Supporting Information

Photochemical Microcontact Printing by Tetrazole Chemistry

Benjamin Vonhören,[‡] Oliver Roling,[‡] Christoph Buten,[‡] Martin Körsgen,[#] Heinrich F. Arlinghaus,[#] Bart Jan Ravoo[‡,*]

[‡]Organisch-Chemisches Institut, Center for Soft Nanoscience and Graduate School of Chemistry, Westfälische Wilhelms-Universität Münster, Corrensstrasse 40, 48149 Münster, Germany

[#]Physikalisches Institut, Westfälische Wilhelms-Universität Münster, Wilhelm-Klemm-Strasse 10, 48149 Münster, Germany

Email: b.j.ravoo@uni-muenster.de

Instrumentation and analysis

NMR spectroscopy

NMR measurements were performed on superconductive Bruker spectrometers (ARX 300, ARX 400). In all experiments deuterated solvents were used. Chemical shifts (δ) are reported in units of parts per million (ppm). The signals of residual non-deuterated solvents were used for reference. Coupling constants (J) are reported in Hertz (Hz). Signal nomenclature was carried out as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, b = broad.

UV/vis spectroscopy

UV/vis spectroscopy was carried out with a V-650 UV/vis double-beam spectrometer (Jasco Germany GmbH, Groß-Umstadt, Germany) using quartz glass cuvettes with a path length of 1 cm.

Fluorescence spectroscopy

The fluorescence spectrum of the supernatant was measured using a JASCO FP6500 spectrofluorometer (Jasco Germany GmbH, Groß-Umstadt, Germany).

Water contact angle measurements

Water contact angles were measured by the sessile drop method on a DSA 100 goniometer (Krüss GmbH Wissenschaftliche Laborgeräte, Germany).

X-ray photoelectron spectroscopy (XPS)

XPS measurements were performed with an Axis Ultra DLD (Kratos Analytical Ltd, UK). A monochromatic Al Kα source (1486.6 eV) at 10 mA filament current and 12 kV filament voltage source energies was used. The pass energy was set to 20 eV for high resolution scans and to 160 eV for survey scans. The charge neutralizer was used to compensate for sample charging. All measurements were carried out in the "hybrid mode". The data were evaluated with CasaXPS (version 2.3.15, Casa Software Ltd, UK) and the spectra were calibrated to aliphatic carbon (C1s = 285 eV).

Time-of-flight secondary ion mass spectrometry (ToF-SIMS)

ToF-SIMS imaging was performed using an instrument that is widely compatible to the commercial ION-TOF ToF-SIMS V instrument (Münster, Germany). A pulsed 30 kV liquid metal ion source (LMIG, ION-TOF) was used for imaging the samples under static conditions with Bi_3^+ primary ions and an analysis current of 0.05 pA (pulse width 100 ns). The beam spot size was approximately 200 nm. To increase the mass resolution delayed extraction for secondary ions was applied. All samples were analyzed with the detection mode for negative secondary ions.

Atomic force microscopy (AFM)

AFM imaging was performed using a Nanowizard 3 from JPK Instruments operated in tapping mode with Veeco RTESP-Tapping Mode Etched Silicon Probes. The AFM was typically operated with a setpoint of 0.900 V and a scan rate of 1.00 Hz with a resolution of 512x512 pixels. The data were analyzed with Gwyddion (version 2.22).

Optical and fluorescence microscopy

For fluorescence microscopy imaging an Olympus BX 53 microscope was operated with an Olympus XC 10 camera and a X-Cite® Series 120Q by Lumen Dynamics as the irradiation source. Data processing was carried out with the software OLYMPUS Stream Start 1.8.

Test reaction with tetrazole and maleimide in solution

The samples were prepared by irradiating 6 mL of a solution of 0.5 mM tetrazole **2** and 2.5 mM maleimide **3** in ethyl acetate. A sample of 0.5 mL was taken for the spectroscopy measurements and diluted by 1 : 20.

Surface modification

PDMS stamp preparation

Poly(dimethylsiloxane) (PDMS) stamps were prepared by mixing PDMS precursor and curing agent (Sylgard 184, Dow Corning) in a 10 : 1 ratio and casting this mixture on a patterned silicon master. The PDMS mixture was cured at 80 °C overnight. Patterned stamps were cut out with a knife and put into a UV ozonizer (PSD-UV, Novascan Technologies Inc.) for 55 min prior to use. If not used immediately, the PDMS stamps were stored in distilled water.

Preparation of tetrazole SAMs

Silicon wafers or glass substrates were first cleaned by sonication for 3 min in pentane, acetone and water. Next, they were activated in piranha solution (H_2SO_4 (conc.) : H_2O_2 (30%) = 2 : 1) for 30 min (Danger!), rinsed with copious amounts of water, dried in a stream of argon and transferred into a solution of ethoxysilane 1 (1 mM, toluene (analytical grade), argon atmosphere). The solution was stirred for 2 h at 80 °C. Next, the samples were taken out and rinsed with toluene, acetone, ethanol and water and dried in a stream of argon. The tetrazole capped SAMs were stored in a Schlenk tube under argon in the fridge, if not used immediately.

Tetrazole reaction by microcontact printing

Oxidized PDMS stamps were covered with solutions of the ink to be patterned (ethanol, 25 mM) for 1 min, dried in a stream of argon and placed on the tetrazole SAMs. Reactions were normally carried out for 10 min under irradiation ($\lambda_{em,max}$ = 320 nm, Compact UV Sun 20 W, Lucky Reptile) if not stated otherwise. The samples were placed on a mirror in order to reflect the light. The stamps were taken off the SAM and the SAM was rinsed with ethanol, acetone, water and dichloromethane, sonicated for 5 min in ethanol and then dried in a stream of argon. Biotin thiol **12** was dissolved in a 1 : 1 mixture of ethanol and methanol (25 mM).

Protein adsorption on PEGylated SAMs

Micropatterned PEGylated surfaces were incubated with tetramethylrhodamine labeled peanut agglutinin (50 μg mL^{-1}) in HEPES buffer (20 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, pH 7.5) for 20 min. Next, the substrates were carefully washed with HEPES-buffer and ultrapure water by exposing the surfaces for 5 min in beakers with the corresponding wash solutions and gently shaking. After drying the substrates in a slow stream of argon, the samples were analyzed by fluorescence microscopy.

Biotin streptavidin recognition

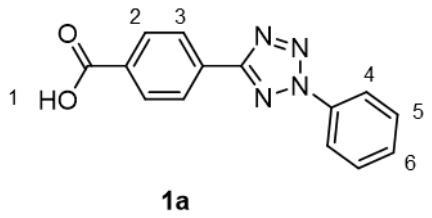
SAMs with patterns of biotin were incubated with a solution of streptavidin labeled with fluorescein isothiocyanate in HEPES buffer for 5 min. A few drops of HEPES buffer were poured on the SAM to displace unbound streptavidin followed by a few drops of ultrapure water to displace the salts of the HEPES buffer. The wet sample was analyzed by fluorescence microscopy.

Preparation of polymer brushes

The initiator for atom transfer radical polymerization (ATRP) was printed on glass substrates (50 mM solution in ethanol). The polymerization was carried out as described elsewhere.[1]

Synthesis

Synthesis of 4-(2-phenyl-2H-tetrazole-5-yl)benzoic acid 1a[2]



1a

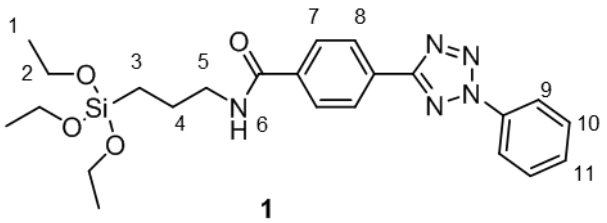
4-formylbenzoic acid (1.5 g, 10 mmol, 1 eq) was dissolved in ethanol (100 mL). Benzenesulfonohydrazide (1.7 g, 50 mmol, 5 eq) was added. After stirring for 1 h, water (200 mL) was added and a white precipitate formed, which was collected in a funnel and dissolved in pyridine (60 mL). In parallel, aniline (0.93 g, 10 mmol, 1 eq) dissolved in water : EtOH (1 : 1, 16 mL) with $HCl_{aq,konz}$ (2.6 mL) was slowly added to a solution of $NaNO_2$ (0.7 g, 10 mmol, 1 eq) in water (2 mL), which was cooled in cooling bath of $NaCl/H_2O$. The second solution was slowly dropped to the solution in aniline while cooling. The reaction mixture was extracted with EtOAc (2 x 40 mL) and HClaq (3 M, 500 mL) was added to the combined organic EtOAc phases. A precipitate formed which was collected by filtration and washed with water and small amounts of EtOH to yield and off-white solid (635 mg, 2.4 mmol, 24 %; lit 34 %). The analytical data is in agreement with the reported data.[2]

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (d, J = 8.1 Hz, 2H, H-4), 8.13 (d, J = 6.6 Hz, 4H, H-2, H-3), 7.68 (t, J = 7.7 Hz, 2H, H-5), 7.62 (t, J = 7.3 Hz, 1H, H-6).

^{13}C NMR (101 MHz, DMSO) δ 166.68, 163.70, 136.01, 132.67, 130.34, 130.20, 130.11, 126.74, 119.94.

MS (ESI (-)) 265.0735 (found), 265.2520 (calculated for $C_{14}H_9N_4O_2$ = mass of the deprotonated product).

Synthesis of tetrazole functionalized silane 1[3]



1

The reaction was performed in dry Schlenk tubes under argon. 4-(2-Phenyl-2H-tetrazole-5-yl)benzoic acid **1a** (200 mg, 0.75 mmol, 1 eq), (3-aminopropyl)triethoxysilane (170 mg, 0.77 mmol, 1 eq) and dimethylaminopyridine (DMAP, 12 mg, 0.1 mmol, 13 mol%) were dissolved in dry DCM (6 mL). A solution of dicyclohexylcarbodiimide (DCC, 190 mg, 0.9 mmol, 1.2 eq) in dry DCM (4 mL) was added dropwise and stirred for 18 h at room temperature. A precipitate formed overnight and was filtered off. The remaining organic solution was concentrated and purified by column chromatography (Cyclohexane/EtOAc = 2/1) to yield a red solid (200 mg, 0.42 mmol, 56 %; lit 35 %). The analytical data is in agreement with the reported data.[3]

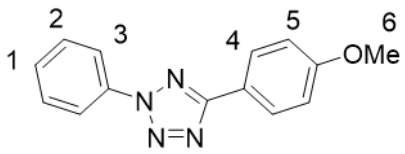
R_f = 0.57

^{1}H NMR (400 MHz, Chloroform-d) δ 8.32 (d, J = 8.4 Hz, 2H, H-7), 8.21 (d, J = 7.3 Hz, 2H, H-8), 7.94 (d, J = 8.3 Hz, 2H, H-9), 7.59 (dd, J = 8.5, 6.7 Hz, 2H, H-10), 7.55 – 7.47 (m, 1H, H-11), 6.69 (d, J = 6.1 Hz, 1H, H-6), 3.84 (q, J = 7.0 Hz, 6H, H-2), 3.51 (q, J = 6.6 Hz, 2H, H-5), 1.86 – 1.73 (m, 2H, H-4), 1.23 (t, J = 7.0 Hz, 9H, H-1), 0.80 – 0.69 (m, 2H, H-3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.87, 164.58, 136.84, 129.98, 129.90, 129.87, 127.74, 127.26, 120.06, 58.71, 49.48, 42.44, 33.96, 25.71, 25.02, 22.99, 18.45, 8.06.

MS (ESI (+)) 492.2045 (found), 492.2043 (calculated for $C_{23}H_{31}N_5NaO_4Si$ = mass of product + Na).

Synthesis of 5-(4-methoxyphenyl)-2-phenyl-2H-tetrazole 2[2]



The synthesis was carried out according to a literature procedure with small changes.[2] To a solution of 4-methoxybenzaldehyde (1.36 g, 10 mmol, 1.0 eq.) in 100 mL ethanol was added benzenesulfonohydrazide (1.72 g, 10 mmol, 1.0 eq.) and the mixture was stirred for 30 min. After addition of 200 mL water a precipitate formed and was collected in a funnel. The solid was dissolved in 60 mL pyridine to give a solution A. In parallel, a solution of $NaNO_2$ (0.69 g, 10 mmol, 1.0 eq.) in 4 mL water was added dropwise to a cooled mixture of aniline (0.93 g, 0.92 mL, 10 mmol, 1 eq.) dissolved in 16 mL water : ethanol (1 : 1) and 2.6 mL concentrated HCl to give solution B. Solution A was cooled with an ice-salt bath and solution B was then slowly added. The reaction was carried out for 1 h and the reaction mixture was dissolved in ethyl acetate and then washed with brine 3 times. The crude product was purified by flash chromatography (CH : DCM : EtOAc=2 : 1 : 1) to obtain the titled compound as a red solid (1.75 g, 6.94 mmol, 69%).

^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 8.22-8.16 (*m*, 3H, H-1, H-2); 7.65-7.44 (m, 4H, H-3, H-4); 7.06-7.02 (m, 2H, H-5); 3.89 (s, 3H, H-6)

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.25, 161.61, 129.77, 129.63, 129.11, 128.73, 119.96, 114.51, 55.55.

MS (ESI(+)) 275.0905 (found), 275.0909 (calculated for $C_{14}H_{12}N_4NaO$ = mass of product + sodium)

Additional analytical data

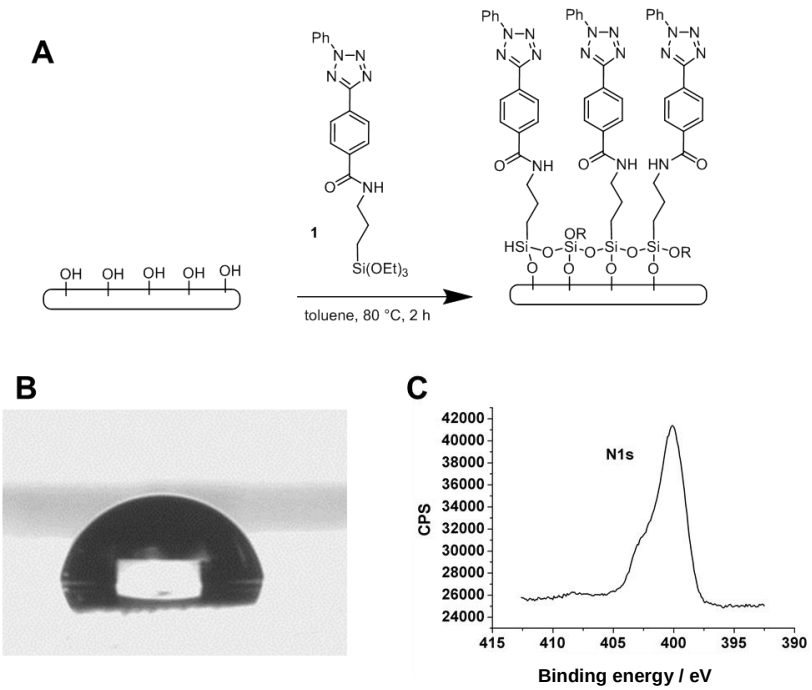


Fig. SI 1: (A) Synthesis of the tetrazole SAM on activated silicon wafers or glass from silane **1**. (B) Static contact angle of the tetrazole SAM (80°). (C) Nitrogen signal in the XPS spectrum of the tetrazole SAM.

Table SI 1: Static water contact angles of substrates that were homogeneously functionalized with flat stamps. The contact angles decrease after microcontact printing indicating the successful deposition of the different molecules.

Substrate functionalized with	Contact angle$_{stat}$/°
tetrazole SAM	80
4-penten-1-ol **6**	67
vinylferrocen **7**	69
nitrobenzoxadiazole substituted acrylate **8**	70
chlorohexyne **9**	68
2-mercaptoethanol **10**	59

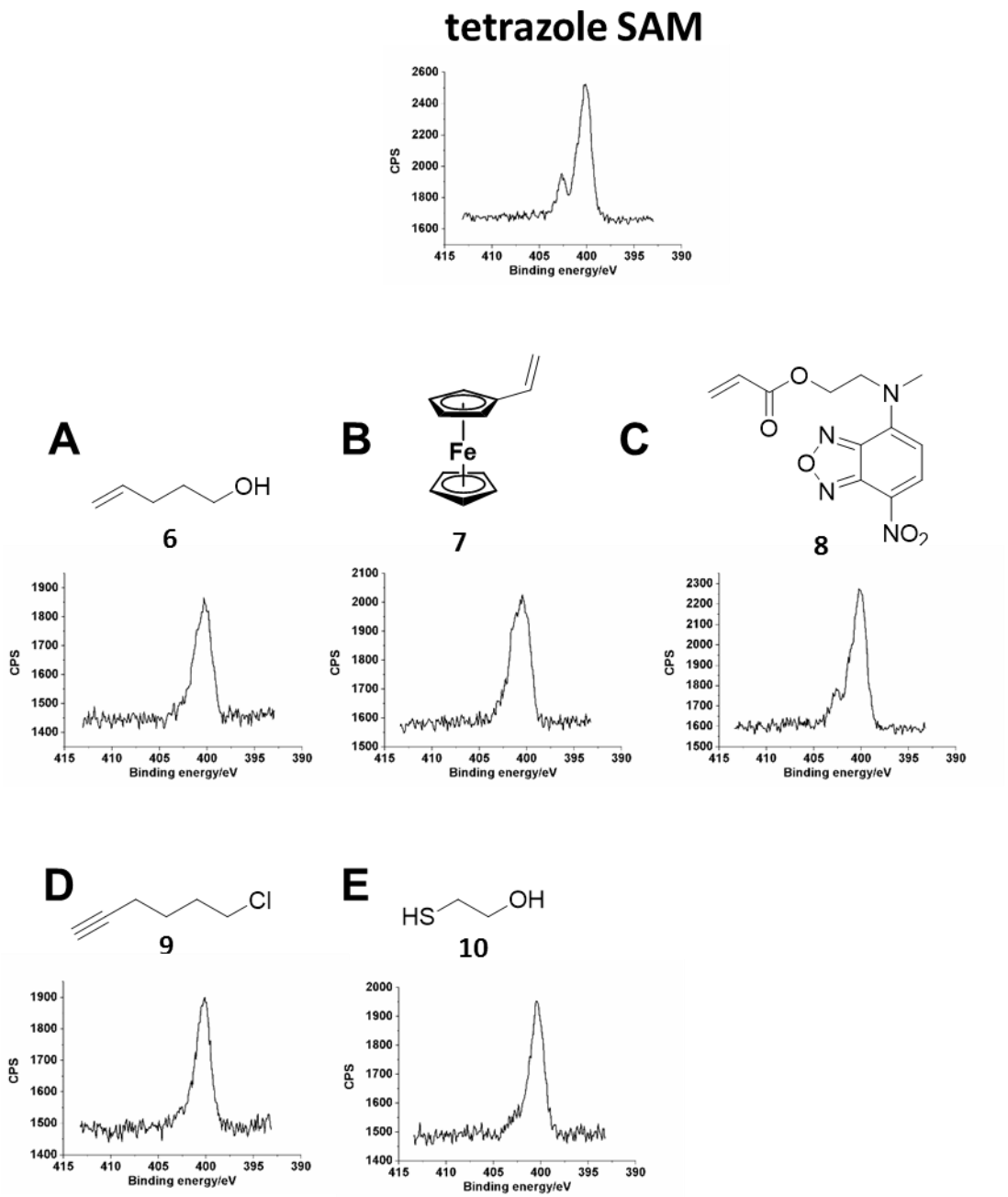


Fig. SI 2: Nitrogen signals in XPS before and after functionalization with various inks employing flat stamps to yield homogeneously functionalized surfaces. The tetrazole SAM exhibits a strong peak at 400.2 eV, which can be attributed to the nitrogen atoms in the tetrazole. The smaller peak at a binding energy of 402.6 eV can be most likely assigned to partially protonated tetrazole species.[3] As expected, the intensities of the nitrogen signals decrease after the reaction, since the products contain less nitrogen the original tetrazole. Moreover, the peak for the protonated tetrazole diminish, which was also observed by Barner-Kowollik et al. and further proves the successful surface modification.[3]

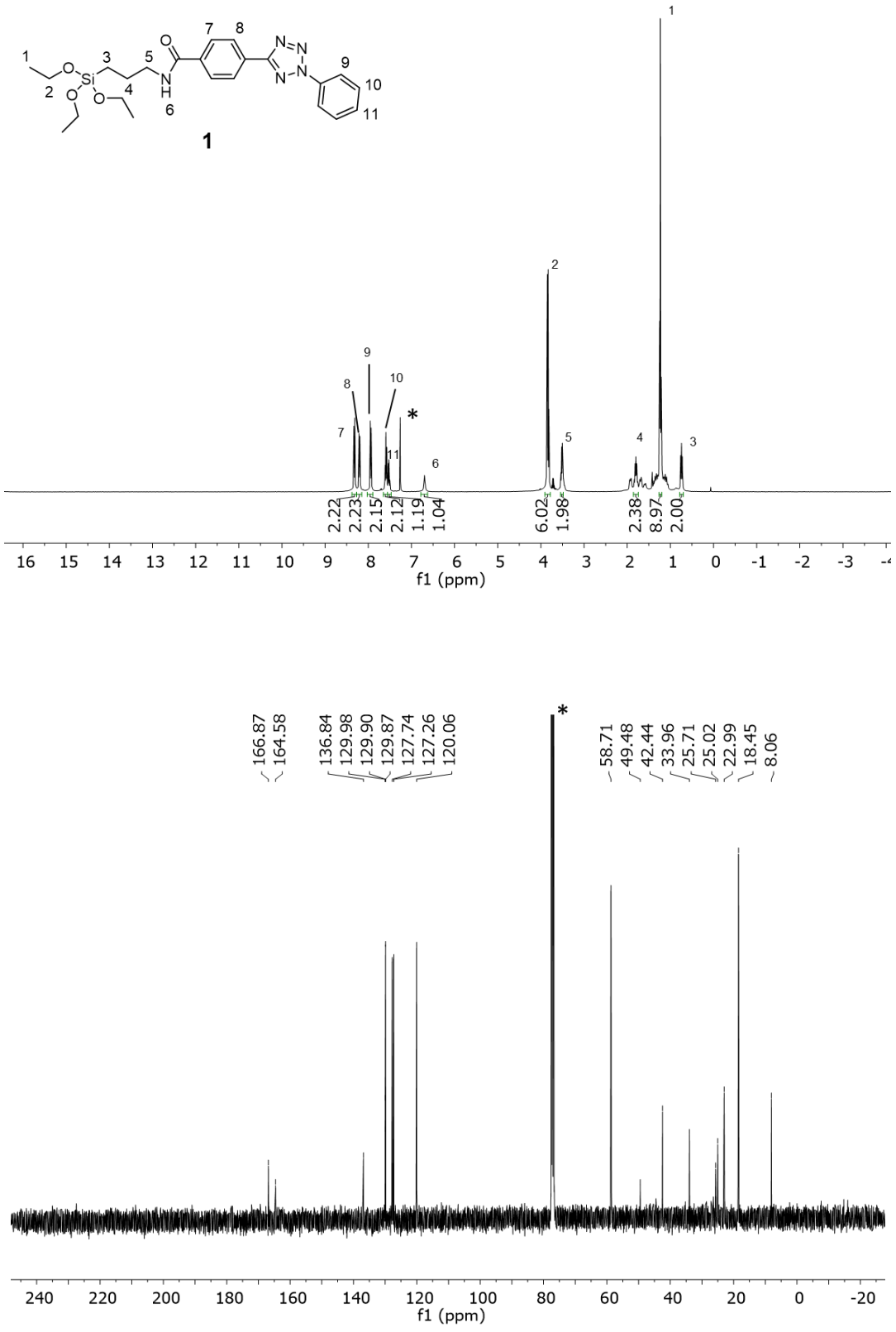


Fig. SI 3: ^{1}H- and ^{13}C-NMR spectra of silane **1** in $CDCl_3$.

References

(1) Roling, O.; Mardyukov, A.; Lamping, S.; Vonhören, B.; Rinnen, S.; Arlinghaus, H. F.; Studer, A.; Ravoo, B. J. Surface Patterning with Natural and Synthetic Polymers via an Inverse Electron Demand Diels–Alder Reaction Employing Microcontact Chemistry. *Org. Biomol. Chem.* **2014**, *12* (39), 7828–7835.

(2) Song, W.; Wang, Y.; Qu, J.; Madden, M. M.; Lin, Q. A Photoinducible 1,3-Dipolar Cycloaddition Reaction for Rapid, Selective Modification of Tetrazole-Containing Proteins. *Angew. Chem. Int. Ed.* **2008**, *47* (15), 2832–2835.

(3) Dietrich, M.; Delaittre, G.; Blinco, J. P.; Inglis, A. J.; Bruns, M.; Barner-Kowollik, C. Photoclickable Surfaces for Profluorescent Covalent Polymer Coatings. *Adv. Funct. Mater.* **2012**, *22* (2), 304–312.