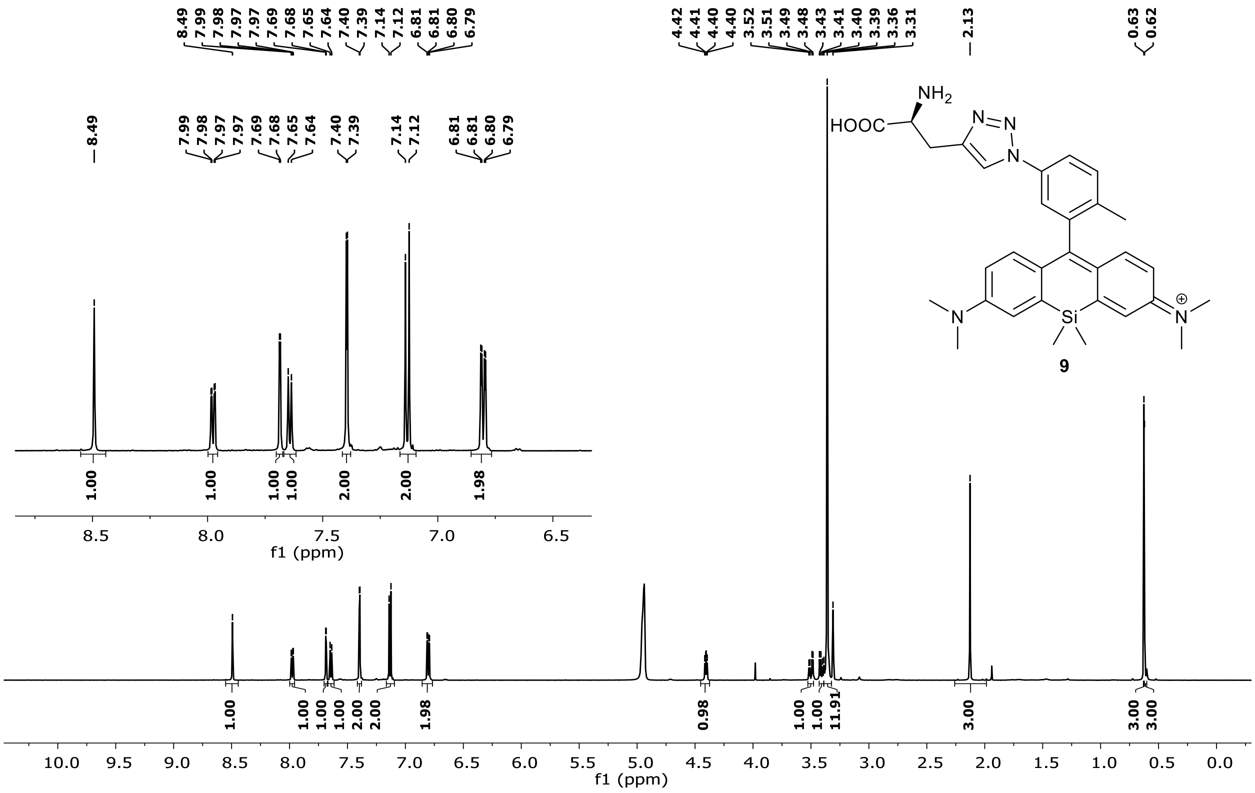


Figure 11: ^{1}H-NMR-spectrum of **9** in methanol-d_4 (600 MHz, 300 K).

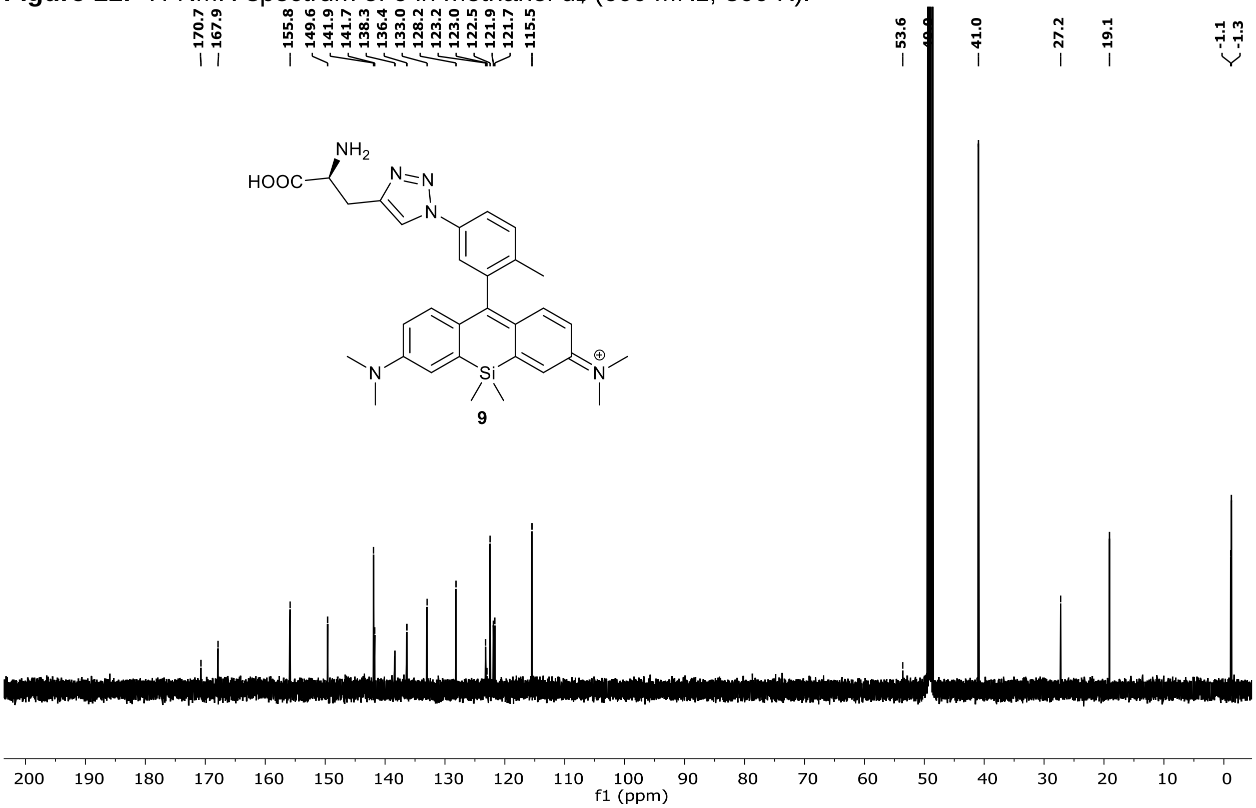


Figure 12: ^{13}C{^{1}H}-NMR-spectrum of **9** in methanol-d_4 (151 MHz, 300 K).

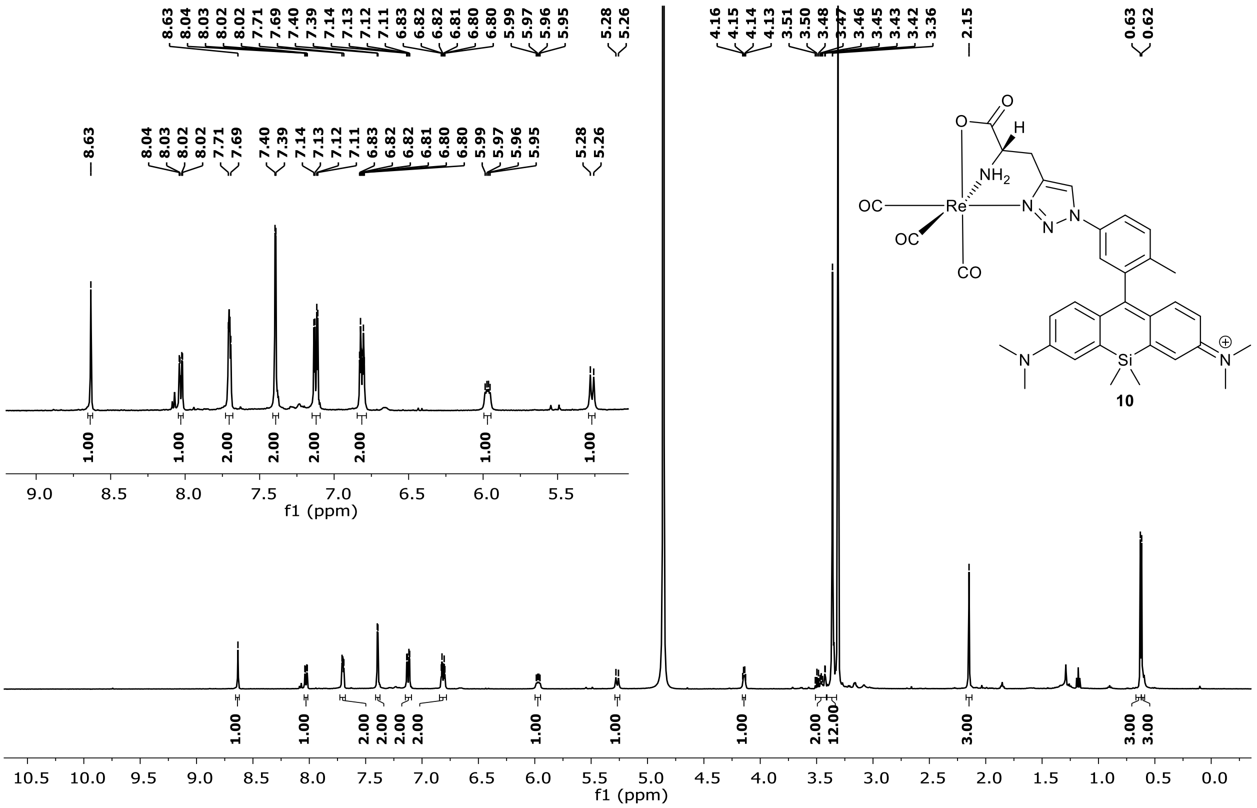


Figure 13: ^{1}H-NMR-spectrum of **10** in methanol-d_4 (500 MHz, 300 K).

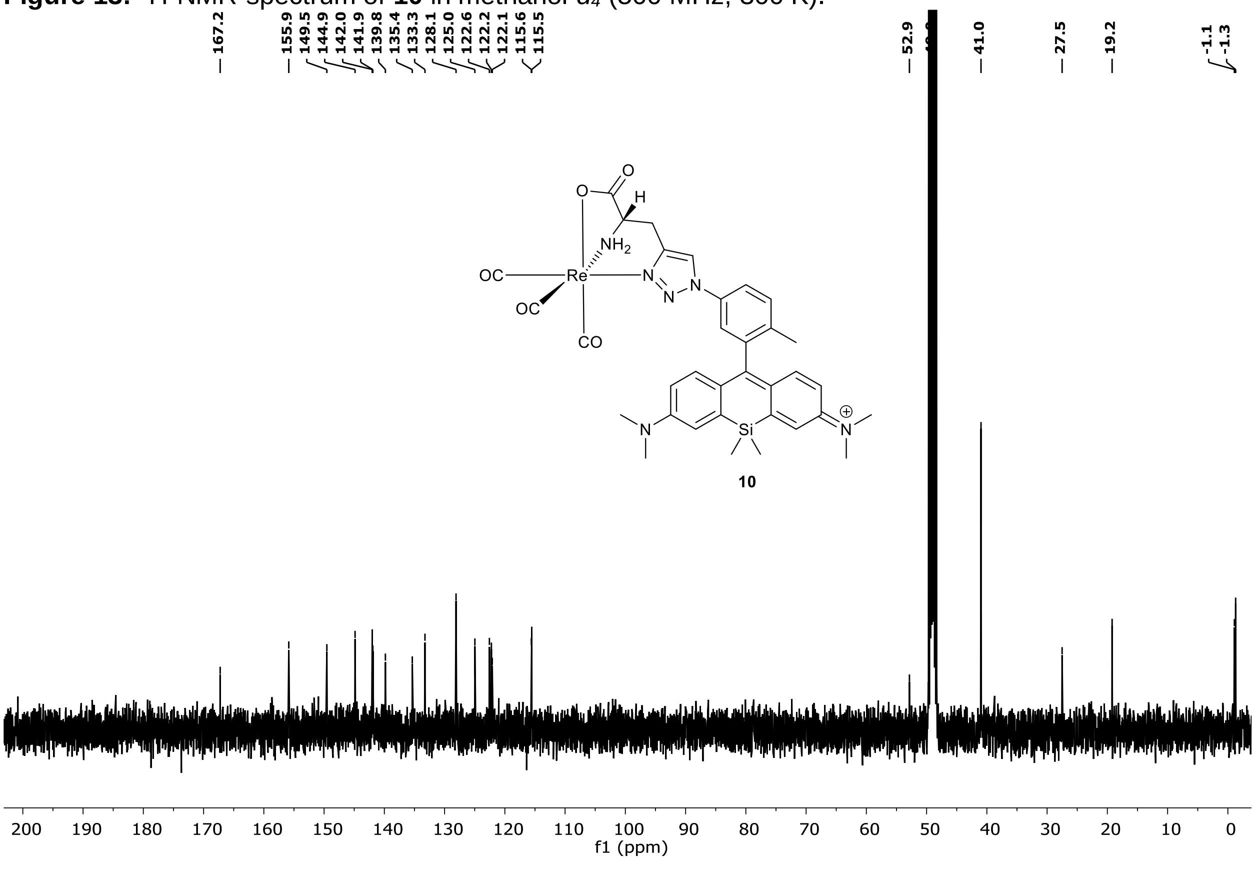


Figure 14: ^{13}C{^{1}H}-NMR-spectrum of **10** in methanol-d_4 (101 MHz, 300 K).

3.2 UV/VIS/NIR spectra

3.2.1 UV/VIS/NIR spectra of amin-functionalized Si-rhodamine 6

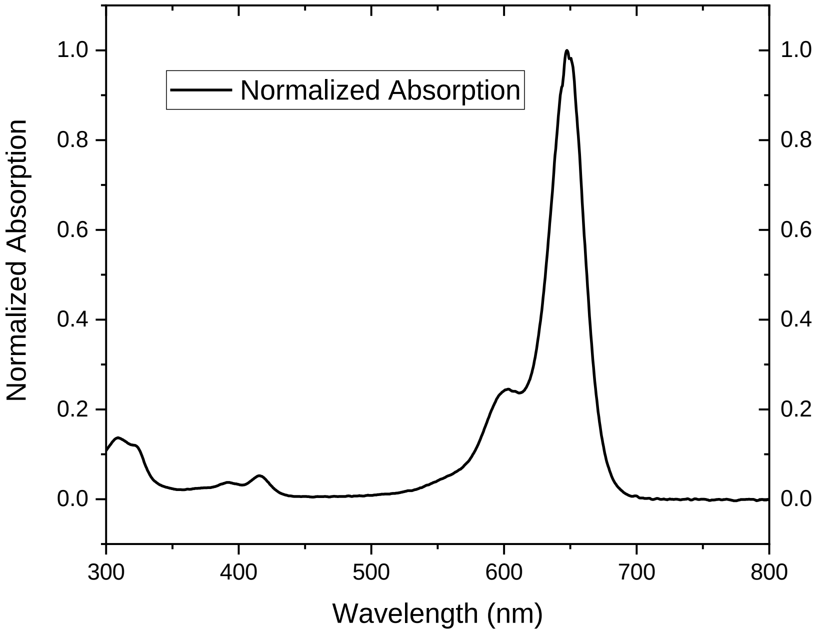


Figure 15: Absorption UV/VIS/NIR spectrum of **6** in methanol at room temperature.

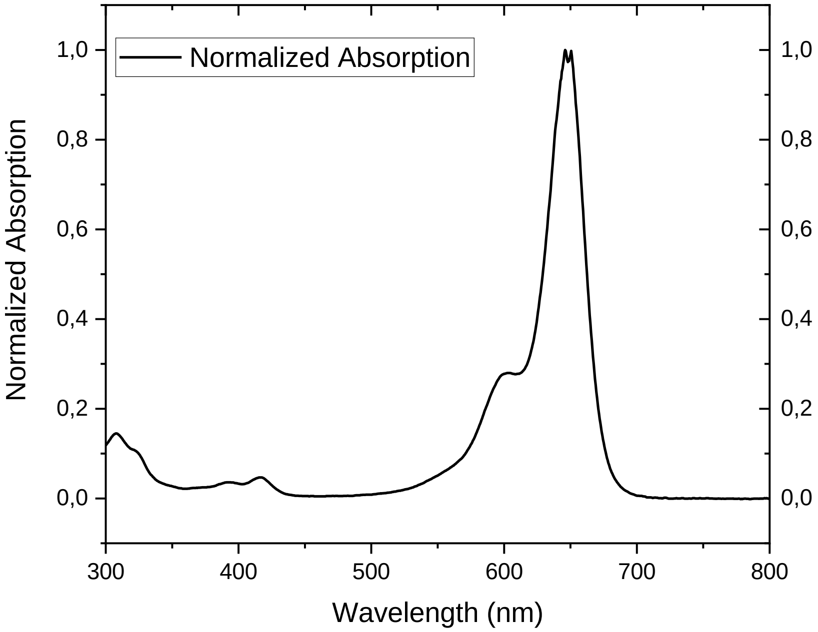


Figure 16: Absorption UV/VIS/NIR spectrum of **6** in PBS (pH = 7.4) at room temperature.

3.2.2 UV/VIS/NIR spectrum of azide-functionalized Si-rhodamine 7

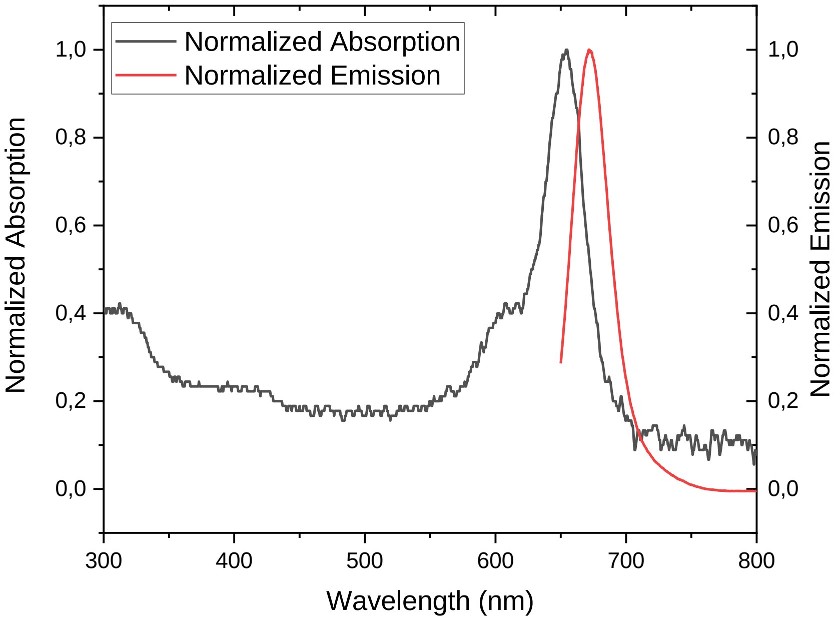


Figure 17: Absorption and emission UV/VIS/NIR spectrum of **7** in methanol at room temperature (λ_{ex} = 650 nm).

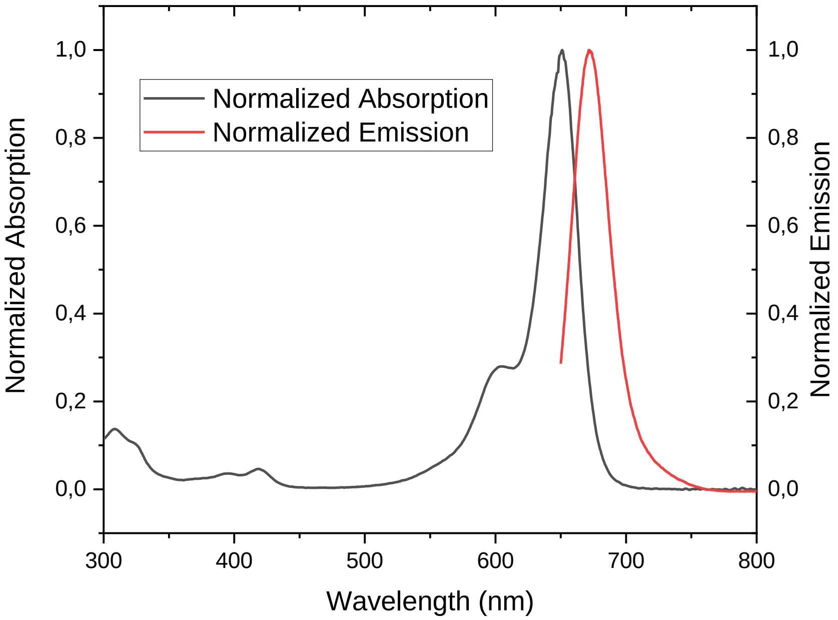


Figure 18: Absorption and emission UV/VIS/NIR spectrum of **7** in PBS (pH = 7.4) at room temperature (λ_{ex} = 650 nm).

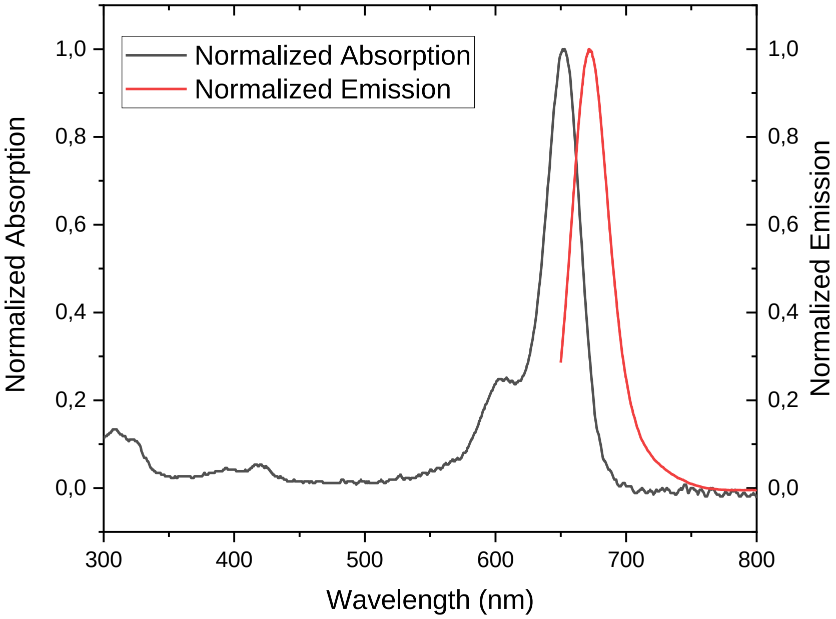


Figure 19: Absorption and emission UV/VIS/NIR spectrum of **7** in water/ethanol (5%) at room temperature (λ_{ex} = 650 nm).

3.2.3 UV/VIS/NIR spectrum of amino acid-functionalized Si-rhodamine 9

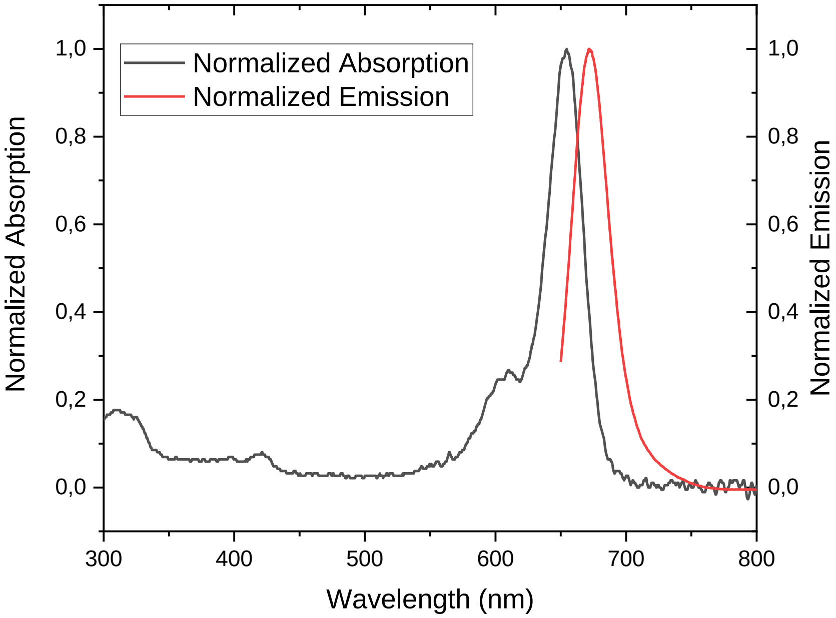


Figure 20: Absorption and emission UV/VIS/NIR spectrum of **9** in methanol at room temperature (λ_{ex} = 650 nm).

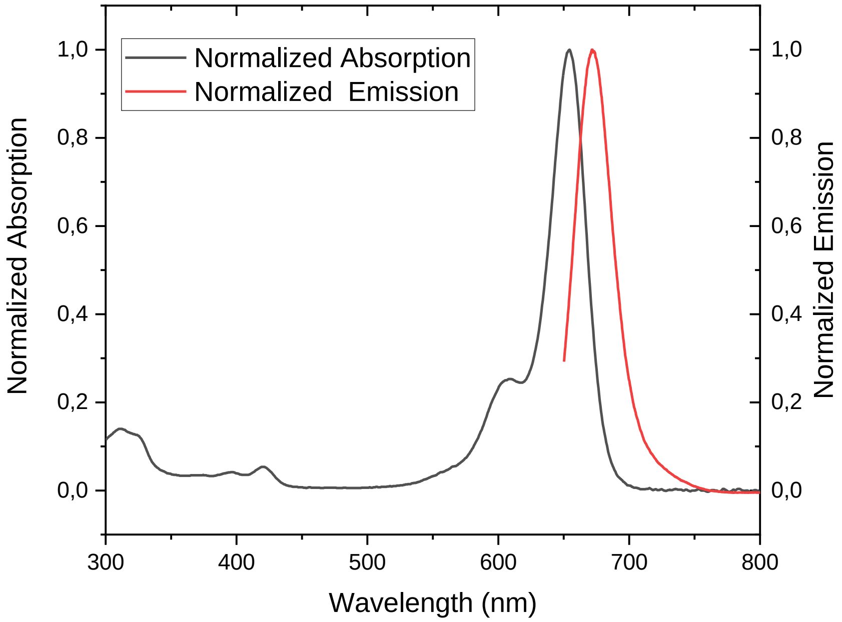


Figure 21: Absorption and emission UV/VIS/NIR spectrum of **9** in PBS (pH = 7.4) at room temperature (λ_{ex} = 650 nm).

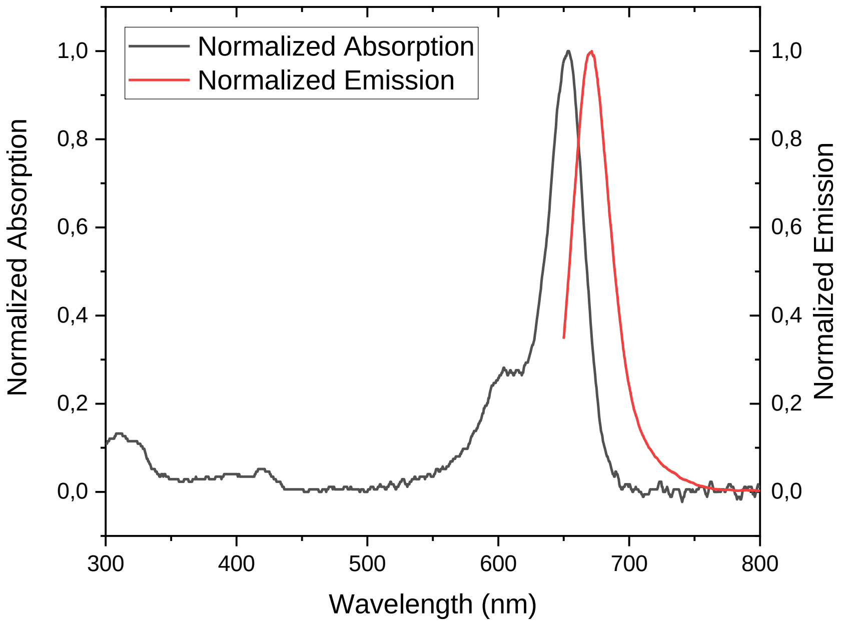


Figure 22: Absorption and emission UV/VIS/NIR spectrum of Si-rhodamine **9** in water/ethanol (5%) at room temperature (λ_{ex} = 650 nm).

3.2.4 UV/VIS/NIR spectrum of rhenium-complex 10

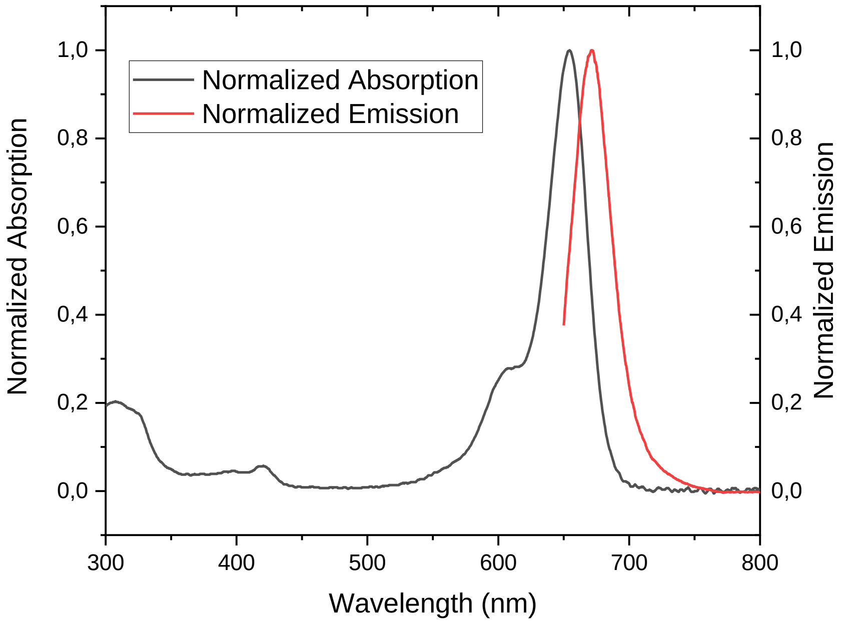


Figure 23: Absorption and emission UV/VIS/NIR spectrum of **10** in methanol at room temperature (λ_{ex} = 650 nm).

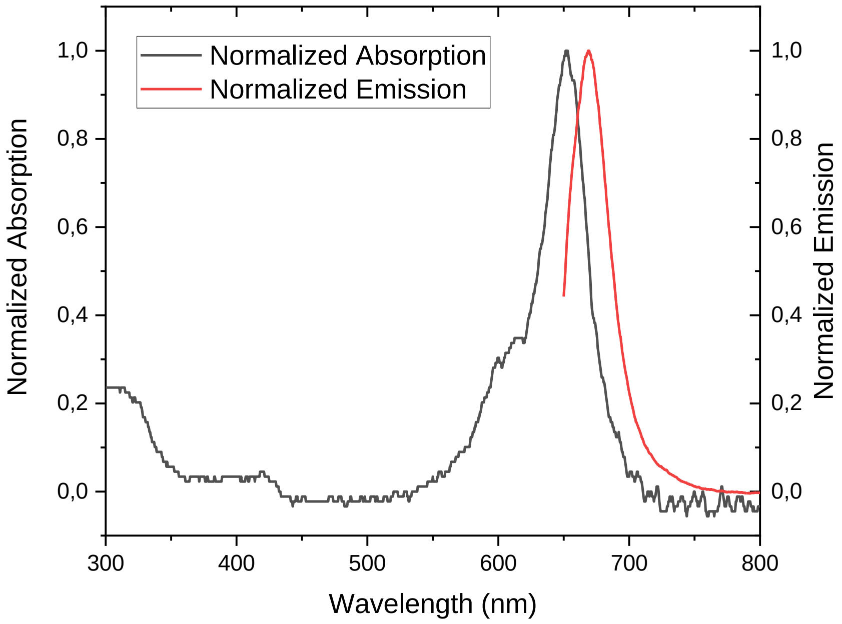


Figure 24: Absorption and emission UV/VIS/NIR spectrum of **10** in PBS (pH = 7.4) at room temperature (λ_{ex} = 650 nm).

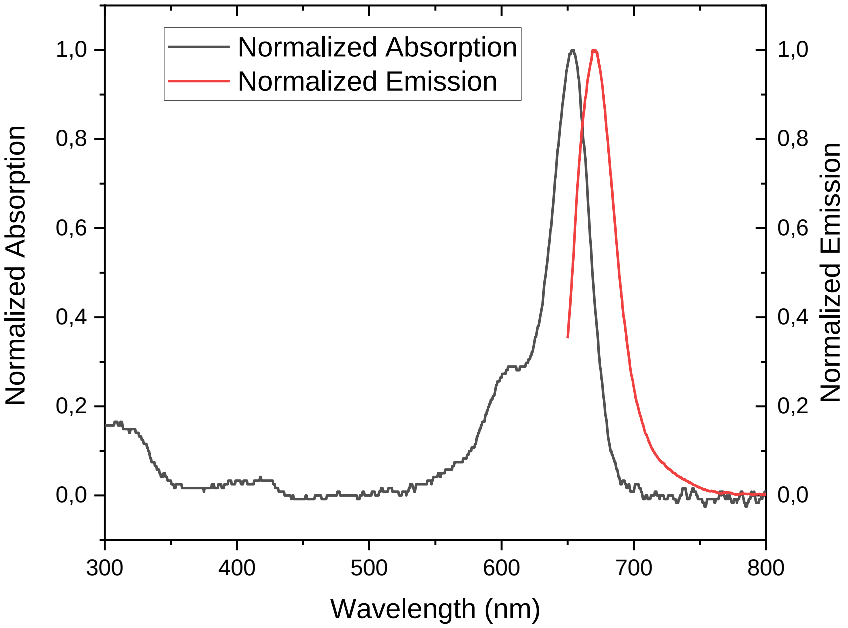
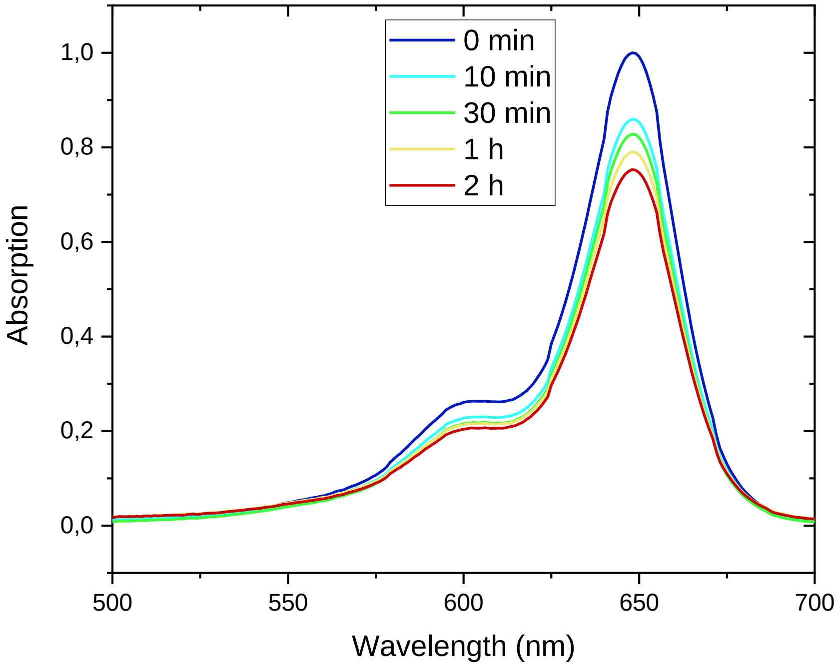
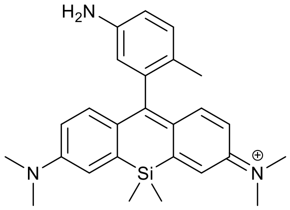


Figure 25: Absorption and emission UV/VIS/NIR spectrum of **10** in water/ethanol (5%) at room temperature (λ_{ex} = 650 nm).

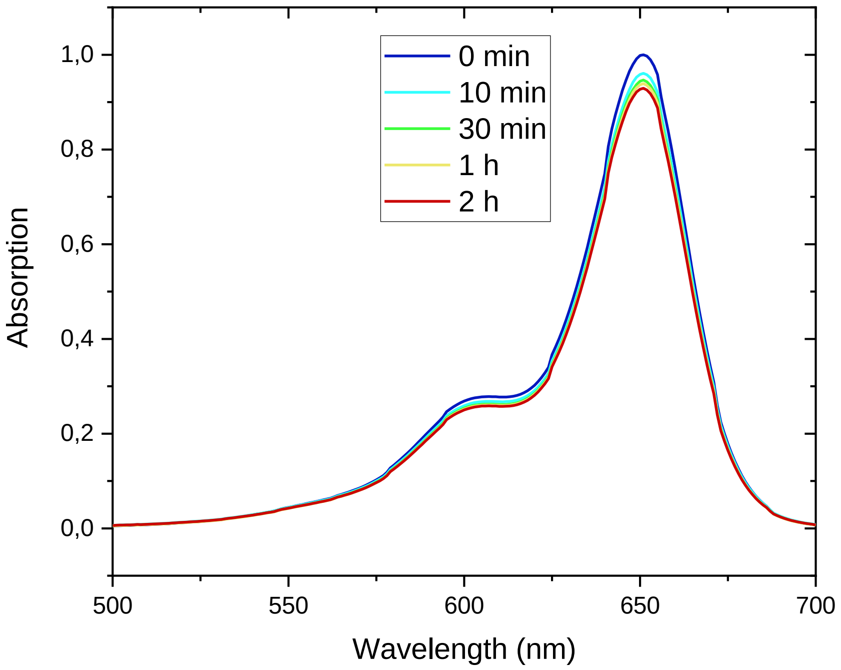
3.3 Photostability tests

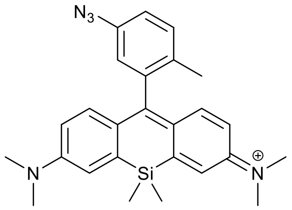




6

Figure 26: Absorption spectra of **6** after irradiation (λ_{ex} = 650 nm) with a pulsed laser (20 kW, pulse width at half peak height < 10 µs) for two hours in water/ethanol (5%). The absorption spectrum for 0 min was normalized and the other absorption spectra are referenced to the spectrum at 0 min.





7

Figure 27: Absorption spectra of **7** after irradiation (λ_{ex} = 650 nm) with a pulsed laser (20 kW, pulse width at half peak height < 10 µs) for two hours in water/ethanol (5%). The absorption spectrum for 0 min was normalized and the other absorption spectra are referenced to the spectrum at 0 min.

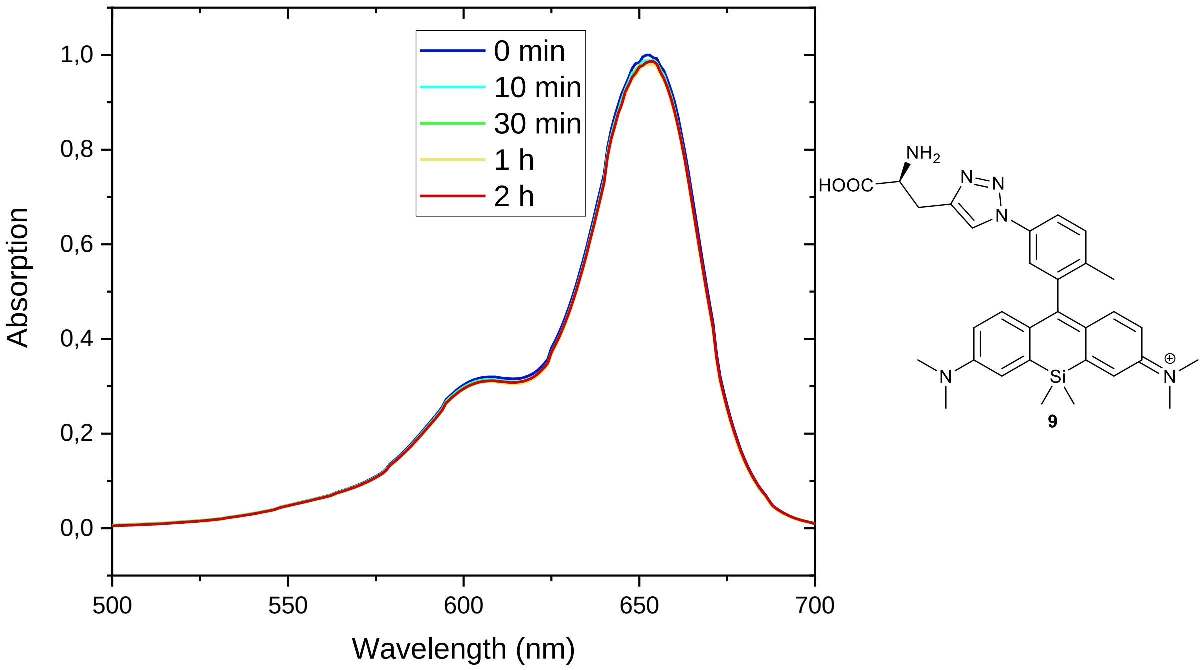
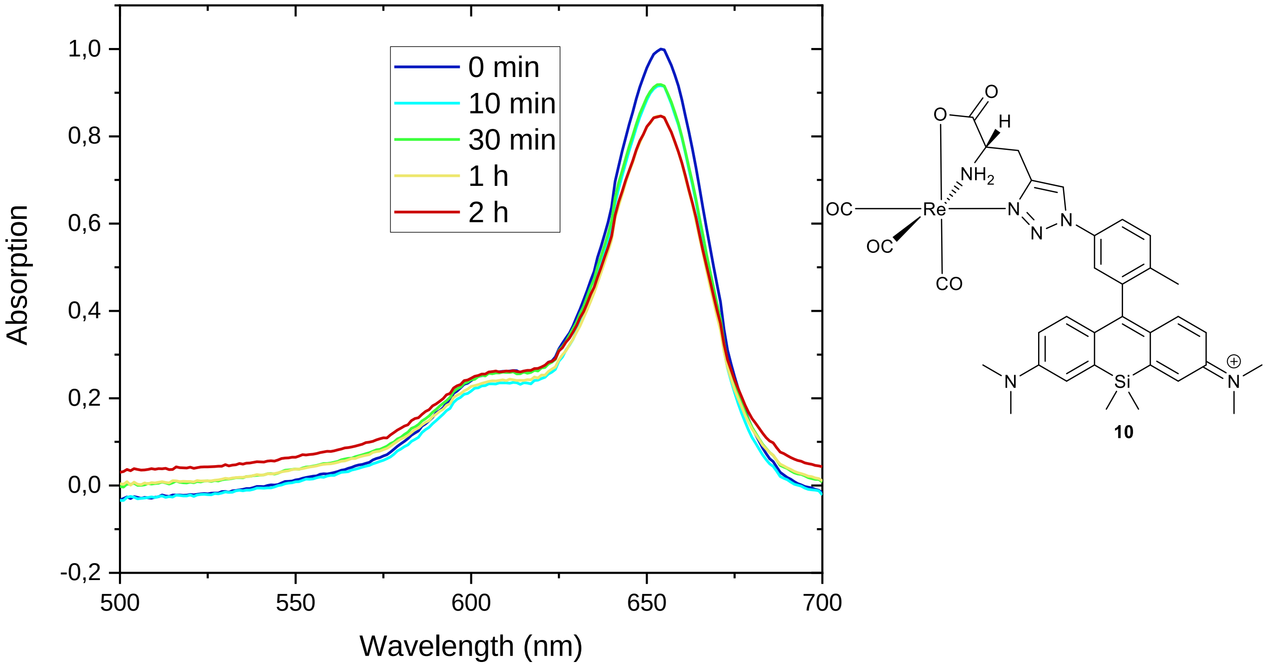


Figure 28: Absorption spectra of **9** after irradiation (λ_{ex} = 650 nm) with a pulsed laser (20 kW, pulse width at half peak height < 10 µs) for two hours in water/ethanol (5%). The absorption spectrum for 0 min was normalized and the other absorption spectra are referenced to the spectrum at 0 min.



10

Figure 29: Absorption spectra of **10** after irradiation (λ_{ex} = 650 nm) with a pulsed laser (20 kW, pulse width at half peak height < 10 µs) for two hours in water/ethanol (5%). The absorption spectrum for 0 min was normalized and the other absorption spectra are referenced to the spectrum at 0 min.

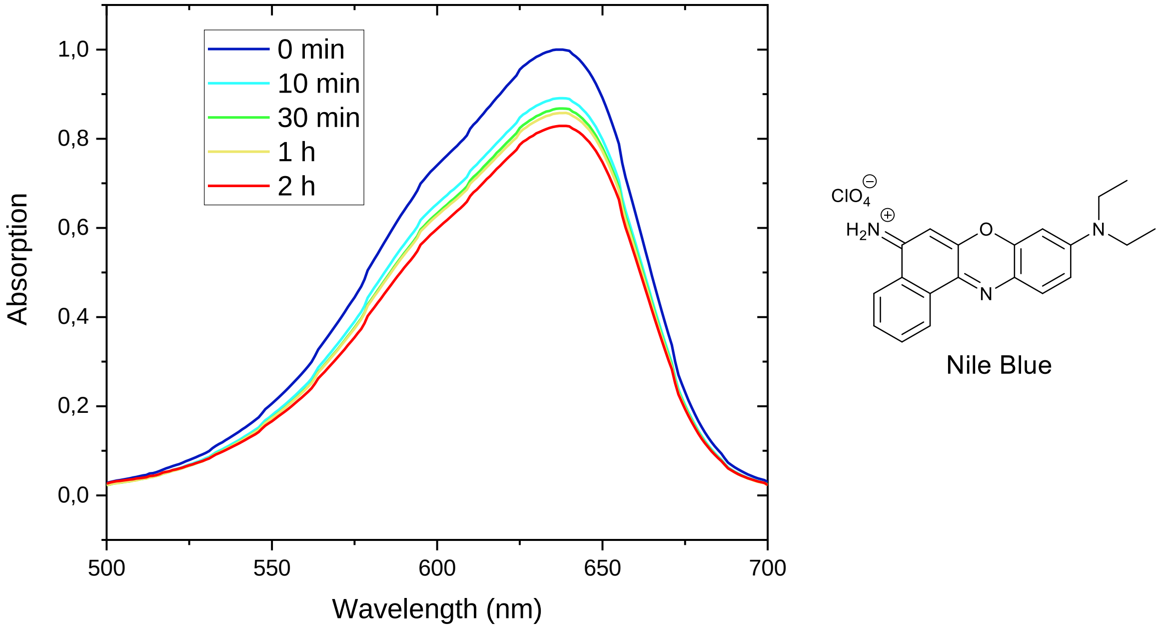


Figure 30: Absorption spectra of Nile Blue after irradiation (λ_{ex} = 650 nm) with a pulsed laser (20 kW, pulse width at half peak height < 10 µs) for two hours in water/ethanol (5%). The absorption spectrum for 0 min was normalized and the other absorption spectra are referenced to the spectrum at 0 min.

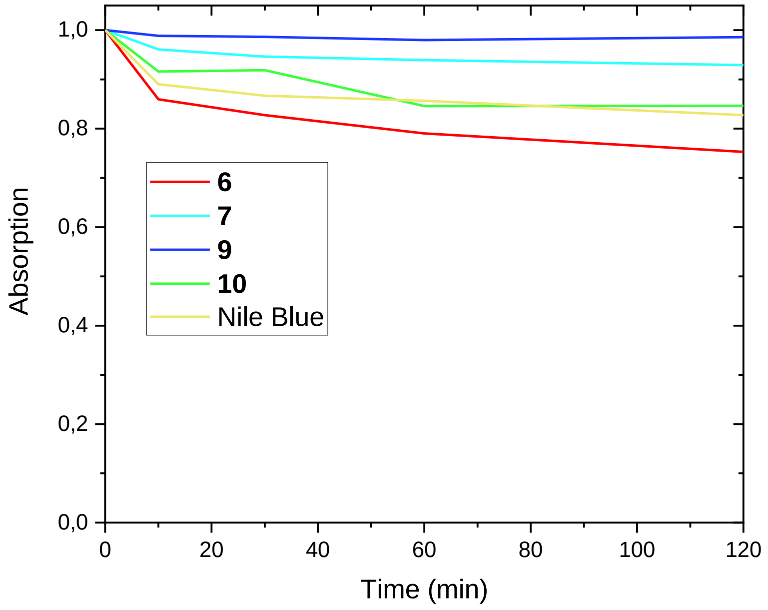
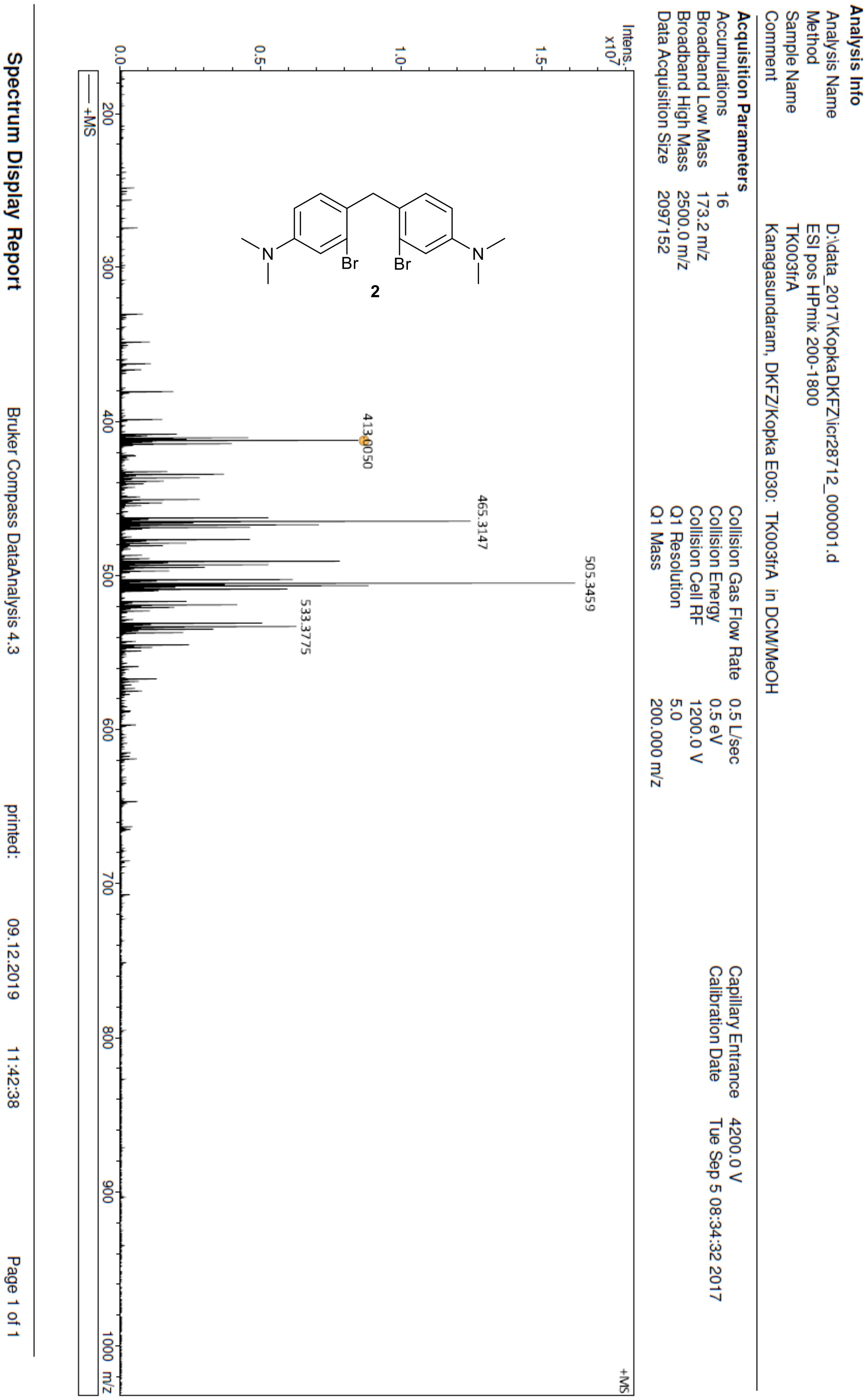


Figure 31: Comparison of the absorption spectra and their photostabilities of **6**, **7**, **9** and **10** with reference fluorophore Nile Blue after irradiation (λ_{ex} = 650 nm) with a pulsed laser (20 kW, pulse width at half peak height < 10 µs) for two hours in water/ethanol (5%).

3.4 HR-ESI-MS-spectra



Analysis Info

Analysis Name	D:\data_2017\KopkaDKFZ\icr28712_000001.d
Method	ESI pos HPmix 200-1800
Sample Name	TK003frA
Comment	Kanagasundaram, DKFZ/Kopka E030: TK003frA in DCM/MeOH

Acquisition Parameters

Accumulations	16	Collision Gas Flow Rate	0.5 L/sec	Capillary Entrance	4200.0 V
Broadband Low Mass	173.2 m/z	Collision Energy	0.5 eV	Calibration Date	Tue Sep 5 08:34:32 2017
Broadband High Mass	2500.0 m/z	Collision Cell RF	1200.0 V		
Data Acquisition Size	2097152	Q1 Resolution	5.0		
		Q1 Mass	200.000 m/z		

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Figure 32: HR-ESI-MS of **2** in DCM/MeOH.

Analysis Info

Analysis Name	D:\data_2018\Kopka DKFZ\icr32780_000001.d
Method	ESI pos HPmix 200-1800
Sample Name	TK025
Comment	Kanagasundaram,DKFZ/Kopka: TK025 in MeOH

Acquisition Parameters

Accumulations	16	Collision Gas Flow Rate	0.5 L/sec	Capillary Entrance	4200.0 V
Broadband Low Mass	173.2 m/z	Collision Energy	0.5 eV	Calibration Date	Tue Nov 20 08:54:54 2018
Broadband High Mass	2500.0 m/z	Collision Cell RF	1200.0 V		
Data Acquisition Size	2097152	Q1 Resolution	5.0		
		Q1 Mass	200.000 m/z		

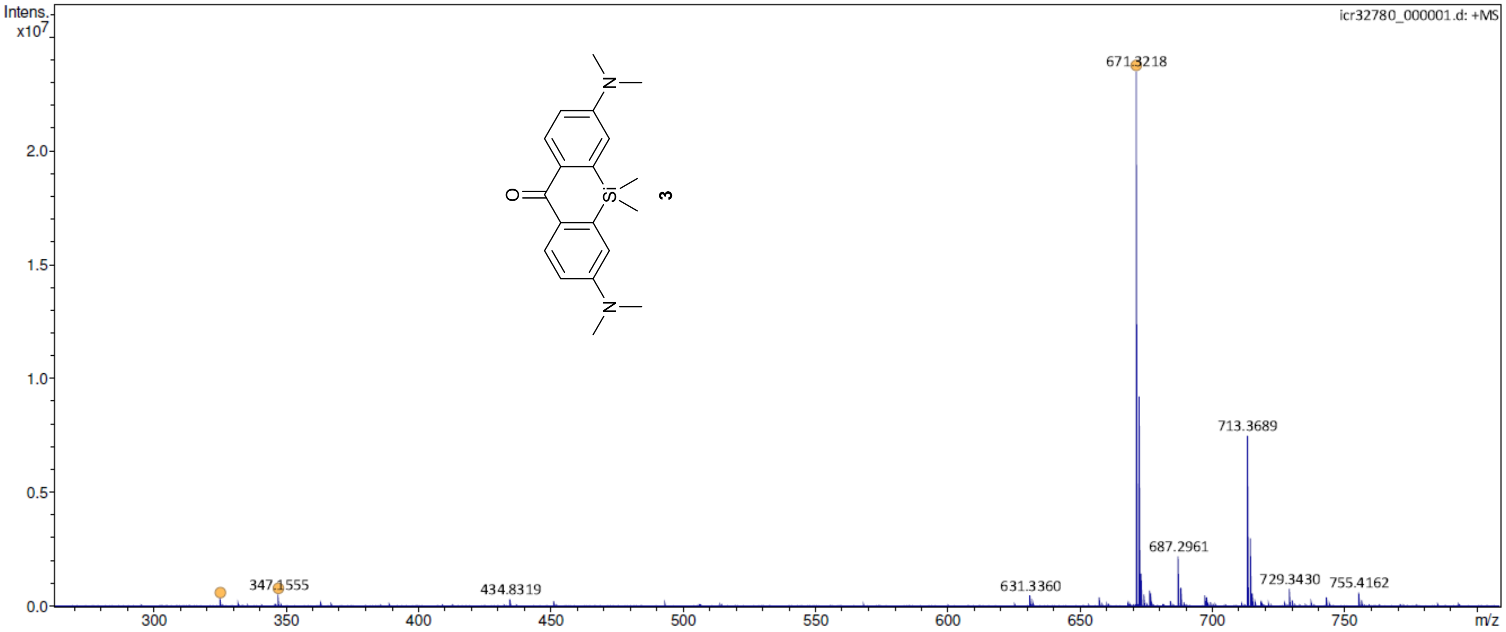

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Figure 33: HR-ESI-MS in positive modus of **3** in MeOH.

Analysis Info

Analysis Name	D:\data_2019\Kopka DKFZ\icr33638_000001.d
Method	ESI pos HPmix 200-1800
Sample Name	TK085
Comment	Kanagasundaram, DKFZ/Kopka: TK085 in DCM/MeOH

Acquisition Parameters

Accumulations	16	Collision Gas Flow Rate	0.5 L/sec	Capillary Entrance	4200.0 V
Broadband Low Mass	173.2 m/z	Collision Energy	0.5 eV	Calibration Date	Wed Jan 9 08:22:35 2019
Broadband High Mass	2500.0 m/z	Collision Cell RF	1200.0 V		
Data Acquisition Size	2097152	Q1 Resolution	5.0		
		Q1 Mass	200.000 m/z		

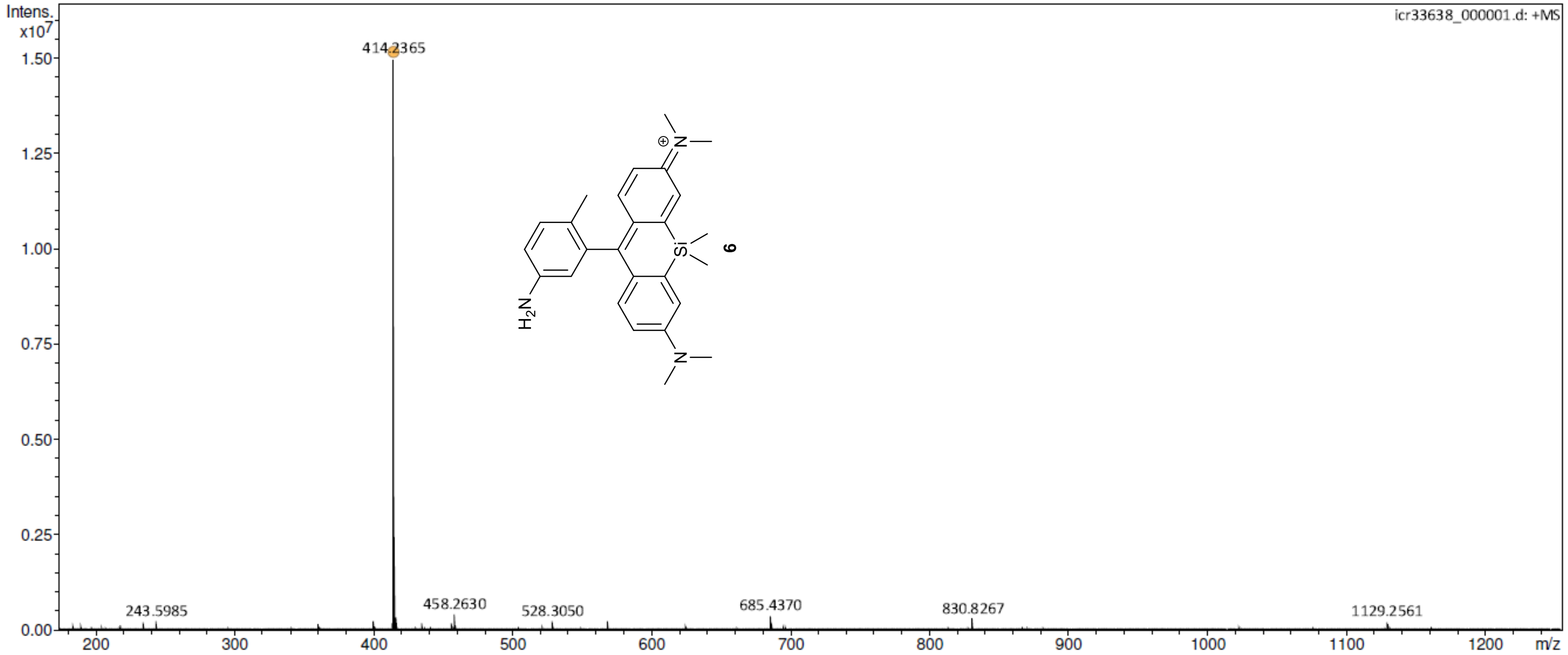

Figure 34: HR-ESI-MS in positive modus of **6** in DCM/MeOH.

Analysis Info

Analysis Name	D:\data_2019\Kopka DKFZ\icr33639_000001.d
Method	ESI pos HPmix 200-1800
Sample Name	TK089
Comment	Kanagasundaram, DKFZ/Kopka: TK089 in DCM/MeOH

Acquisition Parameters

Accumulations	16	Collision Gas Flow Rate	0.5 L/sec	Capillary Entrance	4200.0 V
Broadband Low Mass	173.2 m/z	Collision Energy	0.5 eV	Calibration Date	Wed Jan 9 08:22:35 2019
Broadband High Mass	2500.0 m/z	Collision Cell RF	1200.0 V		
Data Acquisition Size	2097152	Q1 Resolution	5.0		
		Q1 Mass	200.000 m/z		

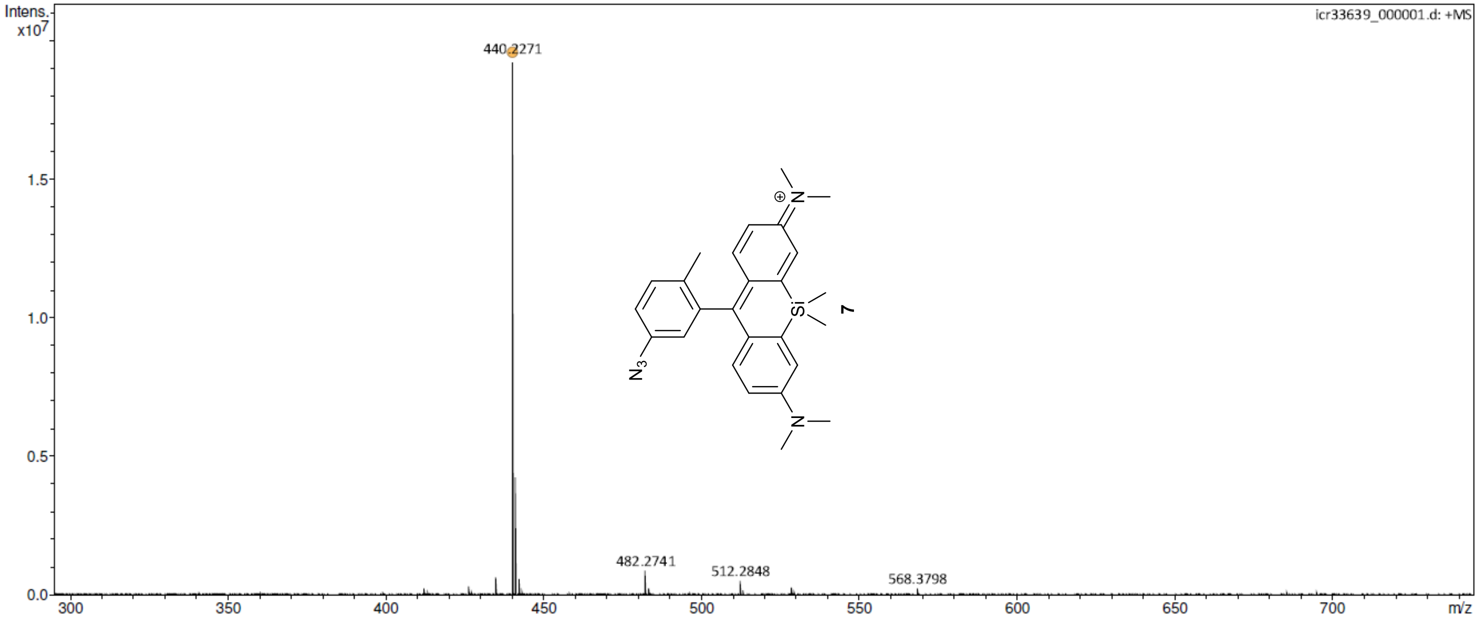

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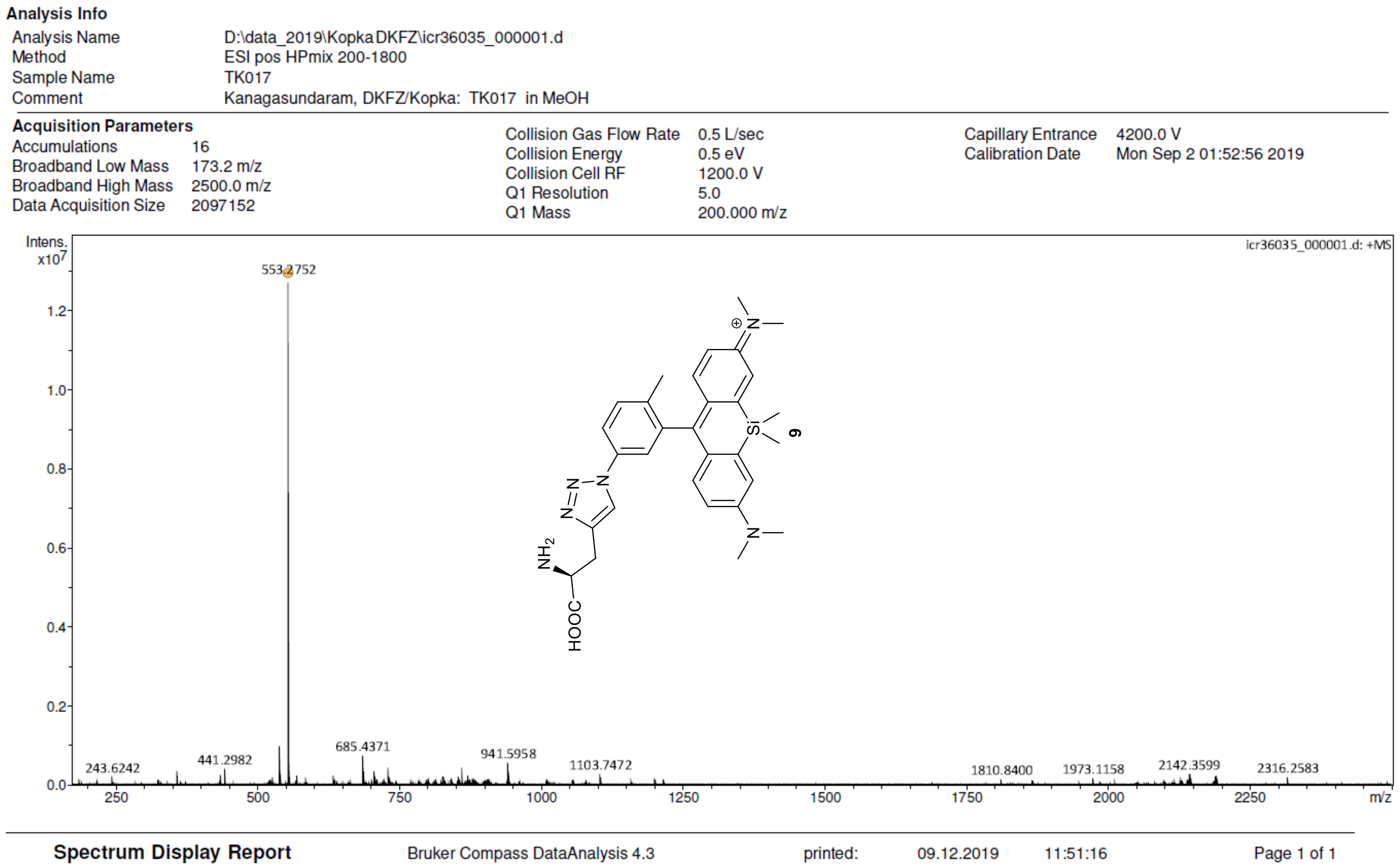
Figure 35: HR-ESI-MS in positive modus of **7** in DCM/MeOH.



Analysis Info

Analysis Name	D:\data_2019\Kopka DKFZ\icr36035_000001.d
Method	ESI pos HPmix 200-1800
Sample Name	TK017
Comment	Kanagasundaram, DKFZ/Kopka: TK017 in MeOH

Acquisition Parameters

Accumulations	16	Collision Gas Flow Rate	0.5 L/sec	Capillary Entrance	4200.0 V
Broadband Low Mass	173.2 m/z	Collision Energy	0.5 eV	Calibration Date	Mon Sep 2 01:52:56 2019
Broadband High Mass	2500.0 m/z	Collision Cell RF	1200.0 V		
Data Acquisition Size	2097152	Q1 Resolution	5.0		
		Q1 Mass	200.000 m/z		

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Figure 36: HR-ESI-MS in positive modus of **9** in MeOH.

Sample Name	TK100HPLC_frA
Comment	Kanagasundaram, DKFZ/Kopka: TK100HPLC_frA in DCM/MeOH

Acquisition Parameters

Accumulations	16	Collision Gas Flow Rate	0.5 L/sec	Capillary Entrance	4200.0 V
Broadband Low Mass	173.2 m/z	Collision Energy	0.5 eV	Calibration Date	Mon Apr 8 01:48:05 2019
Broadband High Mass	2500.0 m/z	Collision Cell RF	1200.0 V		
Data Acquisition Size	2097152	Q1 Resolution	5.0		
		Q1 Mass	200.000 m/z		

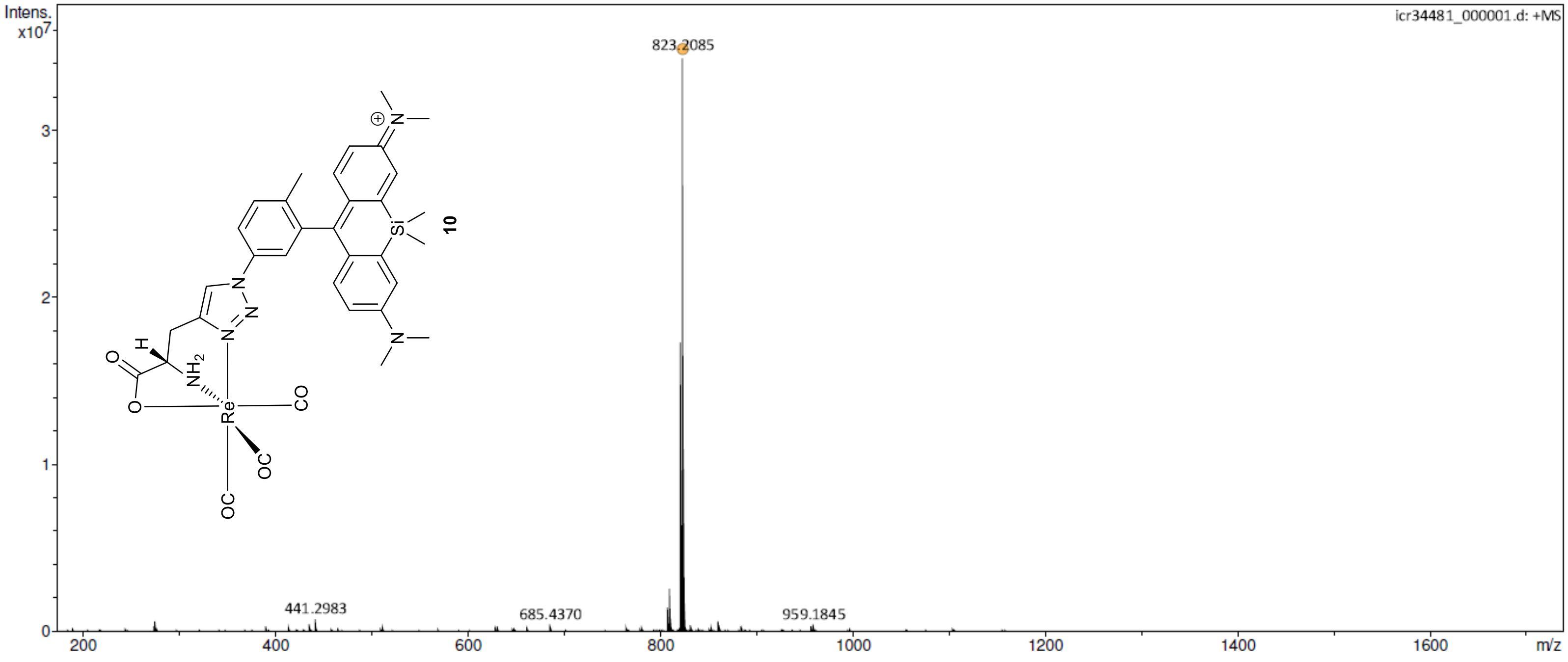

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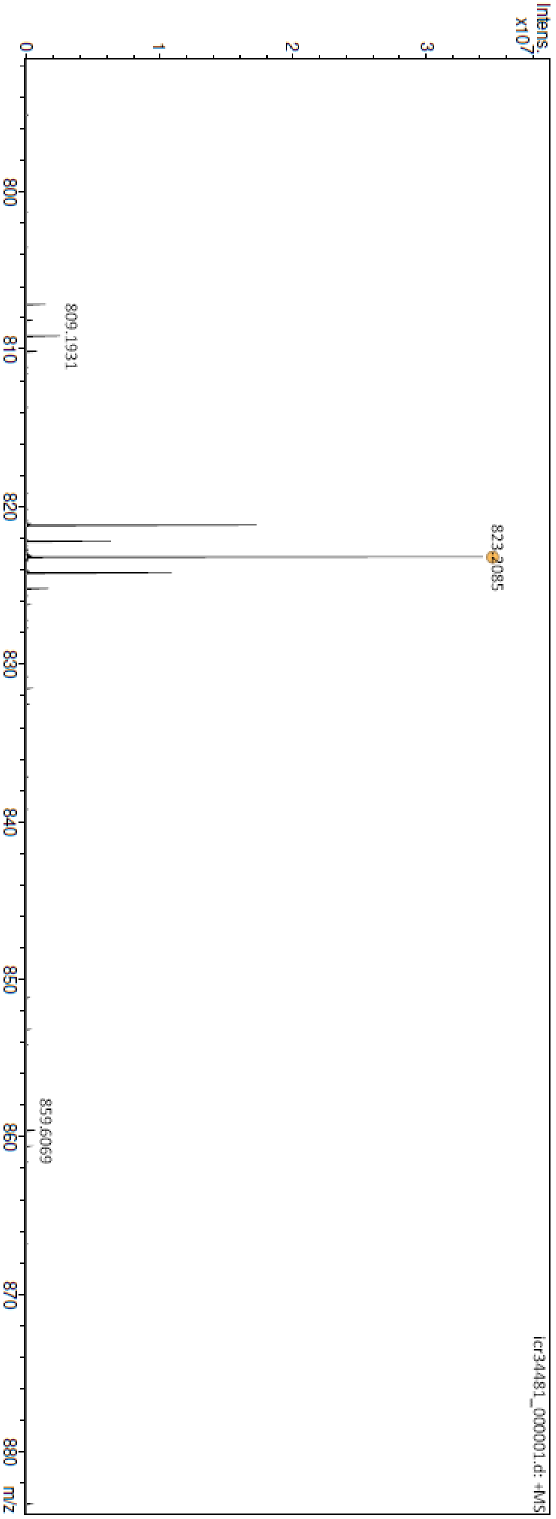
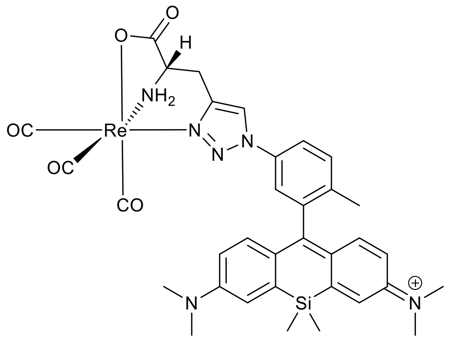
Figure 37: HR-ESI-MS in positive modus of **10** in DCM/MeOH.



Meas. m/z	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
823.2085	C34H36N6O5ReSi	823.2068	-2.0	54.1	22.0	odd	-
	C34H41N3O19Si	823.2098	1.6	61.2	17.0	odd	ok



10

Figure 38: HR-ESI-MS in positive modus of **10** in DCM/MeOH.

3.5 IR-spectra

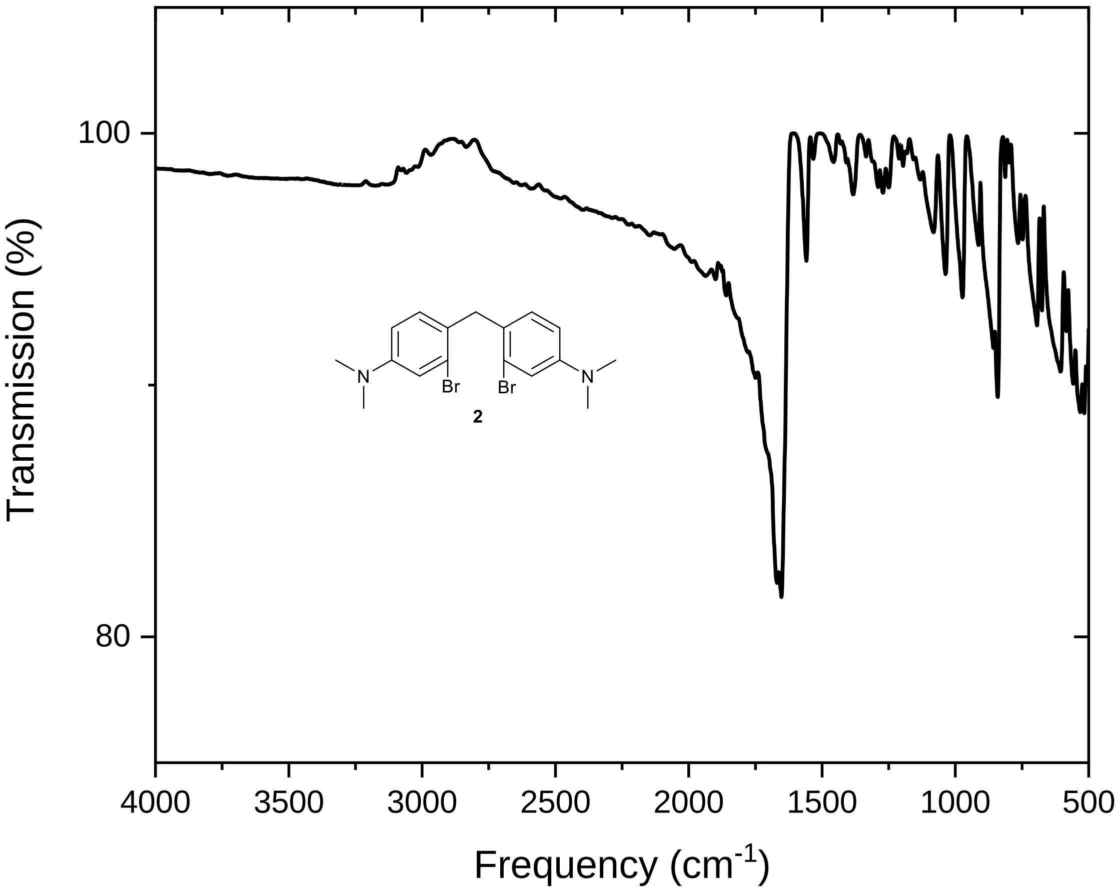


Figure 39: IR-spectrum of **2** pressed in KBr.

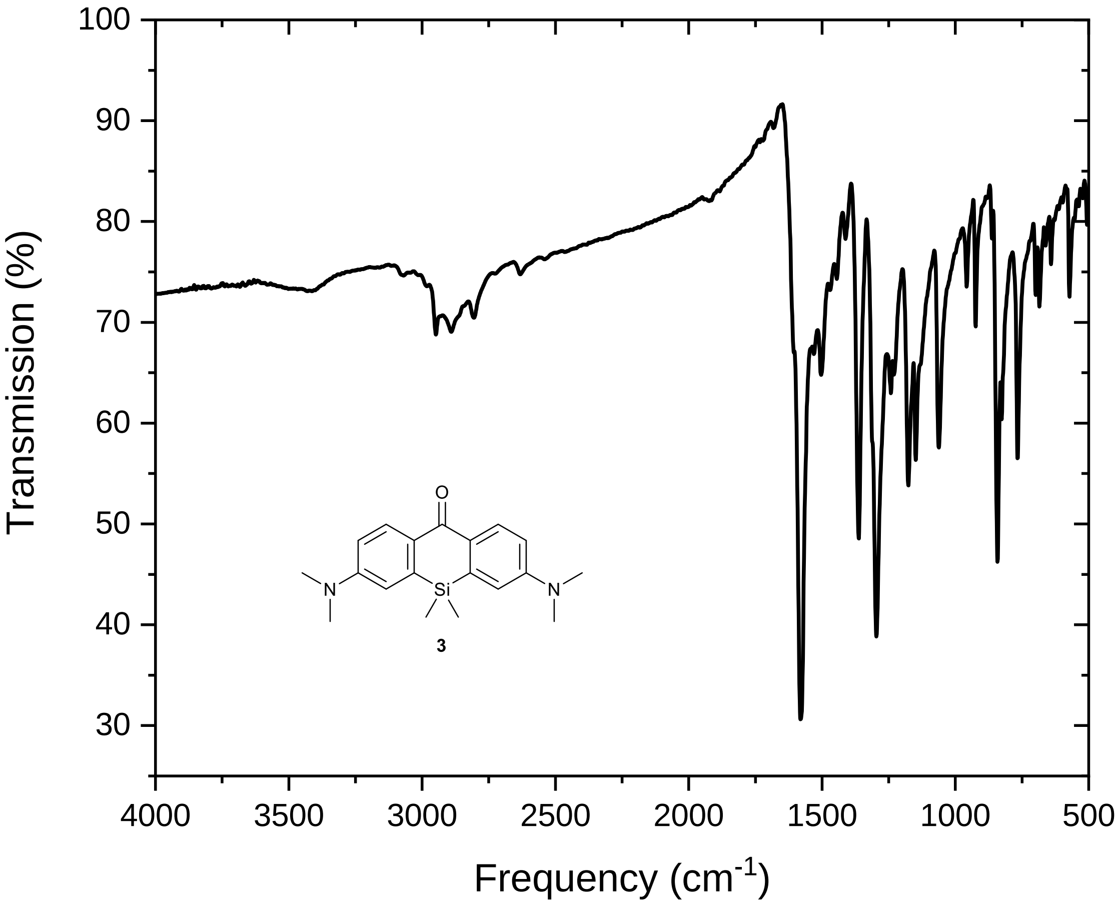


Figure 40: IR-spectrum of **3** pressed in KBr.

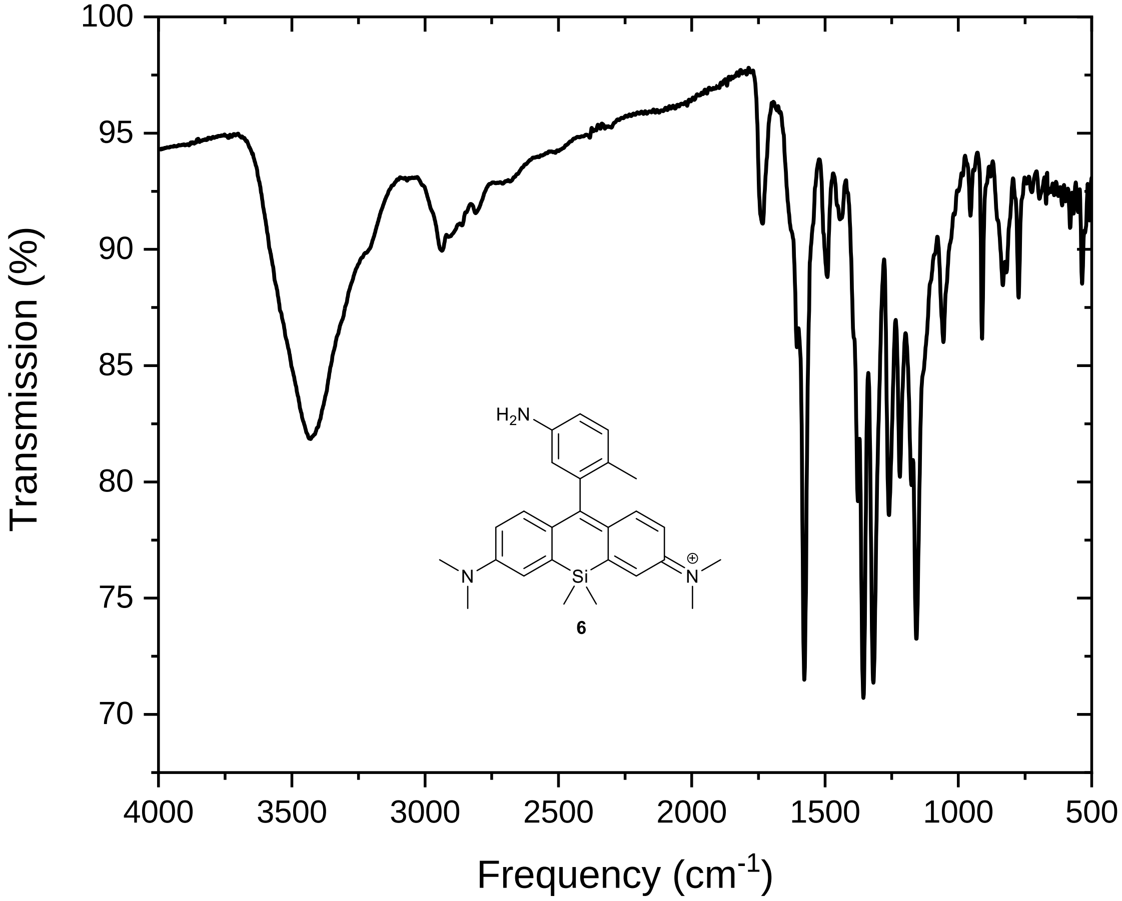


Figure 41: IR-spectrum of **6** pressed in KBr.

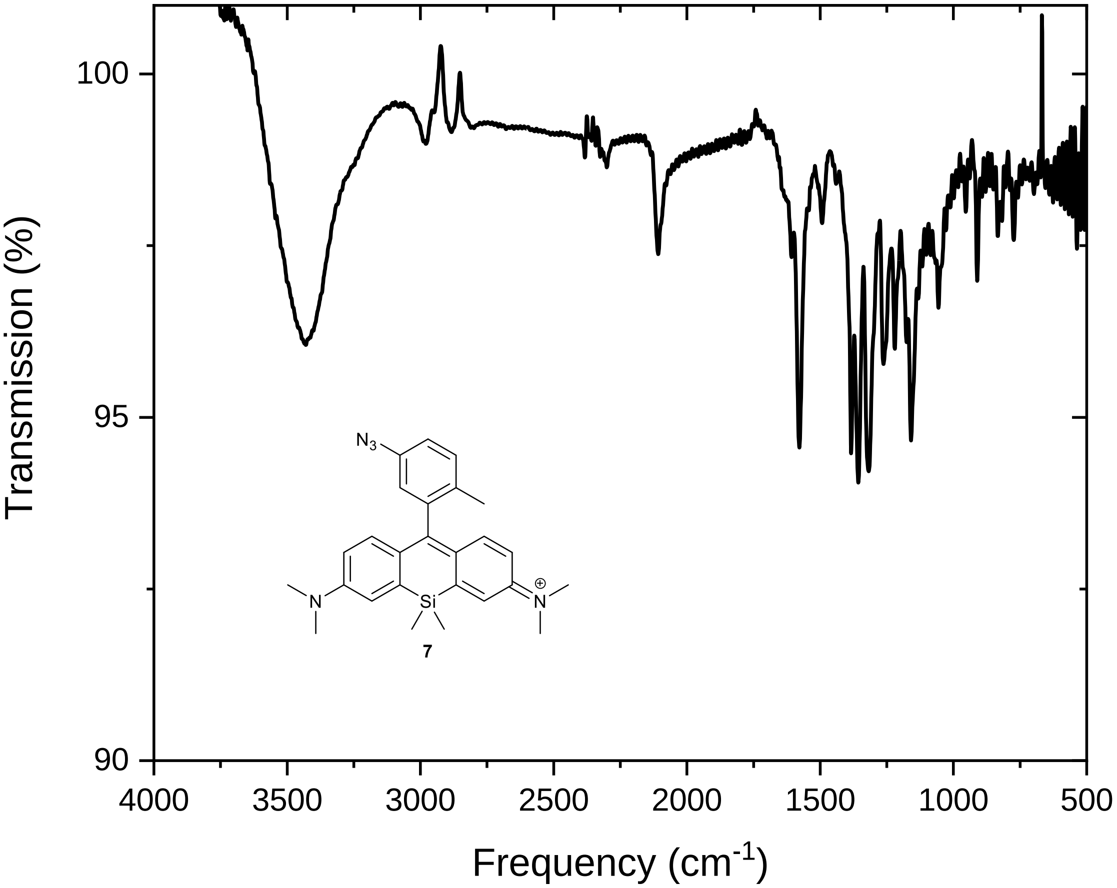


Figure 42: IR-spectrum of **7** pressed in KBr.

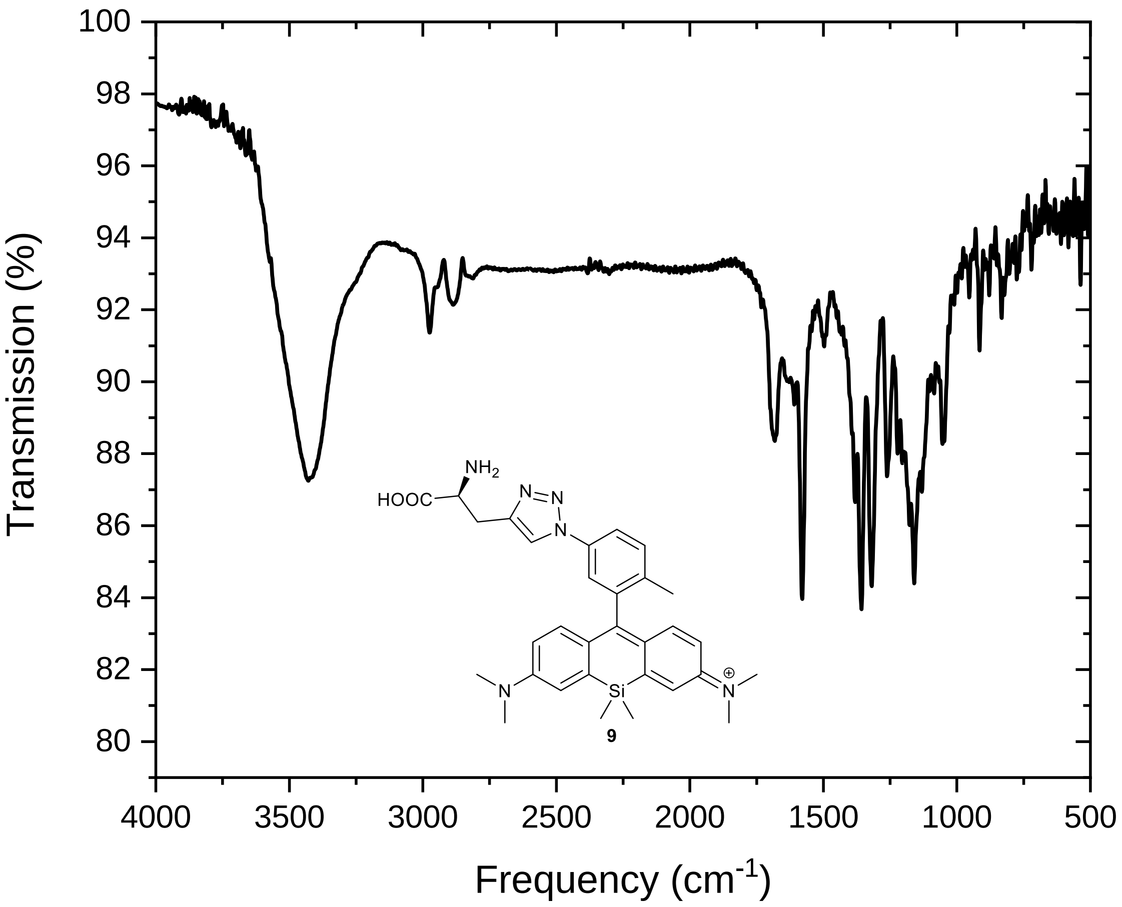


Figure 43: IR-spectrum of **9** pressed in KBr.

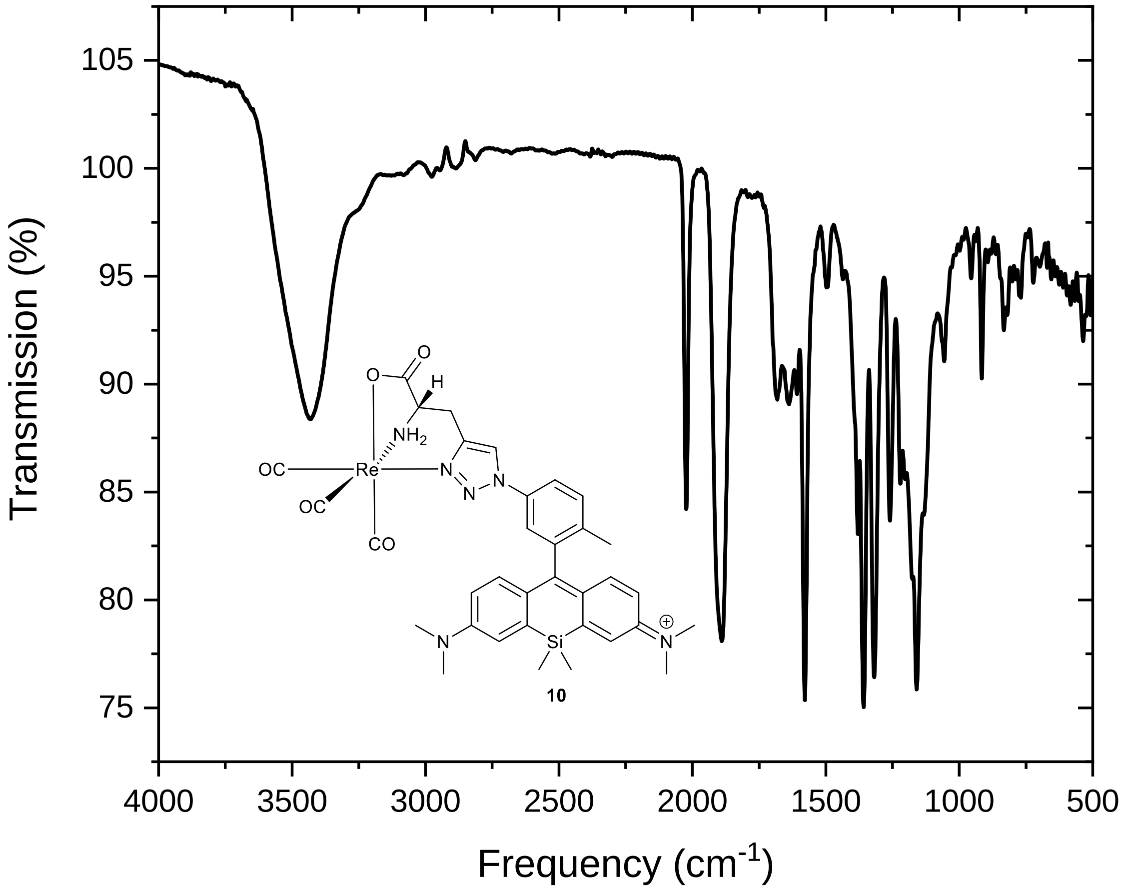
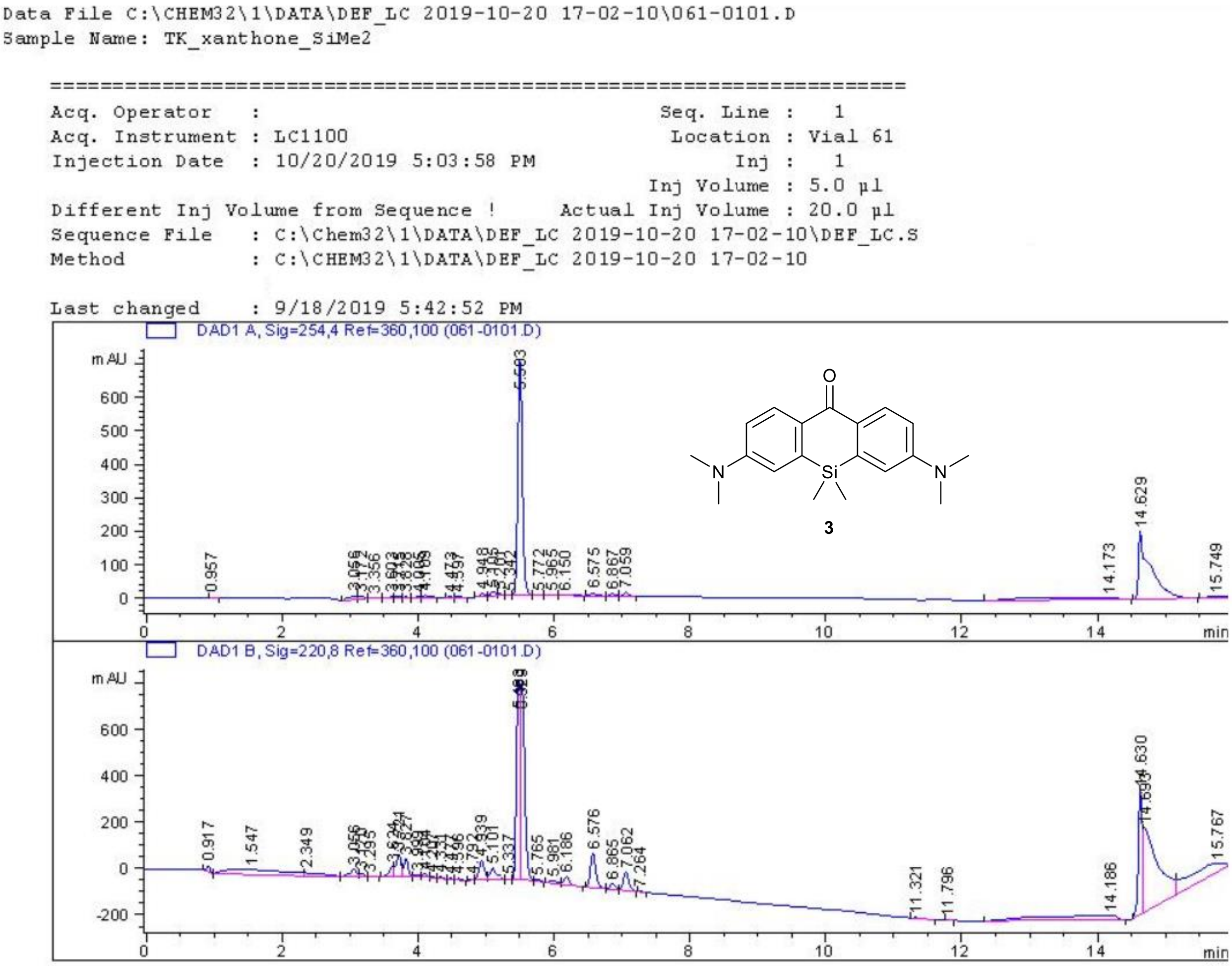


Figure 44: IR-spectrum of **10** pressed in KBr.

3.6 HPLC spectra

The analytical HPLC spectra were measured with a 16 minutes long method (from 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1%; 95% MeCN). The samples were detected with a wavelength of 220.8 nm and 254.4 nm.



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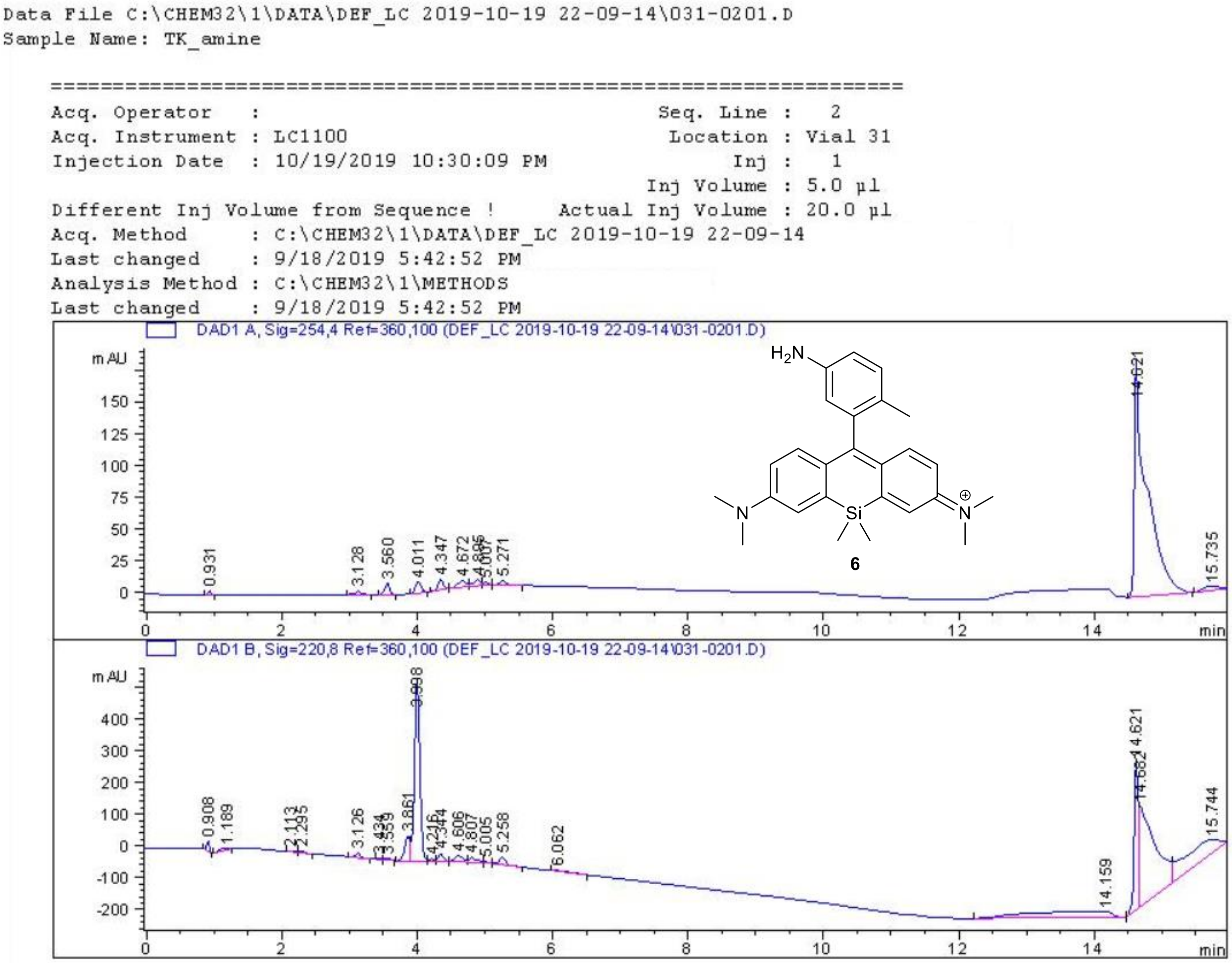
Data File C:\CHEM32\1\DATA\DEF_LC 2019-10-20 17-02-10\061-0101.D
Sample Name: TK_xanthone_SiMe2

    =====================================================================
    Acq. Operator   :                                 Seq. Line :   1
    Acq. Instrument : LC1100                           Location : Vial 61
    Injection Date  : 10/20/2019 5:03:58 PM                 Inj :   1
                                                     Inj Volume : 5.0 µl
    Different Inj Volume from Sequence !      Actual Inj Volume : 20.0 µl
    Sequence File   : C:\Chem32\1\DATA\DEF_LC 2019-10-20 17-02-10\DEF_LC.S
    Method          : C:\CHEM32\1\DATA\DEF_LC 2019-10-20 17-02-10

    Last changed    : 9/18/2019 5:42:52 PM
```

Figure 45: Analytical HPLC-spectrum of **3** (R_t = 5.58 min). Method: 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1% TFA; 95% MeCN in 16 min.



```
Data File C:\CHEM32\1\DATA\DEF_LC 2019-10-19 22-09-14\031-0201.D
Sample Name: TK_amine

    =====================================================================
    Acq. Operator   :                                 Seq. Line :   2
    Acq. Instrument : LC1100                           Location : Vial 31
    Injection Date  : 10/19/2019 10:30:09 PM                Inj :   1
                                                     Inj Volume : 5.0 µl
    Different Inj Volume from Sequence !      Actual Inj Volume : 20.0 µl
    Acq. Method     : C:\CHEM32\1\DATA\DEF_LC 2019-10-19 22-09-14
    Last changed    : 9/18/2019 5:42:52 PM
    Analysis Method : C:\CHEM32\1\METHODS
    Last changed    : 9/18/2019 5:42:52 PM
```

Figure 46: Analytical HPLC-spectrum of **6** (R_t = 4.00 min). Method: 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1% TFA; 95% MeCN in 16 min.

```
)ata File C:\CHEM32\1\DATA\DEF_LC 2019-10-19 22-09-14\061-0101.D
3ample Name: TK_azide

    =====================================================================
    Acq. Operator   :                                  Seq. Line :   1
    Acq. Instrument : LC1100                            Location : Vial 61
    Injection Date  : 10/19/2019 10:12:16 PM                 Inj :   1
                                                      Inj Volume : 5.0 µl
    Different Inj Volume from Sequence !       Actual Inj Volume : 20.0 µl
    Sequence File   : C:\Chem32\1\DATA\DEF_LC 2019-10-19 22-09-14\DEF_LC.S
    Method          : C:\CHEM32\1\DATA\DEF_LC 2019-10-19 22-09-14

    Last changed    : 9/18/2019 5:42:52 PM
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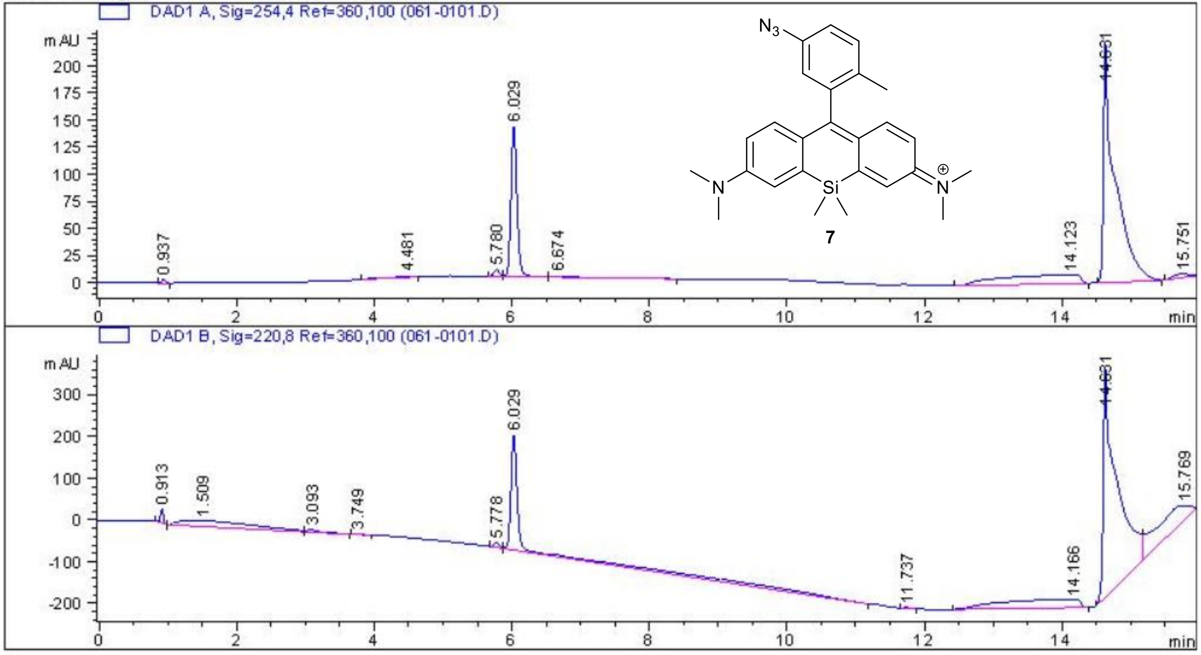
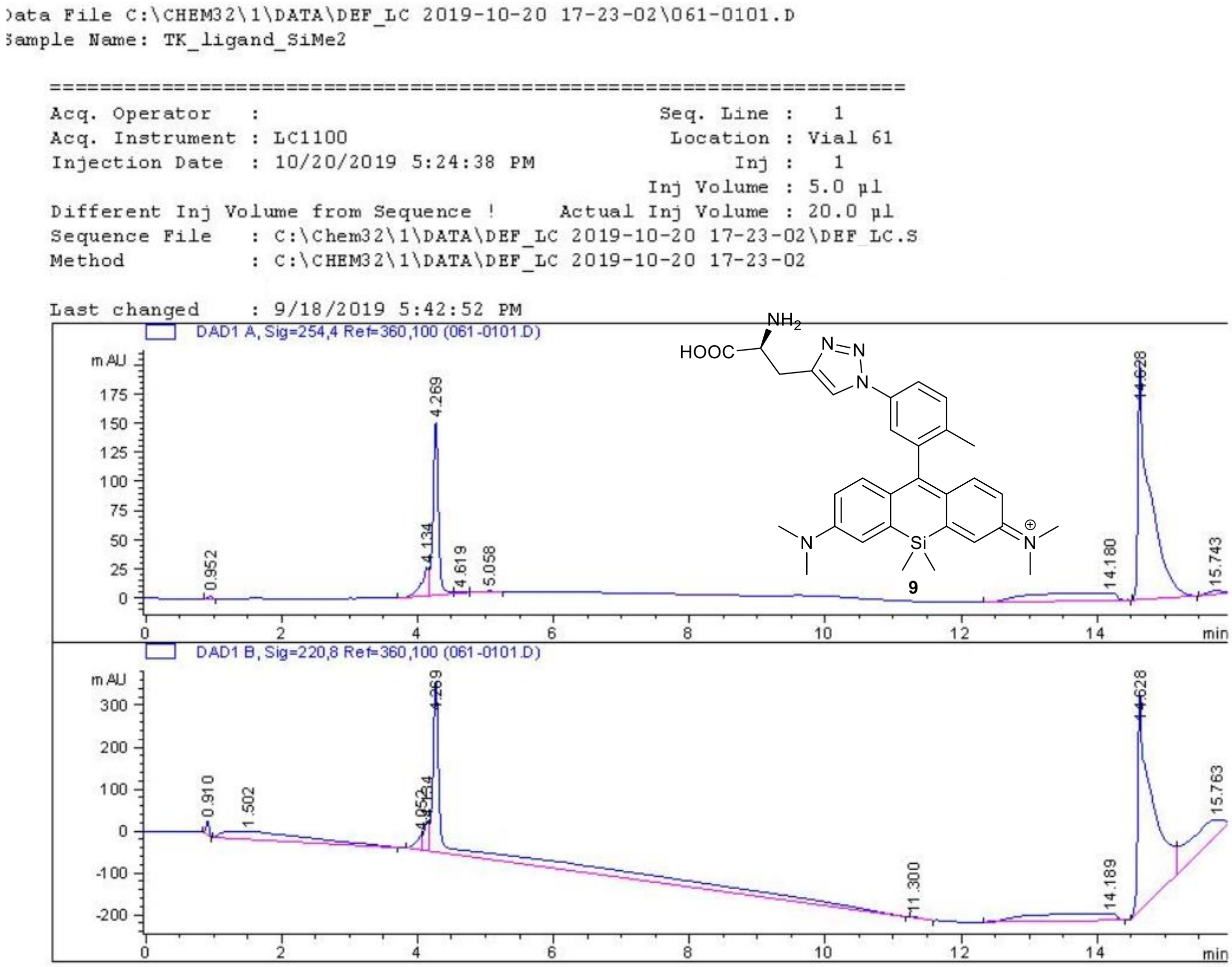


Figure 47: Analytical HPLC-spectrum of **7** (R_t = 6.03 min). Method: 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1% TFA; 95% MeCN in 16 min.



```
Data File C:\CHEM32\1\DATA\DEF_LC 2019-10-20 17-23-02\061-0101.D
Sample Name: TK_ligand_SiMe2

    =====================================================================
    Acq. Operator   :                               Seq. Line :   1
    Acq. Instrument : LC1100                         Location : Vial 61
    Injection Date  : 10/20/2019 5:24:38 PM               Inj :   1
                                                   Inj Volume : 5.0 µl
    Different Inj Volume from Sequence !     Actual Inj Volume : 20.0 µl
    Sequence File   : C:\Chem32\1\DATA\DEF_LC 2019-10-20 17-23-02\DEF_LC.S
    Method          : C:\CHEM32\1\DATA\DEF_LC 2019-10-20 17-23-02

    Last changed    : 9/18/2019 5:42:52 PM
```

Figure 48: Analytical HPLC-spectrum of **9** (R_t = 4.27 min). Method: 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1% TFA; 95% MeCN in 16 min.

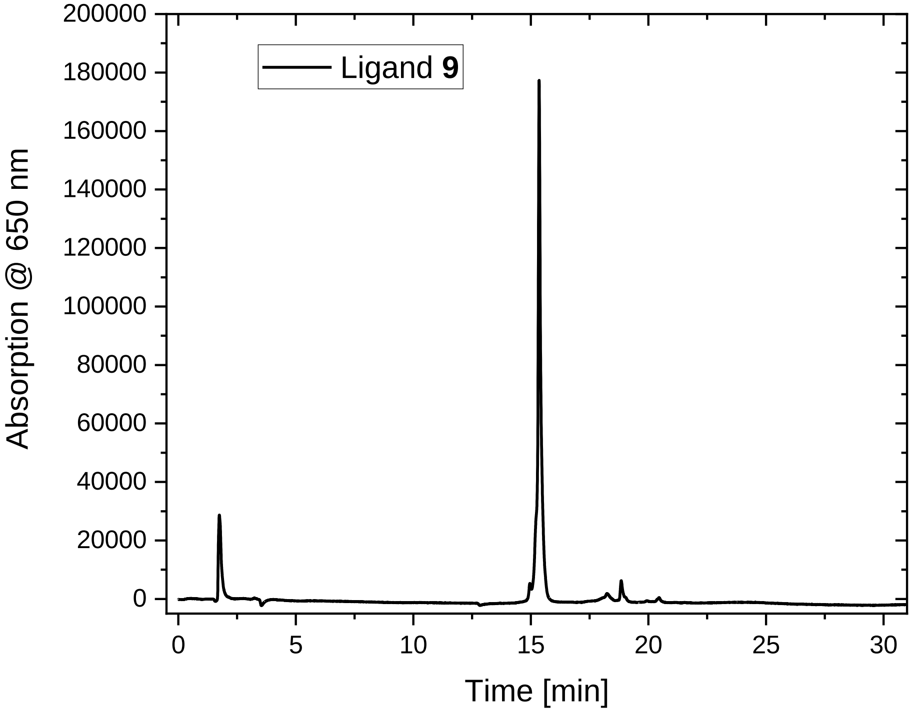


Figure 49: Analytical HPLC-spectrum of **9** (R_t = 15.3 min). Method: 90% H_2O+0.1% TFA; 10% MeCN to 10% H_2O+0.1% TFA; 90% MeCN+0.1% TFA in 30 min. Absorption was detected at a wavelength of 650 nm.

```
Data File C:\CHEM32\1\DATA\DEF_LC 2019-10-20 18-54-44\061-0101.D
Sample Name: TK_Re_CO-complex

    =====================================================================
    Acq. Operator   :                                 Seq. Line :    1
    Acq. Instrument : LC1100                           Location : Vial 61
    Injection Date  : 10/20/2019 6:56:44 PM                 Inj :    1
                                                     Inj Volume : 5.0 µl
    Different Inj Volume from Sequence !      Actual Inj Volume : 30.0 µl
    Sequence File   : C:\Chem32\1\DATA\DEF_LC 2019-10-20 18-54-44\DEF_LC.S
    Method          : C:\CHEM32\1\DATA\DEF_LC 2019-10-20 18-54-44

    Last changed    : 9/18/2019 5:42:52 PM
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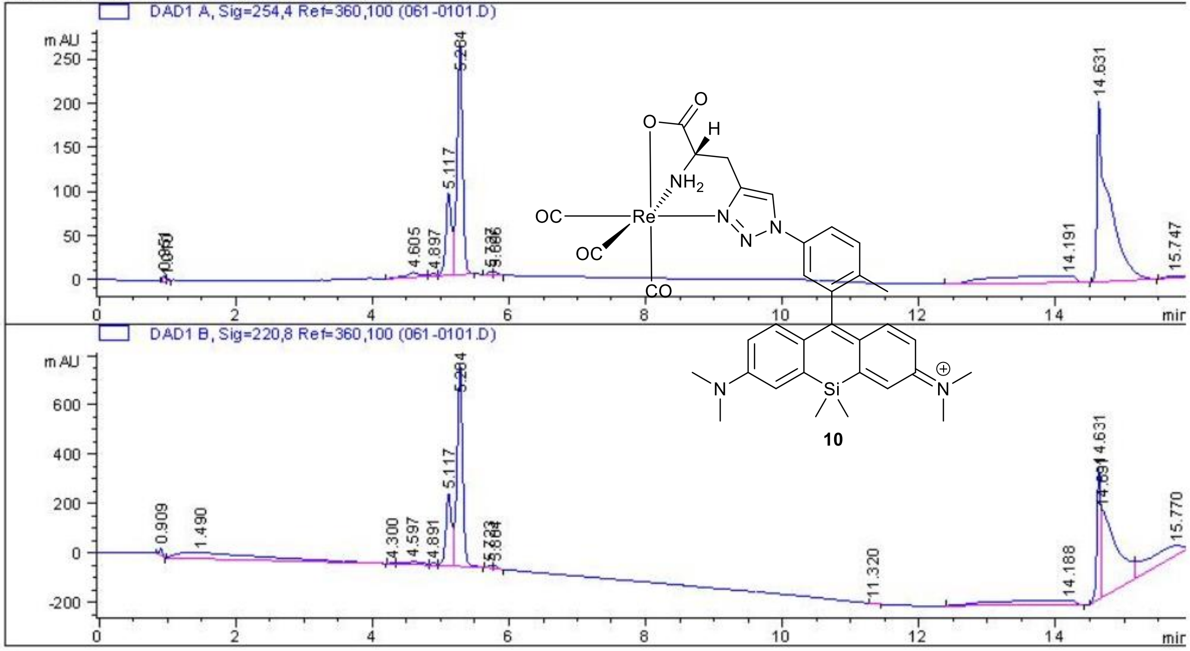

10

Figure 50: Analytical HPLC-spectrum of **10** (R_t = 5.11/5.28 min). Method: 95% H_2O+0.1% TFA; 5% MeCN to 5% H_2O+0.1% TFA; 95% MeCN in 16 min.

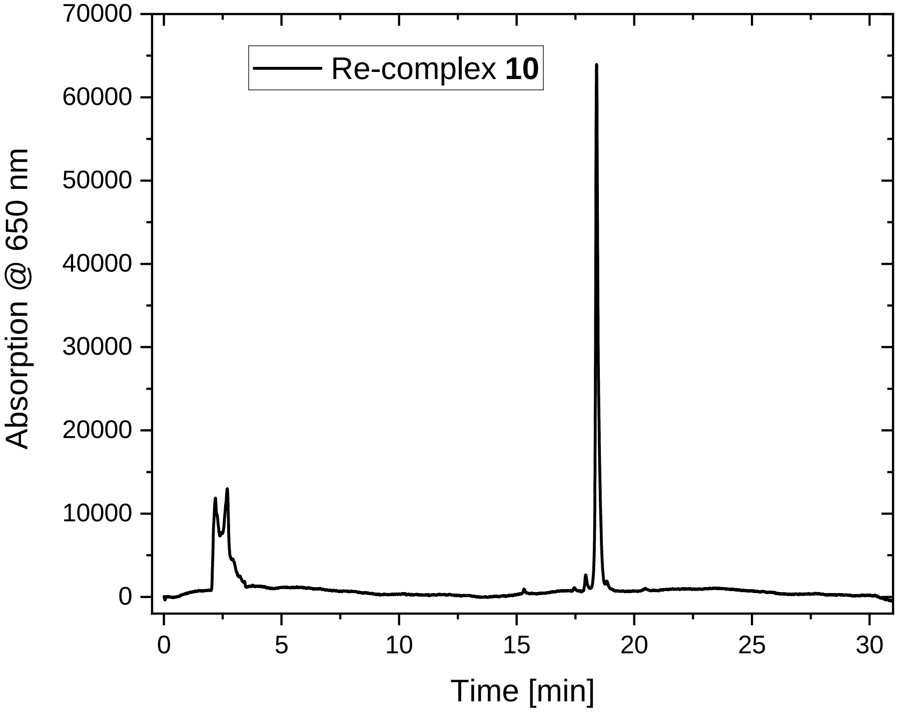

Figure 51: Analytical HPLC-spectrum of **10** (R_t = 18.3/18.4 min). Method: 90% H_2O+0.1% TFA; 10% MeCN+0.1% TFA to 10% H_2O+0.1% TFA; 90% MeCN+0.1% TFA in 30 min. Absorption was detected at a wavelength of 650 nm.