Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

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Phototriggering cell adhesion by caged cyclic RGD peptides

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Chemicals

Chemicals and solvents were purchased from Fluka Chemie AG (D-82024 Taufkirchen), Merck KGaA (D-64271 Darmstadt), ABCR (D-76189 Karlsruhe), Acros Organics (B-2440 Geel) and Sigma-Aldrich Chemie GmbH (D-89555 Steinheim). Solvents had p.a. purity and were used as purchased unless specified. DMF and Pyridine were dried over a 4 Å mole sieve and THF was distilled in the presence of Na using benzophenone as indicator.

Analytical thin layer chromatography was performed with TLC plates (ALUGRAM® SIL G/ UV254) from Macherey-Nagel, Germany. Preparative column chromatography was carried out using Merck silica gel (60Å pore size, 63-200μm particle size). The components were visualized using an UV lamp (254nm) with potassium permanganate.

Cyclo[RGDfK] was purchased from Peptides International, Inc. (Louisville, USA).

Silicon Wafers (100 orientation) were provided by Crystec (Berlin, Germany). Quarzt substrates Suprasil were purchased from Heraeus Quarzglas (Hanau, Germany) and Quarzschmelze Ilmenau (Langewiesen, Germany).

Characterisation methods

Solution ^{1}H-NMR spectra were recorded on a Bruker Spectrospin 250 or 300MHz. All measurements occurred at room temperature, are referenced to TMS (δ= 0 ppm) and calibrated using the deuterated lock-signal of the solvent ($CDCl_3$). The chemical shifts are given in parts per million and the coupling constants in Hertz. The following abbreviations are used: *s*-singlet, *t*-triplet, *q*-quartet, *m*-multiplet.

For contact angle measurements, a Contact Angle System OCA 30 (SCA 202 Software) combined with a High Speed Camera HS for image capturing were used. Substrates were sonicated in Milli-Q water before measurements and dried in a nitrogen stream. A 5 µL droplet of water was suspended on three different sites on each substrate from the tip of a µL syringe supported above the sample stage. Presented values are averaged values from the three measurements.

Layer thickness was measured with an ellipsometer EL X-02C (DRE Dr. Riss Ellipsometerbau, Ratzeburg, Germany) The thickness of the oxidized layer on the silicon wafers was determined before silanization and used subtracted from the calculated layer thickness after silanization. Measurements at five different sites on each substrate were performed.

UV/VIS Spectra were recorded with a Varian Cary 4000 UV/VIS spectrometer (Varian Inc. Palo Alto, USA). Quartz substrates were sonicated in THF and Milli-Q water and dried in a nitrogen stream before measurement.

Mass spectra were recorded with a TRIO-2000 and ZAB 2-SE-FPD (VG-Instruments) using electrospray and atmospheric pressure chemical ionisation.

Irradiation experiments on the surface were carried out using a Polychrome V Monochromator coupled to a Xe-lamp (TILL Photonics GmbH, Gräfelfing, Germany). Irradiation wavelength was 351 nm (1 $mWcm^{-2}$). Masks for site-selective irradiation were provided by ML&C (Jena, Germany) in quartz with chrome patterned fields containing micrometric stripes.

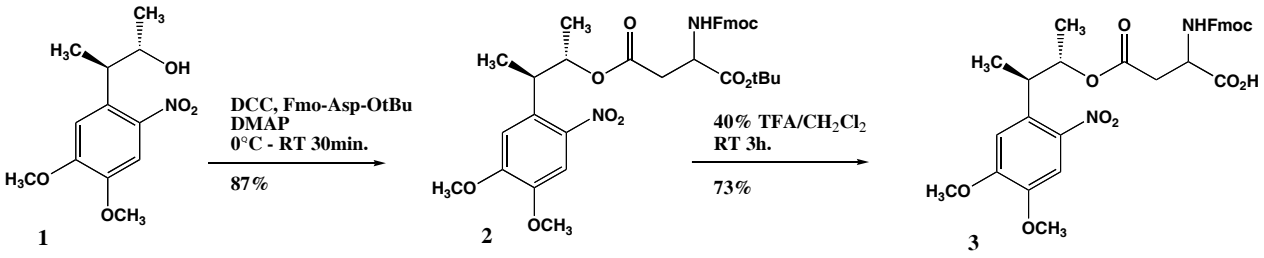
Photolytic experiments in solution were carried out using a 1000W mercury lamp from Hanovia focused on the entrance slit of a monochromator at 364 nm (± 0.2 nm).

The photolysis products in solution were separated and characterized using a Waters 600E HPLC carried out on a Acclaim C18 column (4.6 x 300 nm) coupled to a Waters 2996 PDA detector operating between 200 and 600 nm.

Optical microscopy images were recorded on a Zeiss Axioscope using a digital camera (Zeiss AxioCam) that was attached to the microscope.

1. Synthesis of Fmoc(DMNPB)Asp

The caged Asp derivative, Fmoc(DMNPB)Asp, was obtained in two steps by first coupling Fmoc-Asp-O*t*Bu with the *threo* stereoisomers of a DMNPB alcohol derivative, followed by deprotection of the *t*butyl ester group by acidic treatment



3-(4,5-Dimethoxy-2-nitrophenyl)butan-2-ol *threo stereoisomer,* (1):

Has been synthesized following the method described in A. Specht, J. S. Thomann, K. Alarcon, W. Wittayanan, D. Ogden, T. Furuta, T. Kurakawa, M. Goeldner, *ChemBioChem* **2006**, *7*, 1690.

α-tert-butyl β-3-(4,5-Dimethoxy-2nitrophenyl)-2-butyl N-Fmoc-L-Aspartate (Fmoc-(DMNPB)Asp-O*t*Bu) *threo stereoisomer,* (2)

To a solution of 1 (1.25g, 4.92mmol) in dry CH_2Cl_2(100mL) was subsequently added at 0°C Fmoc-Asp-O*t*Bu (3.04g, 7.38mmol), DCC (1.52g, 7.38mmol), and DMAP (50mg, 0.41mmol). The yellow suspension was allowed to stir 30min at RT then quenched with a saturated aqueous solution of $NaHCO_3$. The two layers were separated and the aqueous layer extracted with EtOAc 3x100mL. The organic extracts were combined, dried on anhydrous Na_2SO_4, filtered and concentrated in vacuo to afford 6.05g of an orange solid as the crude product. The crude product was further purified by flash chromatography (Heptane/EtOAc: 8/2) to give the enantiomerically pure *threo-2* as a yellow solid (2.78g, 87% yield). R_F(Heptane/EtOAc: 1/1) = 0.56. ^{1}H-NMR (300MHz, $CDCl_3$): δ/ppm = 1.27 (6H, m, -CH_3); 1.44(9H, s, -CH_3 O*tBu*); 2.75 (2H, m, CH_2 β);

3.89 (3H, s, OCH_3 meta); 3.93 (3H, s, OCH_3 para); 4.23 (4H, m, CH benz, CH Fmoc9, CH_2 Fmoc); 5.19 (1H, m, CH); 5.64 (1H, m, CH α); 6.88 (1H, s, CH, arom6); 7.32 (5H, m, CH arom3, CH Fmoc2,3,7,6); 7.59 (2H, d, $^3J_{HH}$=6.9Hz, CH Fmoc1,8); 7.76 (2H, d, $^3J_{HH}$=6.8Hz, CH Fmoc4,5)

β-3-(4,5-Dimethoxy-2nitrophenyl)-2-butyl N-Fmoc L-Aspartate (Fmoc(DMNPB)Asp) *threo stereoisomer*, (3)

To a solution of 2 (2.38g 3.67mmol) in dry CH_2Cl_2 (65mL) was added dropwise at RT 43ml TFA. After stirring 3hrs at RT, the mixture was diluted with 300mL H_2O, and the product extracted with EtOAc (3x150mL). The combined organic extracts was dried on anhydrous Na_2SO_4, filtered and concentrated in vacuo to afford 6.09g of a brown oil as the crude product. Repeated trituration in analytical ether afforded a light brown solid as the free acid (1.60g, 73%yield). R_F(Heptane/EtOAc/AcOH: 4.8/4.8/0.4) = 0.2. ^{1}H-NMR (300MHz, $CDCl_3$): δ/ppm = 1.19 (3H, m, CH_3); 1.34 (3H, d $^3J_{HH}$=6.90Hz, CH_3); 2.81 (2H, m, CH_2 β); 3.69 (1H, m, CH benz); 3.84 (3H, s, OCH_3 meta); 3.92 (3H, s, OCH_3 para); 4.24-4.31 (3H, m, CH Fmoc9, CH_2 Fmoc); 4.55 (1H, broad s, CH α); 5.24 (1H, m, CH); 6.68 (1H, s, CH arom6); 7.41-7.27 (5H, m, CH arom3, CH Fmoc2,3,6,7); 7.67 (2H, d, $^3J_{HH}$=6.6Hz, CH Fmoc 1,8); 7.83ppm(2H, d, $^3J_{HH}$=67.2Hz, CH Fmoc4,5). ^{13}C-NMR (300MHz, $CDCl_3$): δ/ppm = 18.24 (s, CH_3CH); 18.71 (s, CH_3CHO); 37.44 (s, CH_2 β); 39.28 (s, CH_3CH); 47.91 (s, CH Fmoc9); 51.84 (s, CH α); 56.50 (s, OCH_3 meta); 56.60 (s, OCH_3 para); 67.52 (s, CH_2 Fmoc); 75.02 (s, CH_3CHO); 108.41 (s, CH arom3); 111.34 (s, CH arom6); 120.81 (s, CH Fmoc2,7); 126.22 (s, CH Fmoc1,8); 128.0 (s, CH Fmoc4,5); 128.57 (s, CH Fmoc3,6); 132.28 (s, CH arom1); 142.08 (s, C-NO_2 arom2); 144.07 (s, C_{quart} Fmoc); 145.04 (s, C_{quart} Fmoc); 148.40 (s, C-OCH_3 arom5); 153.65 (s, C-OCH_3 arom4); 157.08 (s, C=O amide); 170.99 (s, C=O ester); 174.57 (s, C=O acid). ESI-MS: 637.2 $[M^{3-} + 2Na]^+$, 656.9$[M^{3-} + Na+K]^-$

The DMNPB-ester was proven to be stable under the reaction conditions required in the posterior peptide synthesis for the removal of protecting groups, i.e. treatment in TFA/H_2O/Phenol (90/5/5 vol.) for 2.5h at room temperature.

2. Synthesis of cyclo[RGD(DMNPB)fK]

The synthesis of cyclo[RGD(DMNPB)fK] was performed by Neo MPS Laboratories (Strasbourg, France) using standard solid phase synthesis and final HPLC purification as follows.

Synthesis of linear H-Asp(DMNPB)-D-Phe-Lys(Boc)-Arg(Pbf)-Gly-OH

The preparation of the linear peptide was started from 1.5 mmole of H-Gly-2-Chlorotrityl PS resin. The coupling of Fmoc-amino acids (Arg(Pbf), Lys(Boc), Asp(DMNPB)) and deprotection are described below.

2 eq of Fmoc-Amino acid and 2 eq of HOBt were dissolved in DMF (5 mL per mmole of amino acid). 2 eq of DIC was added into the reaction vessel containing the resin.

Step	Solvent	Time	cycle
1	Coupling/DMF	(*) min	Coupling
2	DMF	3x1 min	washing
3	Piperidine/DMF (25%)	1 min (**)	Deprotection
4	Piperidine/DMF (25%)	2x15 min	Deprotection
5	DMF	7x1 min (**)	Washing

* Completion of coupling was determined by the Kaser test.

** The solvent volume for washing and deprotection was 10 mL/gramme of peptide-resin.

The cleavage of protected peptide from the resin was performed using 0.5% of TFA in DCM. 1.97 g of crude product was obtained from 1.5 mmoles of H-Gly-2-Chlorotrityl PS resin.

Synthesis of Cyclo[-Asp(DMNPB)-D-Phe-Lys(Boc)-Arg(Pbf)-Gly-]

The cyclization of protected linear peptide was performed in solution using HATU as coupling reagent. The protected peptide (1.8 g, 1.5 mmole) was dissolved in DMF (15 mL) and added dropwise onto a mixture of HATU (0.86 g, 2.25 mmole) dissolved in DMF (60 mL) containing NMM (1.35 mL, 12.3 mmole) over a period of 1 h. The resulting mixture was stirred at room temperature for 1h. The solvent was removed under vacuum and the residue was precipitated by addition of a solution of $NaHCO_3$ (5%). The precipitate was filtrated and washed with water to give 1.38 g of protected cyclic peptide as a solid. Yield 78%.

Deprotection

Cyclo[-Asp(DMNPB)-D-Phe-Lys(Boc)-Arg(Pbf)-Gly-] (1.38 g, 1.16 mmole) was dissolved in a mixture TFA/H_2O (6.65 mL/0.35 mL) and stirred for 2 h. IPE was added and the precipitate was filtrated and washed with IPE to give 1.18 g of crude product. Yield 95.9%

Purification

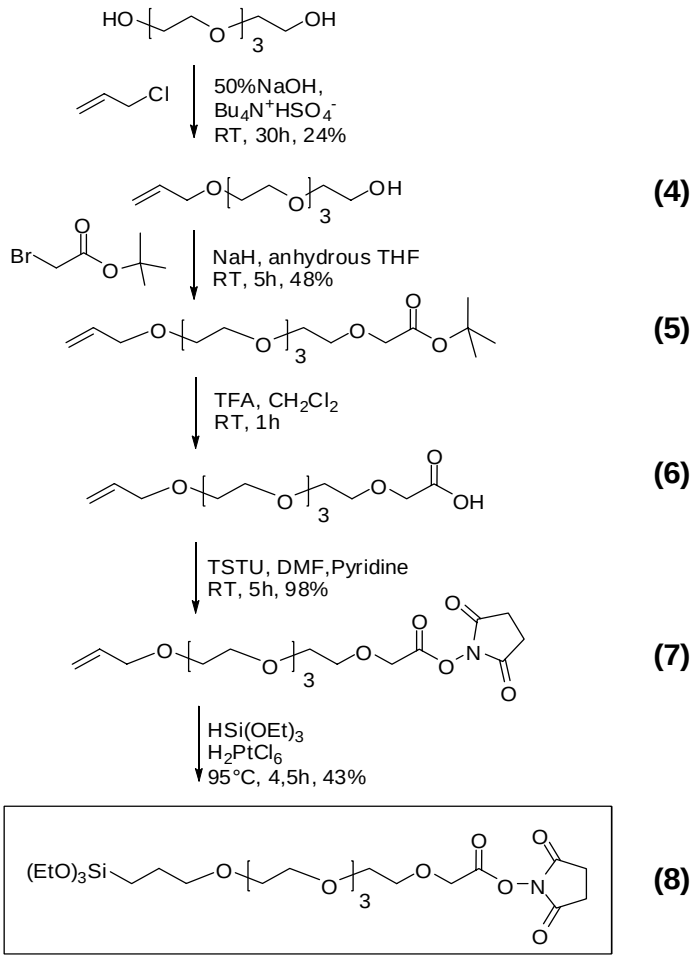
The crude product was purified by RP-HPLC using water/acetonitrile containing 0.1% TFA as eluents. The pure fractions were collected and freeze dried to give a powder. 544 mg of solid were obtained from 1.48 g of crude product. Yield 36.7%. HPLC analysis: 97.98% (mixture of two diastereoismers); MS ES^+: 841.4 $(M+1)^+$;

Stability tests

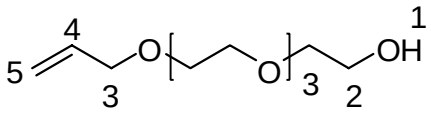
The stability of the peptide in physiological conditions was checked by HPLC analysis of a 1.28×10^{-4} M solution of cyclo[RGD(DMNPB)fK] in 0.1M phosphate buffer pH 7.2 at room temperature in the absence of light. After 3h, one single peak was still observed in the chromatogram, indicating that neither hydrolysis of the ester, nor β–elimination processes (as in photolytic decomposition) occurred in these conditions. The peptide is also expected to be stable under normal lighting conditions for a few days.

3. Synthesis of the linker

The synthesis of the linker molecule, 2,5-dioxopyrrolidin-1-yl 4,4-diethoxy-3,7,10,13,16,19-hexaoxa-4-silahenicosan-21-oate, starts by the introduction of a double bond to TEG by a Williamson etherification reaction with allyl chloride. A second etherification process introduces a *t*-butylbromoacetate unit at the remaining free end of TEG, yielding a TEG-monoallyl-*t*-. butylester after isolation. Acid hydrolysis using TFA yields the corresponding carboxylic acid, which was then activated into a NHS-ester via TSTU coupling. Silanization of the double bound using triethoxysilane and H_2PtCl_6 yielded the target molecule in good yields. Synthetic details and structural characterisation information are given below.



3,6,9,12-tetraoxapentadec-14-en-1-ol, (4)

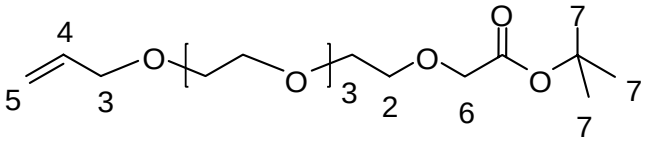


[234.29]

Tetraethylene glycol (20 g, 102 mmol), an equimolar amount of allyl chloride (7.8 g, 51 mmol) and tetrabutyl ammonium hydrogen sulfate (2.2g, 6 mmol) were dissolved in dichloromethane (80 ml). A 50% (w/V) NaOH solution (0.5 mol NaOH) was slowly added under vigorous stirring. The reaction was allowed to proceed for other 30 hours at room temperature. Then the organic phase was separated, and the aqueous phase was washed with three times with dichloromethane. The organic fractions were collected, dried over sodium sulphate and filtrated. After evaporation

of the solvent 18,6 g of a mixture of unreacted tetraethylene glycol, monoallyl ether and diallyl ether was obtained. The monoallyl ether as compound 4 was isolated by column chromatography on silica using ethyl acetate/ ethanol (9:1) as eluent to give a colourless oil (5.73 g, 24.48 mmol, 24% yield). ^{1}H-NMR (250.1 MHz, CDCl3): δ/ ppm = 2.82 (1H, s, H1), 3.51 – 3.66 (16H, m, H2), 3.94 (2H, d, H3), 5.13-5,26 (2H, m, H4); 5.87-5.99 (1H, m, H5)

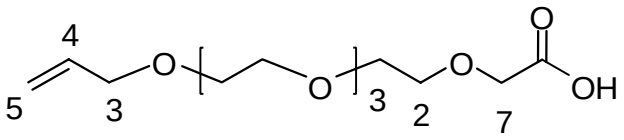
tert-butyl 3,6,9,12,15-pentaoxaoctadec-17-en-1-oate, (5)



[348.44]

Compound 4 (5.73 g, 24.48 mmol) was treated with 1.2 equivalents of NaH (705 mg, 29.38 mmol) in 10 ml anhydrous THF at 0°C under an argon atmosphere. After stirring for 30 minutes, 1.2 equivalents of *tert*-butylacetate (5.63 g, 29.38 mmol) were added dropwise to the reaction mixture within 10 min. The reaction was allowed to proceed for other 5 hours at room temperature. The volatiles were removed in vacuum, 10 g of a mixture of unreacted compound 4, tert butyl bromo acetate and compound 5 were obtained. The ester was isolated by column chromatography on silica using ethyl acetate as eluent to give a colourless oil (4.08 g, 11.75 mmol, 48% yield). ^{1}H-NMR (250.1 MHz, CDCl3): δ/ ppm = 1.48 (9H, s, H7), 3.51 – 3.66 (16H, m, H2), 3.94 (2H, H3), 3.94 (2H, s, H6), 5.13 – 5.26 (2H, m, H5), 5.87 - 5.99 (1H, m, H4).

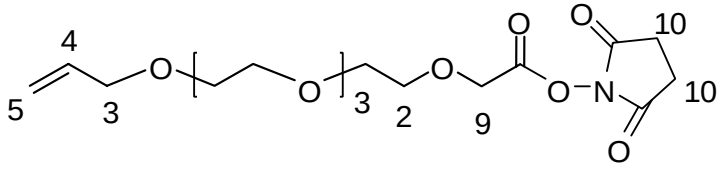
3,6,9,12,15-pentaoxaheptadecan-1-oic acid, (6)



[292.33]

Compound 5 (4.08 g, 11.75 mmol) was diluted in 20 ml of a solution of CH_2Cl_2 and TFA (1:1). After stirring at room temperature for 1 hour, the volatiles were removed to yield the carboxylic acid 6 in quantitative yields, which was used for the next step without further purification. Complete conversion was visualized by TLC. ^{1}H-NMR (250.1 MHz, CDCl3): δ/ ppm = 3.51–3.66 (16H, m, H2), 3.94 (2H, d, H3), 4.19 (1H, s, H8), 5.13 – 5.26 (2H. m, H5), 5.87 - 5.99 (1H, m, H4)

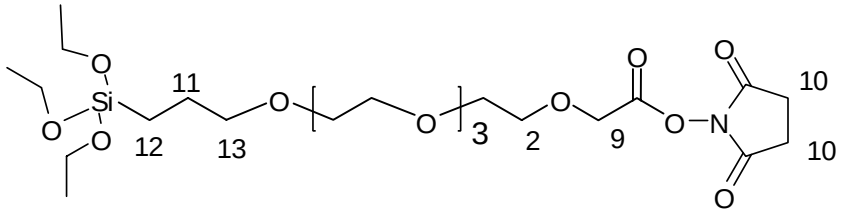
2,5-dioxopyrrolidin-1-yl 3,6,9,12,15-pentaoxaoctadec-17-en-1-oate, (7)



[389.41]

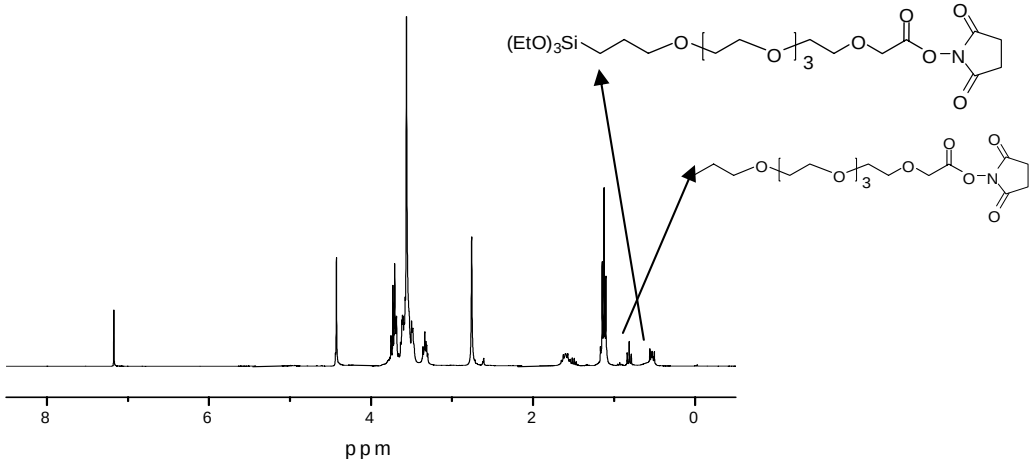
Compound 6 (2.06 g, 7.04 mmol) was dissolved in 10 ml DMF and dry Pyridine (10 ml) was added. 1 equivalent of (O-(N-succinimidyl)-N,N,N`,N`-tetramethyluronium tetrafluoroborate) (TSTU) (2.12 g; 7.04 mmol) was separately dissolved in 10 ml DMF and added under stirring. After 5h of stirring at room temperature under Ar, the THF was evaporated and the DMF was eliminated. The residue was dissolved in 10 ml of CH_2Cl_2 and solids were removed by filtration. The solution was washed with once 10 ml 5% HCl and 10 ml of water. CH_2Cl_2 was evaporated to yield 2.8 g, 7.18 mmol (98 %) of compound 7 with a small impurity of free carboxylic acid. ^{1}H-NMR (250.1 MHz, CDCl3): δ/ ppm =2.85 (2H, s, H10), 3.51 – 3.66 (16H, m, H2), 3.84 (2H, d, H3), 4.19 (s, remaining free carboxylic acid), 4.52 (2H, s, H9), 5.13 – 5.26 (2H, m, H5), 5.87 - 5.99 (1H, m, H4). Purification of this product by flash chromatography using silica gel or alumina caused partial hydrolysis of the NHS ester. For this reason, it was used in the next step without purification.

2,5-dioxopyrrolidin-1-yl 4,4-diethoxy-3,7,10,13,16,19-hexaoxa-4-silahenicosan-21-oate, (8)



[552.41]

Into a round bottom flask previously passivated with hexamethyldisilazane (HMDS), a solution of 7 (2.8 g, 7.18 mmol) in 10 equivalents of triethoxysilane (11.8 g, 71.8 mmol) was added. The mixture was heated under anhydrous conditions to 95 °C and $3.3*10^{-3}$ equivalents of H_2PtCl_6 (2.7% in i-PrOH) were added and the solution was allowed to stir for further 4.5 h at 95°C. Excess $HSi(OEt)_3$ was removed under vacuum, and the crude product was purified by filtration over celite. A mixture of 2.3 g containing the reduced product and the silane 8 (28% to 72%) was obtained. ^{1}H-NMR (300.1 MHz, CDCl3): δ/ppm = 0.5 (1.46H, t, H12), 0.8 (0.85H, t, reduced product), 1.15 (6.2H, t, Ethoxy-CH_3), 1.54 (2H, m, 11 and reduced product), 2.85 (2H, s, H10), 3.4 (2H, m, 13 and reduced product) 3.51 – 3.66 (16H, m, H2), 3.7 (4.7, q, Ethoxy-CH_2), 4.52 (2H, s, H9).



The stability of the obtained mixture of reduced product and silane with time, even if stored under argon, is limited. Hydrolysis and condensation of the triethoxysilane were observed by NMR after storage for a few days in some batches (See NMR spectrum: the decrease in intensity

of the ethoxy peaks at 1.15 ppm and 4.7 ppm and the broadening of the signal at 0.5 ppm indicate hydrolysis and condensation reactions respectively). This is probably caused by partial hydrolysis of the NHS-ester, which liberates free carboxylic acid groups that can catalyse the hydrolysis of the silane. In the condensed state, compound 8 could no longer serve for controlled surface deposition. For this reason, it was used immediately for surface modification after synthesis.

4. Surface modification with the linker 8

Quartz or glass substrates were modified with the linker molecule by solution phase silanisation in THF as follows.

Silicon wafers (25×10 mm) with a 2.2 nm native oxide layer and quartz substrates (25×10mm) were first cleaned by placing them into fresh Piranha solution (H_2SO_4 : 30%H_2O_2 (5:1) v/v) overnight and then rinsing with deionised Milli-Q water and drying at 90°C under vacuum. The cleaned substrates were immediately used for silanization.

For optimizing the silanisation conditions to obtain dense and homogenous layers, silicon wafers were immersed in a 1% solution of the silane and removed after different reaction times. The thickness of the deposited layer was measured by ellipsometry on different sites of each substrate.

Optimised silanisation conditions: 233 mg of crude product 8 were prehydrolised in 17 ml THF and 20 µl of 1N NaOH as catalyst for 5 hours. The reduced compound does not contain any anchoring group for surface coupling, permitting that only compound 8 will be covalently bound to substrates during silanization with the obtained mixture. The resulting 1% w/v silane solution was then filtrated through a 0.2 µm PTFE (Teflon) filter. The clean substrates were immersed into the silane solution for 24h. After deposition, the substrates were gently rinsed successively with THF and Milli-Q water and backed for 1 h at 95 ◦C in a vacuum oven. Sonication in THF in an ultrasound bath was performed to remove any physically adsorbed silane, and then the substrates were blow dry in a nitrogen stream for further use.

Layers of up to 1.75 ± 0.05 nm were obtained after 24 h of surface modification. Surface reaction times above 30 h result in a thick and visible layer of silane, which remains on the surface after washing thoroughly. This suggests the formation of large condensates in solution which attach to the surface and result in thick, inhomogeneous layers. On the basis of these results, a deposition

time of 24 h was chosen for standard reaction conditions. The static contact angle of such layers is 40°.

The stability of the NHS activated carboxylic acid in the conditions used for surface modification was tested by ^{1}H-NMR. A new signal at 4.19 ppm in the NMR spectrum, assigned to methylene groups next to a free carboxylic acid, appeared after four hours and evidenced undesired partial hydrolysis of the NHS ester under these conditions. This means that part of the carboxylic acid groups at the surface are no longer NHS activated, and that linking the peptide through the amino group to the surface required an additional EDC/NHS activation step (see next section for details).

5. Coupling of RGD peptide to the surfaces modified with the linker 8

Coupling of the RGD peptide to the carboxylic surfaces was performed through the free amine of the Lys residue. Having observed partial hydrolysis of the NHS-ester during silanization, an additional TSTU-activation of the carboxyl groups on the surface was carried out before peptide coupling on substrates. Reaction was performed in aqueous medium at pH 7.2 to avoid competing reaction of the arginine side chain guanidine group with the activated carboxyl group on the surface.

110 µl of 0.05M TSTU in DMF containing (0.5% (v/v) of Pyridine were spotted on the substrates and left 2 hours. The substrates were then washed with DMF and PBS, prior to 2 hours incubation with 110 µl of 2 $mgml^{-1}$ solution of cyclo[RGD(DMNPB)fK] in PBS. After incubation, substrates were washed four times in PBS, gently rinsed with Milli-Q water and then blown dry in a nitrogen stream.

Phosphate-Buffered Saline (PBS): NaCl (8 g), KCl (0.2 g), Na_2HPO_4 (1.44 g), KH_2PO_4 (0.24 g) were dissolved in 800 ml ofMilli-Q water. The pH was adjusted to 7.2 with HCl and further water was added to make up a 1 L solution that was autoclaved.

6. Surface characterisation

Surface layers were characterized by ellipsometry and water contact angle measurements. UV spectroscopy was also used in the case of substrates modified with caged RGD.

7. Photolytic studies of cyclo[RGD(DMNPB)fK] in solution

HPLC and UV analysis of the photolytic products of cyclo[RGD(DMNPB)fK] in solution: A 0.128 mM solution (4 mL) of cyclo[RGD(DMNPB)fK] in 100 mM phosphate buffer (pH 7.2) was exposed to irradiation at 364±0.2 nm for 2 hours. Aliquots of the irradiated sample (200 μL) were extracted after different exposure times (0.5, 1 and 2 hours) and characterized by reverse phase HPLC (Acclaim C18 column (4.6 x 300 nm) coupled to a Waters 2996 PDA detector operating between 200 and 600 nm. Flow rate was 1 mL/min, using a linear gradient of acetonitrile in an aqueous solution of TFA (0.1%) from 0 to 100% (v/v) over 30 min as eluent. According to HPLC, the peak corresponding to cyclo[RGD(DMNPB)fK] (t_R=18.0min) in solution looses intensity upon exposure at 364 nm. In parallel to the disappearance of the cyclo[RGD(DMNPB)fK] from solution, a new major product with increasing intensity could be detected in the chromatogram at shorter retention times (t_R=12.8). MAS analysis of the chromatographic fractions containing this new component gave a mass of 604 Dalton, which was assigned to the $[M+H]^+$ of uncaged cyclo[RGDfK]. UV analysis of these fractions confirmed this hypothesis (see spectra in Figure 2 of the manuscript). Quantification of the released cyclo[RGDfK] was performed based on the value of UV absorbance at 257.5 nm (corresponding to the phenyl group of the phenylalanine residue) after calibration with the linear RGDfK peptide (200 μL at concentrations 1.64×10^{-4} M, 1.17×10^{-4} M, 0.82×10^{-4} M, 0.41×10^{-4} M and 0.16×10^{-4} M).

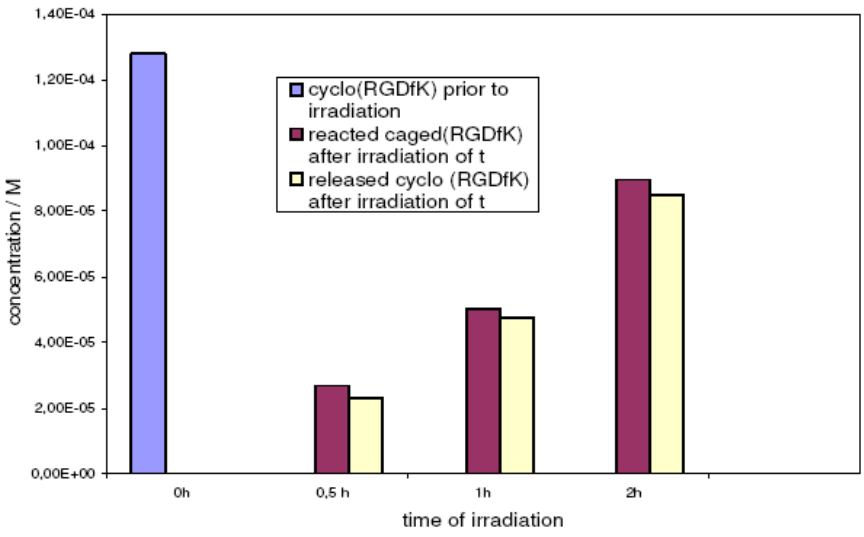


Figure 1A: Concentration of cyclo[RGD(DMNPB)fK] and uncaged cyclo[RGDfK] in solution after irradiation at 364 nm for different times

Quantum yield determination of cyclo[RGD(DMNPB)fK] in solution: The quantum yield of the photolytic reaction was determined by HPLC and UV analysis of the photolyzed solution after exposure at 315 nm using 2-(nitrophenyl)ethyl-ATP (NPE-ATP) ($\Phi = 0.63$) in phosphate buffer (0.1 mM, pH 7.2) at 25°C as reference molecule. Both compounds were tested at identical optical densities at 315 nm as follows. A mixture of 0.07 mM of cyclo[RGD(DMNPB)fK] and 0.2 mM of NPE-ATP was photolysed during 20 minutes, and aliquots were taken after 5, 10, 15 and 20 minnutes and subjected to reverse phase HPLC to determine the extent of the photolytic conversion (Acclaim C18 column (4.6 x 300 nm); flow rate 1 mL/min with a linear gradient of acetonitrile in an aqueous solution of TFA (0.1%) from 0 to 100% (v/v) over 30 min). The retention times of cyclo[RGD(DMNPB)fK] and NPE-ATP were 18.0 and 11.7 min respectively. Quantum yields were calculated by considering the conversions up to 20%, in order to avoid errors due to undesired light absorption during photolysis.

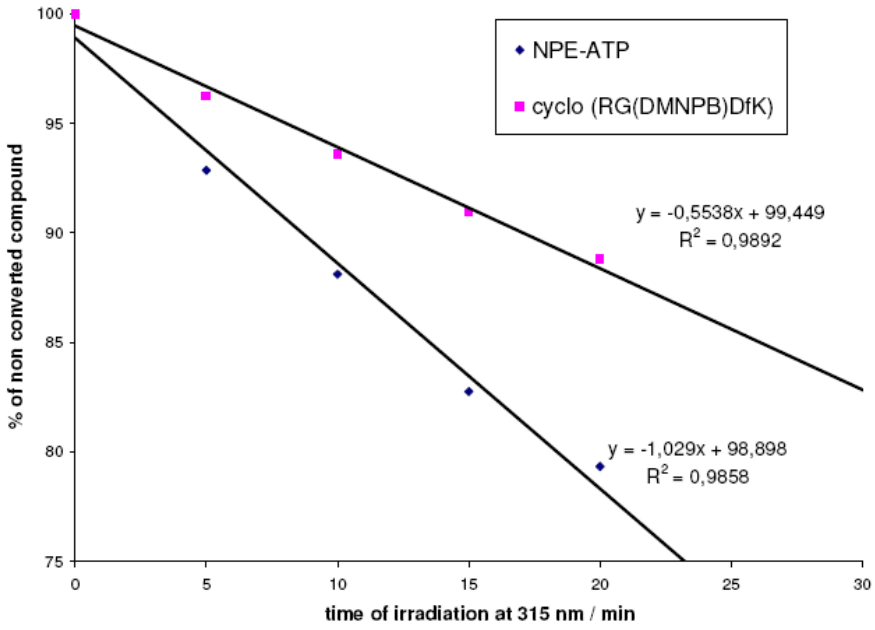


Figure 2A: Loss of cyclo[RGD(DMNPB)fK] and NPE-ATP as a function of irradiation time.

8. Photolytic study of cyclo[RGD(DMNPB)fK] on the surface

Quartz substrates modified with cyclo[RGD(DMNPB)fK] were irradiated at 351 nm for increasing times. After irradiation, substrates were washed three times in THF and sonicated for two minutes. Other washing conditions (eg. water or detergents) did not improve the washing results any further. The progress of the uncaging reaction was followed by UV-spectroscopy analysis of the substrates after light exposure. The decay in the absorbance indicates the removal of the chromophore.

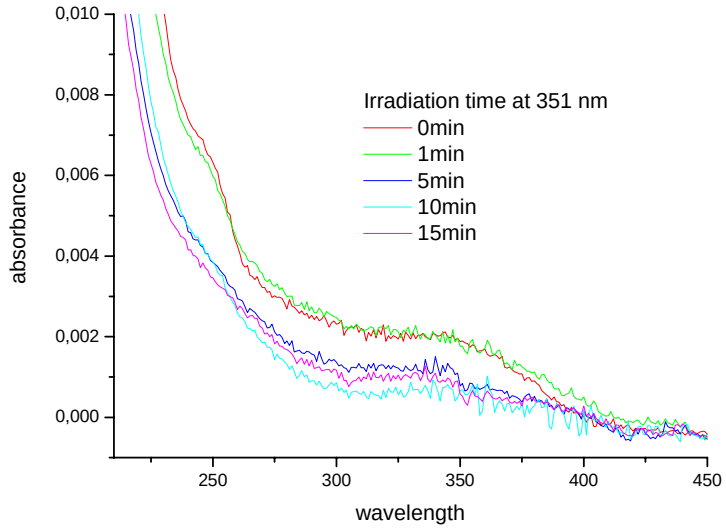


Figure 3A: UV spectra (raw data) corresponding to substrates modified with cyclo[RGD(DMNPB)fK] after irradiation at 351 nm during increasing times.

9. Cell experiments

Mouse fibroblasts (NIH3T3) were cultured in DMEM medium (Invitrogen, Germany) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine (Invitrogen) at 37 °C and 10% CO2. Before plating on the quartz substrates, cells were trypsinized with 0.25 % trypsin and 1mM EDTA in Hanks' buffered salt solution.

Quartz slides (25 mm x 10 mm x 1 mm) were sterilized in 70 % ethanol and washed with phosphate-buffered saline (PBS) at room temperature. 150 µL of DMEM culture medium containing $2x10^5$ cells/mL (30000 cells/sample) were plated in the substrate. After 1 h of incubation at 37 °C, 5% CO_2, the medium containing unattached cells was removed and new DMEM medium was added to the substrates. The culture was maintained for 24 hours. Cells were counted in three different fields on each substrate, acquired through a 10x objective recorded by a camera 3 hours, 6 hours and 21 hours after plating (Zeiss Axioscope – ZeissAxioCam).

10. In-situ masked irradiation and generation of cell patterns

Quartz slides (25 mm x 10 mm x 1 mm) were sterilized in 70 % ethanol and washed with phosphate-buffered saline (PBS) at room temperature. 150 μL of DMEM culture medium containing $2x10^5$ cells/mL (30000 cells/sample) were deposited in a previously sterilized polydimethylsiloxane (PDMS) reservoir (25 mm x 10 mm x 3 mm). The substrate was placed over the PDMS piece so that one of the faces was completely in contact with the culture medium. Irradiation (10 min, 351 nm) was accomplished from the back side of the substrate, through a chrome mask (200 μm thick stripes separated by 100 μm gap). After irradiation, the mask was removed and the substrate turned over. The sample was incubated at 37 °C, 5% CO_2 for three hours, the medium containing unattached cells was removed and new DMEM medium was added to the substrates.

Images were acquired through an x10 objective recorded by a Zeiss Axioscope – ZeissAxioCam after 3, 6 and 21 hours of incubation. To prevent bacterial growth during the manipulation of the sample 1% penicillin/streptomycin was added to the medium.