Chemistry–A European Journal

Supporting Information

Green Algae as a Drug Delivery System for the Controlled Release of Antibiotics

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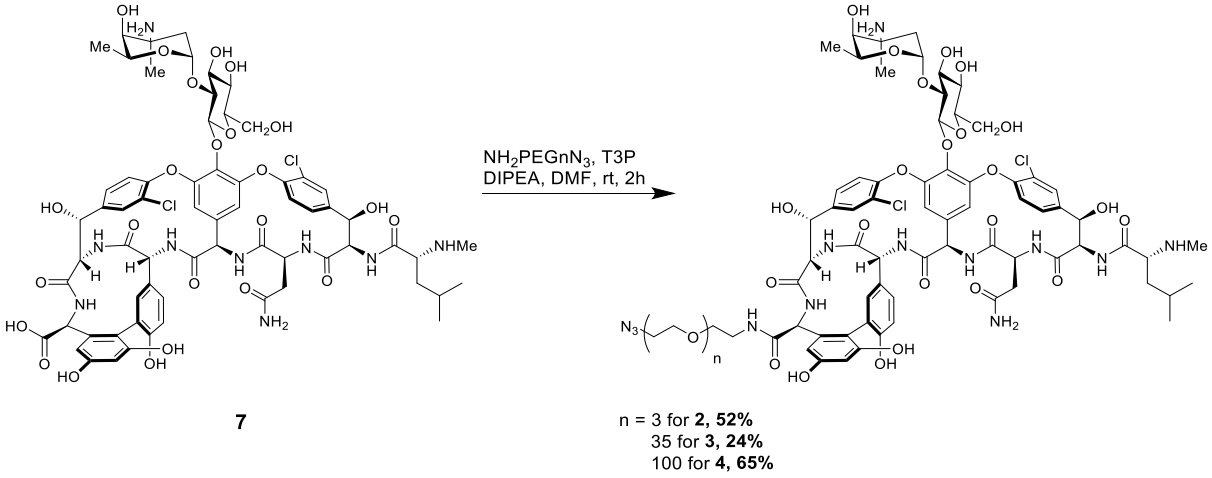
Experimental Part

1. General

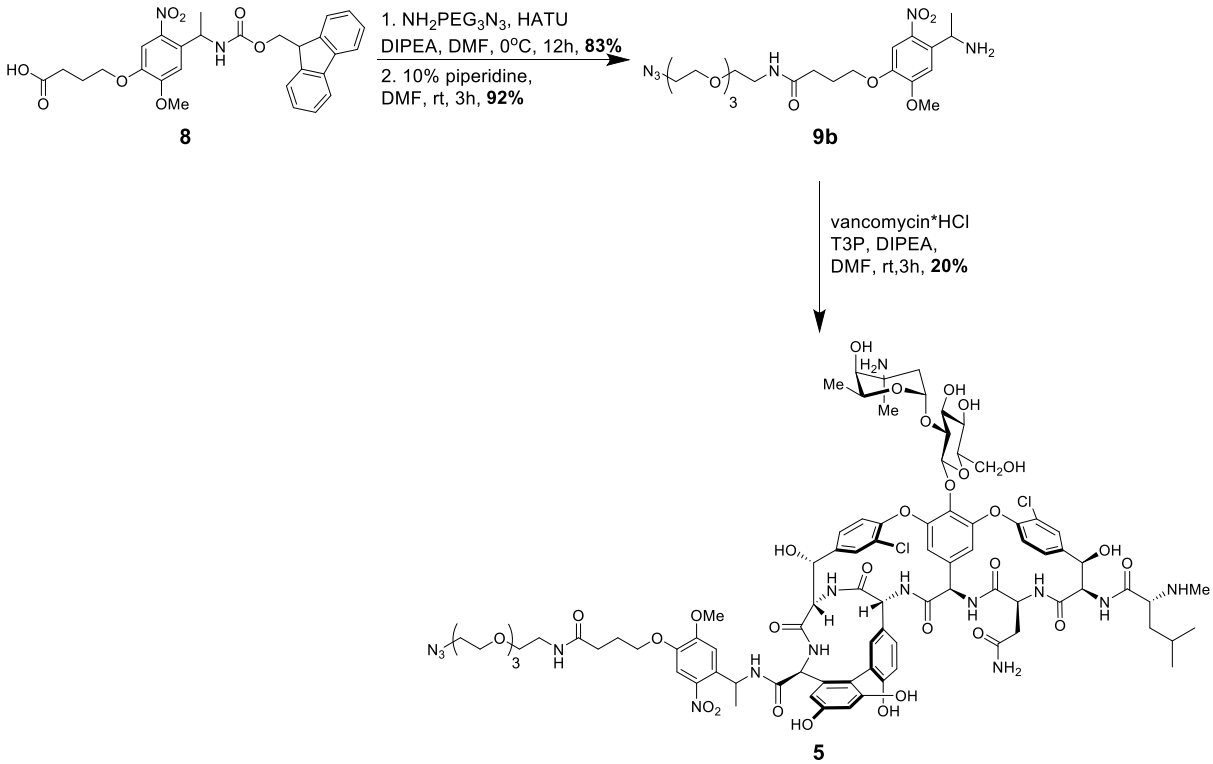
Reactions were carried out under inert gas (N_2 or Ar) in oven-dried (120°C) glass equipment and monitored for completion by TLC or UHPLC−MS (ESI). Solvents for reactions and analyses were of analytical grade. Evaporation of solvents in vacuum was done with a rotary evaporator at 40°C bath temperature and appropriate pressure or by overnight lyophilisation. Merck TLC plates silica gel 60 F254 on glass plate with the indicated solvent system were used; the spots were visualized by UV light (365 nm), and stained by anisaldehyde, ninhydrin, phosphomolybdic acid (PMA) or $KMnO_4$ stain. Silica gel column chromatography was performed using silica gel 60 (230 - 400 Mesh) purchased from Sigma-Aldrich with the solvent mixture indicated. The SPE columns used were DSC-18 (Supelco, Sigma). Analytica, semi-preparative and preparative HPLC separations were performed on a Shimadzu HPLC system (LC-20AP dual pump, CBM-20A Communication Bus Module, SPP-20, A UV/VIS Detector, FRC-10A Fraction collector) using reverse-phase (RP) columns Gemini-NX C18 (150 mm x 4.6 mm; 3 µm, 10 Å), or Synergi Hydro-RP (150 mm x 4.6 mm; 4 µm, 80 Å) or Synergi Hydro-RP (250 mm x 4.6 mm; 4 µm, 80 Å) or Synergi Hydro-RP (10 µm, 80 Å, 150 mm x 10 mm) or Gemini-NX C18 (250 mm x 21.2 mm; 10 µm, 110 Å) or Synergi Hydro-RP (250 mm x 21.2 mm; 10 µm, 80 Å). Ultra-high-performance liquid chromatography (UHPLC) coupled to mass spectrometer (MS) experiments were performed on an Ultimate 3000 LC system (HPG-3400 RS pump, WPS-3000 TRS autosampler, TCC-3000 RS column oven, Vanquish DAD detector from Thermo Scientific) coupled to a triple quadrupole (TSQ Quantum Ultra from Thermo Scientific). The separation was performed using a RP column (Kinetex EVO C18; 50×2.1 mm; 1.7µm; 100 Å, Phenomenex), a flow of 0.4 ml/min, a solvent system composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1% HCO_2H) and an elution gradient starting with 5% B, increasing from 5% to 95% B in 3.5 min, from 95% to 100% B in 0.05 min, and washing the column with 100% B for 1.25 min. UHPLC-MS measurements after the photolysis experiments the fragments ions were monitored by SIM mode focusing on the *m*/*z* 725.20 Da and 965.40 Da. IR-spectroscopy was performed on a *Varian 800 FT-IR ATR Spectrometer*. Lyophilisation was performed on a *Christ Freeze dryer ALPHA 1-4 LD plus.* High-resolution mass spectra were recorded by the Mass Spectrometric Service of Zurich University on a QExactive instrument (Thermo Fisher Scientific, Bremen, Germany) equipped with a heated electrospray (ESI) ionization source and connected to a Dionex Ultimate 3000 UHPLC system (ThermoFischer Scientifics, Germering, Germany). The samples were dissolved in MeOH, MeOH/CH_2Cl_2 3:1, MeOH/H_2O 1:1, DMSO/H_2O 1:10, or H_2O at a concentration of ca. 50 µg•ml^{-1} there of 1µl was injected on-flow with a XRSauto-sampler (CTC, Zwingen, Switzerland). The mobile phase with the flow rate of 0.12 ml/min consisting of a solvent systems MeOH + 0.1 % HCO_2H or acetonitrile/H_2O 2:8 + 0.1% HCO_2H were chosen according to the solubility. Full scan MS were acquired in the alternating (+)/(–)-ESI mode and over the ranges m/z 100-1500, or 200-3000 at 70000 resolution (full width half-maximum) and with automatic gain control (AGC) target of 3.00E+06. The maximum allowed ion transfer time (IT) was 30ms. Masses were calibrated below 2 ppm accuracy between m/z 130.06619 and

1621.96509 in the positive and between 265.14790 and 1779.96528 in the negative ESI mode using the Pierce® ESI calibration solutions (Thermo Fisher Scientific, Rockford, USA). Additionally, contaminations of erucamide (m/z 338.34174, (+)-ESI) and palmitic acid (m/z 255.23295, (–)-ESI) were used as lock masses in (+)-and (–)-ESI, respectively. ^{1}H NMR spectra were recorded in DMSO-d_6 at 298 K on Avance II or III-500 (500 MHz with Cryo-BBO, TXI, BBI or BBO probe), or AvanceII-400 (400 MHz with QNP, BBO or BBFO probe); δ in ppm relative to solvent signals (δ = 2.50 ppm for DMSO-d_6). ^{13}C NMR spectra were recorded in the indicated solvents and on the same instruments; δ in ppm relative to solvent signals (δ = 39.52 ppm for DMSO-d_6). Data are reported as follows: chemical shift (δ, ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet or not resolved signal; br, broad signal), coupling constant (s) (*J*, Hz) and integration. The melting point was measured using Buchi Melting point B-545. Confocal fluorescence microscopy was performed on an inverse confocal laser scanning microscope (Leica SP5) equipped with argon laser (488 nm), objective (HCX PL APO lambda blue 63.0x1.40 OIL UV) and two hybrid detectors (HyD) for emission detection (500-530 nm, 600-700 nm). The microscope was operated using Leica Advanced Fluorescence software and images were processed by adjusting the brightness/contrast using Fiji. Photolysis experiments were performed using a Sina UV lamp (SI-MA-032-W; equipped with UV lamps 4x9, 365 nm) at the distance of ~5 cm. The filtration before HPLC analysis were performed using Syringe Filters, Nylon 66, 0.22 µm. Flow-cytometry experiments were performed on a LSR II Fortessa (BD Biosciences). For the assessment of cells autofluorescence, the samples were irradiated with a laser at 488 nm and the fluorescence was detected using a PerCP-Cy5.5 filter (695/40). The experiments done with propidium iodide were irradiated at 561 nm and the signal was detected using a PE-Texas Red (610/20) filter. The samples were recorded under medium speed for 120 s. Analysis of the flow cytometry data were performed with the software FlowJo 10.0. The microplate reader used for the experiments was the Synergy H1 apparatus from BioTek. The Incubation assays were performed using an Eppendorf Thermomixer Compact with 1.5 mL blocks at 25ºC with a mixing speed of 700 rpm. Optical density for bacterial suspension adjustment was measured by Biochrom Cell Density Meter Ultrospec 10.

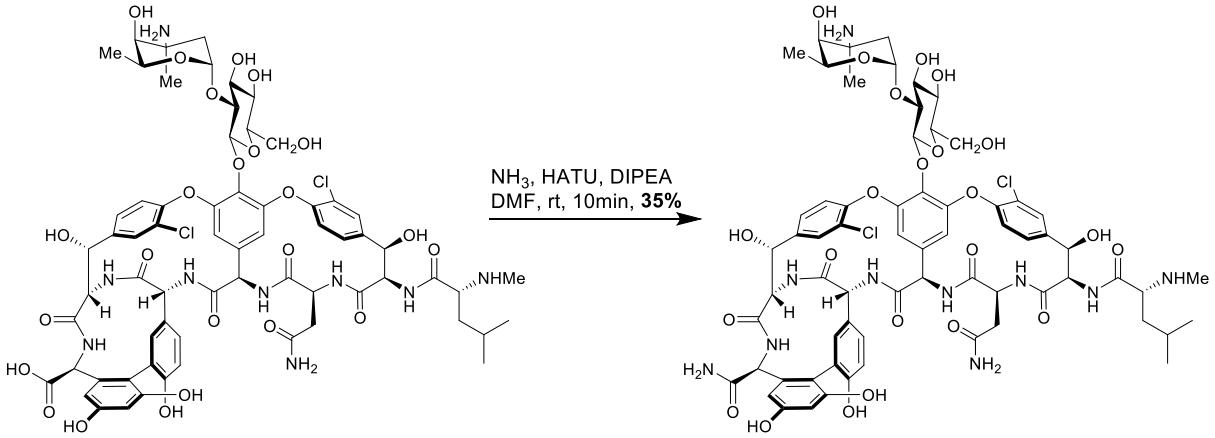
2. Synthesis



Scheme S**1**. Synthetic route for the vancomycin derivative with PEG linkers VancPEG$_3$N$_3$ (**2**), VancPEG$_{35}$N$_3$ (**3**) and VancPEG$_{100}$N$_3$ (**4**)

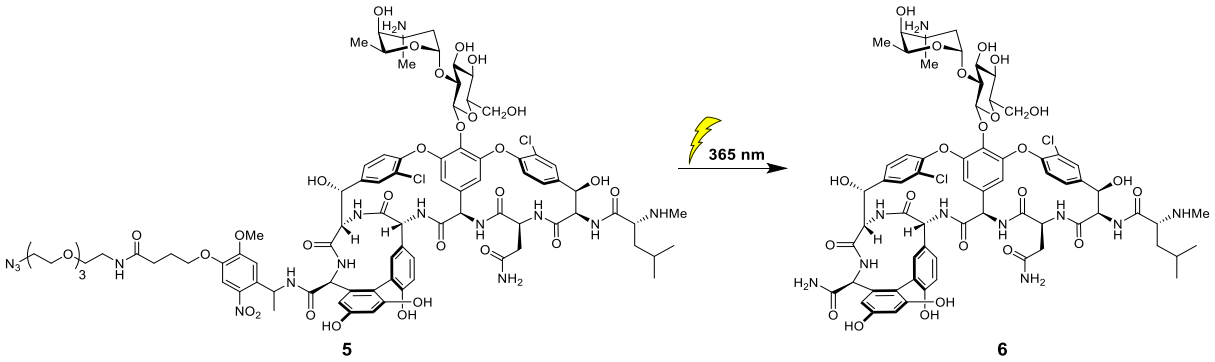


Scheme S**2**. Synthetic route for the vancomycin derivative with photocleavable linker VancphotoN$_3$ (**5**)



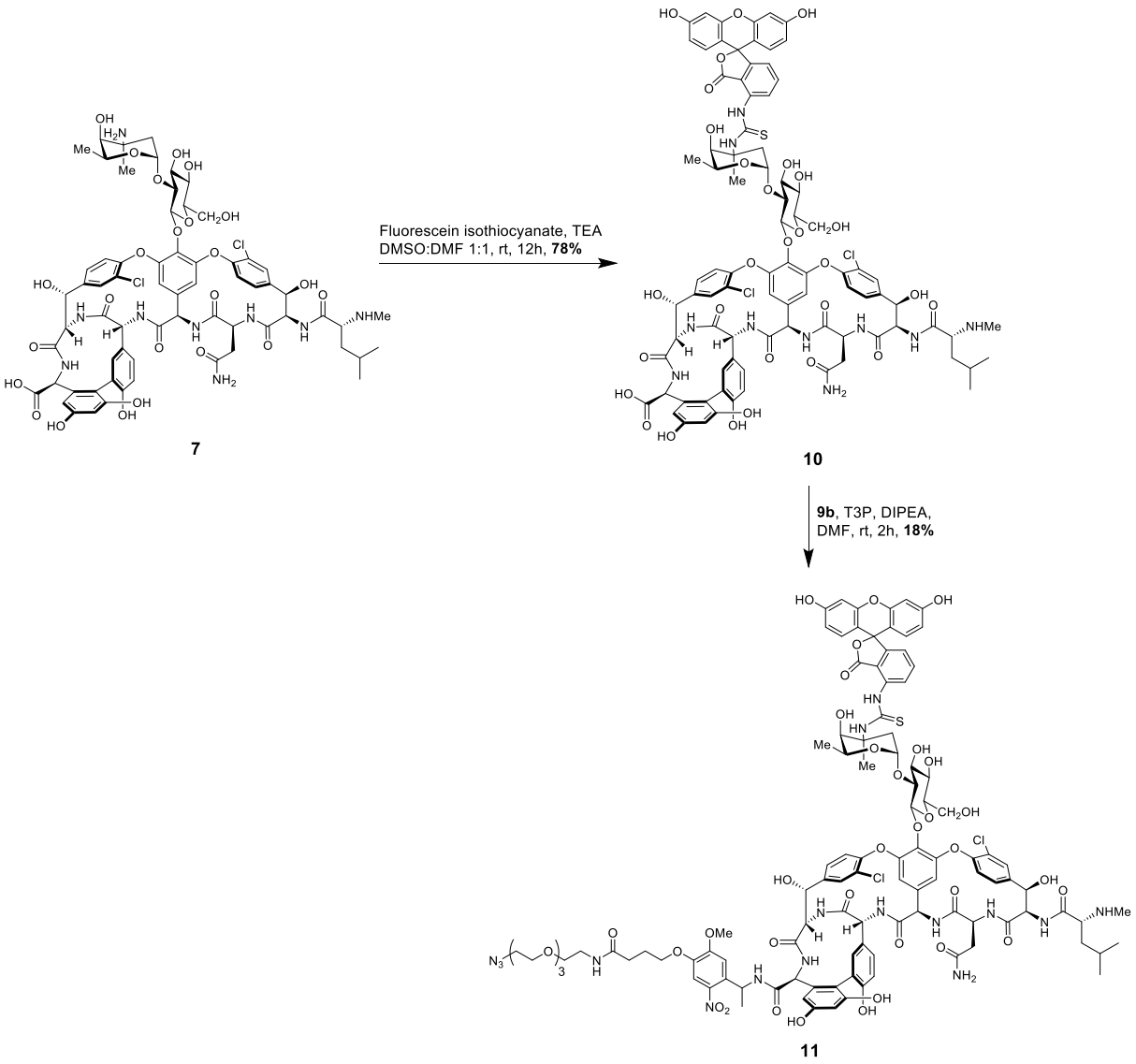
7 **6**

Scheme S**3**. Synthetic route for the amide vancomycin derivative $VancNH_2$ (**6**)



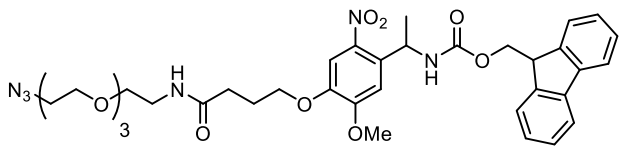
5 **6**

Scheme S**4**. Formation of the amide vancomycin derivative $VancNH_2$ (**6**) by UV-irradiation of $VancphotoN_3$ (**5**)



Scheme S**6**. Synthetic route for vancomycin fluorescein derivative with photocleavable linker **10** (VancFITCphotoN$_3$)

Synthesis of (9*H*-fluoren-9-yl) methyl (1-(4-((1-azido-13-oxo-3,6,9-trioxa-12-azahexadecan-16-yl) oxy)-5-methoxy-2-nitrophenyl) ethyl) carbamate (9a)



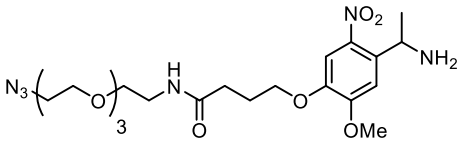
To a solution of **8** (100 mg, 0.192 mmol) in anhydrous DMF (3 mL) at 0°C, distilled *N*,*N*-diisopropylethylamine (0.095 ml, 0.576 mmol) and HATU (146 mg, 0.384 mmol) in DMF (1 mL) were added. The reaction turned dark brown. After 10 min 11-azido-3,6,9-trioxaundecan-1-amine (42 µl, 0.211 mmol) was added. The reaction mixture was stirred in the dark for 12 h at 5°C. The solvents were evaporated followed by purification of crude product by flash silica column chromatography, (EtOAc:MeOH 100:1) to obtain the product **9a** (115 mg, 83%) as a slightly yellow solid.

R*f* = 0.54 (EtOAc:MeOH 20:1); **IR (film)**: ν_{max} = 3336, 2934, 2873, 2103, 1687, 1642, 1521, 1451, 1335, 1281, 1218, 1119, 1086, 1051, 873, 758, 738 cm^{-1}; **^{1}H NMR (500 MHz, DMSO-*d*$_6$)** δ 8.05 (d, *J* = 8.2 Hz, 1H), 7.90 (t, *J* = 5.5 Hz, 1H), 7.86 (d, *J* = 7.4 Hz, 2H), 7.63 (d, *J* = 7.4 Hz, 2H), 7.46 (s, 1H), 7.38 (td, *J* = 2 Hz, 7.2 Hz, 2H), 7.30 – 7.22 (m, 3H), 5.19 (p, *J* = 7.4 Hz, 1H), 4.28 – 4.23 (m, 2H), 4.17 (t, *J* = 6.3 Hz, 1H), 4.01 (t, *J* = 6.3 Hz, 2H), 3.86 (s, 3H), 3.57 (t, *J* = 4.7 Hz, 2H), 3.55 - 3.46 (m, 8H), 3.39 – 3.33 (m, 4H), 3.20 - 3.15 (m, 2H), 2.22 (t, *J* = 7.4 Hz, 2H), 1.96 (p, *J* = 6.9 Hz, 2H), 1.40 (d, *J* = 6.9 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 171.49, 155.25, 153.41, 146.36, 143.88, 143.62, 140.74, 139.93, 135.38, 127.60, 126.91, 125.01, 120.13, 109.36, 108.11, 69.77, 69.75, 69.67, 69.54, 69.23, 69.10, 68.25, 65.24, 56.18, 49.97, 46.70, 46.70, 45.95, 38.52, 31.50, 24.67, 21.92.

ESI-HRMS: *m/z* 721.31852 Da ($C_{36}H_{45}N_6O_{10}^+$; $[M+H]^+$; calc. 721.31917); **Mp** : 104.0-108.0 °C.

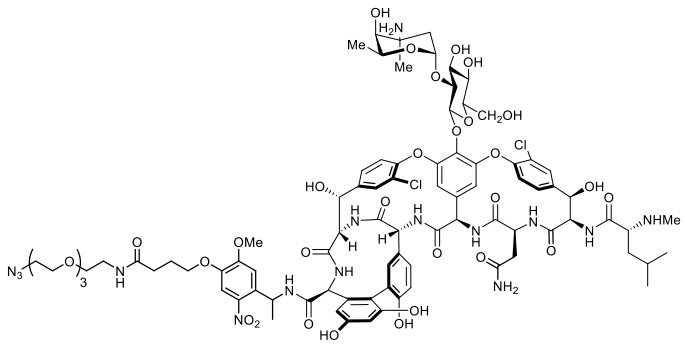
Synthesis of 4-(4-(1-aminoethyl)-2-methoxy-5-nitrophenoxy)-*N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)butanamide (9b)



(9*H*-fluoren-9-yl) methyl (1-(4-((1-azido-13-oxo-3,6,9-trioxa-12-azahexadecan-16-yl) oxy)-5-methoxy-2-nitrophenyl) ethyl) carbamate (110 mg, 0.530 mmol) was treated with 20 percent of piperidine in DMF (3 mL). The reaction mixture was stirred for 3 h at rt and purified by flash silica chromatography (EtOAc:MeOH 10:1) to afford the product **9b** (70 mg, 92%) as a yellow oil.

R_f = 0.28 (EtOAc:MeOH 10:1); **IR (film)**: ν_{max} = 3300, 2924, 2870, 2106, 1650, 1518, 1469, 1337, 1274, 1215, 1119, 1051, 936, 844, 758, 558 cm^{-1}; **^{1}H NMR (500 MHz, DMSO-d_6)** δ 7.93 (t, *J* = 5.5 Hz, 1H), 7.51 (d, *J* = 9.9 Hz, 2H), 4.04 (t, *J* = 6.5 Hz, 2H), 3.93 (s, 3H), 3.60 – 3.57 (m, 2H), 3.56 – 3.47 (m, 10H), 3.42 – 3.36 (m, 5H), 3.20 (q, *J* = 5.8 Hz, 3H), 2.24 (t, *J* = 7.4 Hz, 2H), 1.94 (p, *J* = 6.9 Hz, 2H), 1.44 (d, *J* = 6.6 Hz, 3H); **^{13}C NMR (126 MHz, DMSO-d_6)** δ 171.94, 153.69, 147.01, 140.73, 110.43, 109.00, 70.25, 70.22, 70.15, 70.01, 69.71, 69.56, 68.76, 56.89, 50.45, 46.18, 40.58, 40.49, 40.41, 40.32, 40.16, 39.99, 39.82, 39.66, 39.49, 38.98, 31.92, 25.07, 23.39, 21.60; **ESI-HRMS**: *m/z* 499.25160 Da ($C_{21}H_{35}N_6O_8^+$; $[M+H]^+$; calc. 499.25109 Da).

Synthesis of vancomycin derivative with photocleavable linker VancphotoN$_3$ (5)

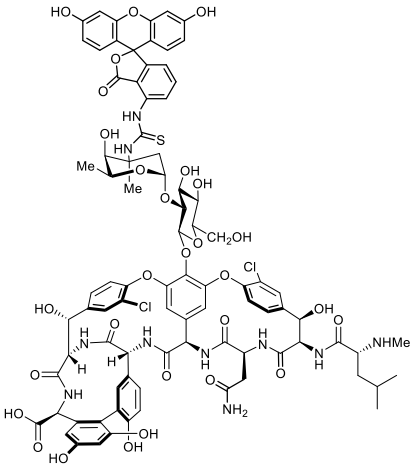


Vancomycin hydrochloride **7** (32.8 mg, 0.022 mmol) was dissolved in dry DMF (0.5 mL). To this mixture, distilled *N*,*N*-diisopropylethylamine (0.010 mL, 0.061 mmol) and propylphosphonic anhydride (0.018 mL, 0.060 mmol) were added, followed by the photocleavable linker **9b** (10 mg, 0.020 mmol). The reaction was stirred for 3h at rt. The reaction mixture was dissolved in MeCN:H_2O (1:3, with 0.1% HCO_2H) and filtered through a SPE column. The solvent was evaporated and the compound was purified by preparative RP-HPLC using a Synergi Hydro-RP column. The solvent system was composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), the flow was 20 mL/min and the gradient was kept at 5% A for 6 min, was changed from 5% to 35% A in 52 min and from 35% to 100% A in 7 min. The desired product, eluting at 34.2 min, was collected and lyophilized to afford VancphotoN$_3$ (**5**) (7.86 mg, 20%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.31 (s, 1H), 9.04 (s, 1H), 8.62 (s, 2H), 8.50 – 8.34 (m, 5H), 7.92 (t, J = 5.6 Hz, 1H), 7.85 (s, 1H), 7.53 – 7.43 (m, 3H), 7.37 – 7.29 (m, 2H), 7.28 – 7.20 (m, 2H), 7.02 (s, 1H), 6.78 – 6.72 (m, 1H), 6.72 – 6.61 (m, 2H), 6.35 (d, J = 2.2 Hz, 1H), 6.06 (d, J = 2.2 Hz, 1H), 5.76 – 5.70 (m, 1H), 5.60 – 5.50 (m, 2H), 5.37 – 5.24 (m, 3H), 5.21 (s, 1H), 5.18 – 5.11 (m, 2H), 5.10 - 5.03 (m, 1H), 4.90 – 4.84 (m, 1H), 4.70 – 4.62 (m, 1H), 4.48 – 4.41 (m, 1H), 4.33 (d, J = 5.3 Hz, 1H), 4.30 – 4.24 (m, 1H), 4.04 – 3.98 (m, 3H), 3.79 (s, 3H), 3.70 – 3.64 (m, 1H), 3.60 – 3.56 (m, 3H), 3.55 – 3.46 (m, 12H), 3.42 – 3.35 (m, 4H), 3.19 (q, J = 5.8 Hz, 2H), 2.29 (s, 3H), 2.23 (t, J = 7.4 Hz, 2H), 2.16 – 2.10 (m, 1H), 2.04 – 1.89 (m, 4H), 1.75 – 1.67 (m, 2H), 1.58 – 1.52 (m, 1H), 1.51 (d, J = 6.8 Hz, 2H), 1.48 – 1.43 (m, 2H), 1.42 – 1.35 (m, 1H), 1.30 – 1.20 (m, 10H), 1.18 – 1.12 (m, 3H), 1.03 (d, J = 6.3 Hz, 3H), 0.89 (d, J = 6.6 Hz, 3H), 0.86 – 0.82 (m, 4H). **ESI-HRMS**: *m/z* 964.83900 Da ($C_{87}H_{109}O_{31}N_{15}Cl_2^{2+}$; $[M+2H]^{2+}$; calc. 964.83899 Da).

The purity of the compound was analysed by analytical RP-HPLC using a Synergi Hydro-RP column, a solvent system composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1% HCO_2H), a flow of 1 mL/min and a gradient which was kept at 5% A for 4 min and was changed from 5% to 100% A in 11 min. The product **5** was eluted at 12.4 min and was detected at 280 nm.

Synthesis of fluorescein derivative of vancomycin VancFITC (10)

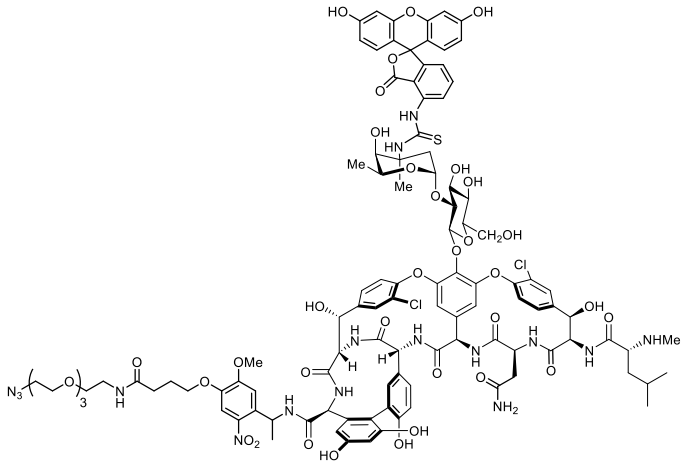


Into the solution of vancomycin hydrochloride **7** (73 mg, 0.049 mmol) in a mixture of DMSO (0.3 mL) and DMF (0.4 mL), fluorescein isothiocyanate (28.6 mg, 0.073 mmol) and triethylamine (35 µl, 0.245 mmol) were added. The reaction was stirred overnight at rt. The residue was dissolved in MeCN:H_2O (1:1, with 0.1% HCO_2H) and filtered through a SPE column. MeCN was evaporated and the compound was purified by preparative RP-HPLC using a Gemini-NX C18 column. The solvent system was composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), the flow was 20 mL/min and the gradient was kept at 15% A for 15 min, was changed from 15% to 50% A in 30 min and from 50% to 100% Ain 5 min. The desired product, eluting at 31 min, was collected and lyophilized to afford a product VancFITC (**10**, 70.4 mg, 78%) as a yellow foam.

^{1}H NMR (500 MHz, DMSO-*d*$_6$) ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.69 – 8.82 (m, 1H), 8.68 – 8.53 (m, 1H), 8.36 (s, 1H), 8.32 (d, J = 8.6 Hz, 1H), 8.19 – 8.11 (m, 1H), 7.93 – 7.83 (m, 1H), 7.77 – 7.66 (m, 1H), 7.65 – 7.55 (m, 1H), 7.47 – 7.38 (m, 1H), 7.37 – 7.33 (m, 1H), 7.32 – 7.23 (m, 2H), 7.21 – 7.14 (m, 2H), 7.08 – 7.03 (m, 1H), 6.80 – 6.53 (m, 10H), 6.38 – 6.25 (m, 2H), 5.97 – 5.85 (m, 1H), 5.76 – 5.67 (m, 1H), 5.55 – 5.40 (m, 1H), 5.35 – 5.15 (m, 4H), 5.15 – 5.04 (m, 2H), 4.75 – 4.59 (m, 2H), 4.57 – 4.47 (m, 1H), 4.49 – 4.40 (m, 1H), 4.37 (d, J = 5.6 Hz, 1H), 4.23 – 4.12 (m, 1H), 4.10 - 3.95 (m, 1H), 3.67 (d, J = 10.6 Hz, 1H), 3.60 – 3.55 (m, 2H), 3.15 (s, 3H), 2.30 – 2.21 (m, 1H), 2.20 -2.09 (m, 1H), 2.03 – 1.92 (m, 1H), 1.90 – 1.81 (m, 1H), 1.80 – 1.73 (m, 1H), 1.67 – 1.57 (m, 2H), 1.56 – 1.46 (m, 1H), 1.24 (d, J = 7.9 Hz, 3H), 1.07 (d, J = 6.2 Hz, 1H), 1.01 (d, J = 6.2 Hz, 2H), 0.97 – 0.90 (m, 6H). **ESI-HRMS**: *m/z* 919.24066 Da ($C_{87}H_{88}O_{29}N_{10}Cl_2S^{2+}$; $[M+2H]^{2+}$; calc 919.24027 Da).

The purity of the compound was analyzed by analytical RP HPLC using a Synergi Hydro-RP column, a solvent system composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), a flow of 1 mL/min and a gradient which was kept at 5% A for 4 min and was changed from 5% to 100% A in 11 min. The product **10** was eluted at 13 min and was detected at 440 nm.

Synthesis of fluorescein derivative of vancomycin with photo cleavable linker VancFITCphotoN$_3$ (11)

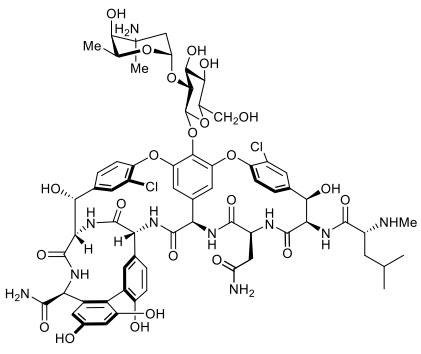


Freshly distilled *N*,*N*-diisopropylethylamine (13.5 µl, 0.081 mmol) and propylphosphonic anhydride (24 µl, 0.081 mmol) were added to a solution of compound **10** (30 mg, 0.016 mmol) in dry DMF (0.2 mL) following by the compound **9b** (9 mg, 0.018 mmol) in dry DMF (0.2 mL). The reaction was stirred for 3h at rt. The solvent was evaporated and the reaction mixture was dissolved in MeCN:H_2O (1:1, with 0.1% HCO_2H) and filtered through a SPE column. The solvent was evaporated and the compound was purified by preparative RP-HPLC using a Synergi Hydro-RP column. The solvent system was composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), the flow was 20 mL/min and the gradient was kept at 5% for 12 min, was changed from 5% to 40% A in 44 min and from 40% to 100% A in 13 min. The desired product, eluting at 17.7 min, was collected and lyophilized to afford the desired product VancFITCphotoN$_3$ (**11**, 7 mg, 18% yield) as a yellow solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.33 (s, 1H), 9.06 (s, 2H), 8.70 – 8.62 (m, 2H), 8.34 (s, 5H), 8.16 (s, 1H), 7.97 – 7.91 (m, 1H), 7.91 – 7.80 (m, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.54 – 7.40 (m, 2H), 7.39 – 7.22 (m, 4H), 7.21 – 7.16 (m, 1H), 7.16 – 7.12 (m, 1H), 7.08 (d, *J* = 8.2 Hz, 1H), 6.79 – 6.73 (m, 1H), 6.73 – 6.64 (m, 3H), 6.64 – 6.55 (m, 5H), 6.41 – 6.33 (m, 2H), 6.07 (d, *J* = 2.2 Hz, 1H), 5.95 – 5.79 (m, 1H), 5.74 (d, *J* = 8.2 Hz, 1H), 5.61 – 5.55 (m, 1H), 5.54 – 5.51 (m, 1H), 5.50 – 5.45 (m, 1H), 5.42 – 5.35 (m, 1H), 5.34 – 5.26 (m, 2H), 5.26 – 5.20 (m, 2H), 5.20 – 5.16 (m, 1H), 5.08 (s, 1H), 4.73 – 4.62 (m, 2H), 4.58 (d, *J* = 5.8 Hz, 1H), 4.51 – 4.44 (m, 1H), 4.33 – 4.27 (m, 1H), 4.06 – 3.98 (m, 3H), 3.90 (s, 2H), 3.82 (s, 2H), 3.70 – 3.63 (m, 1H), 3.61 – 3.56 (m, 3H), 3.56 – 3.48 (m, 10H), 3.20 (t, *J* = 5.8 Hz, 2H), 3.16 (s, 4H), 3.13 – 3.08 (m, 1H), 2.33 – 2.26 (m, 1H), 2.24 (t, *J* = 7.4 Hz, 2H), 2.18 – 2.09 (m, 1H), 2.07 (s, 1H), 2.04 – 1.90 (m, 4H), 1.89 – 1.82 (m, 1H), 1.81 – 1.73 (m, 1H), 1.71 – 1.58 (m, 1H), 1.58 – 1.52 (m, 2H), 1.52 – 1.42 (m, 2H), 1.41 – 1.33 (m, 1H), 1.31 – 1.17 (m, 30H), 1.04 – 0.97 (m, 3H), 0.94 (d, *J* = 6.2 Hz, 3H), 0.91 (d, *J* = 6.2 Hz, 3H) 0.85 (t, *J* = 6.7 Hz, 3H). **ESI-HRMS**: *m/z* 780.56770 Da ($C_{108}H_{120}O_{36}N_{16}Cl_2NaS^{3+}$; $[M+2H+Na]^{3+}$; calc 780.56767 Da).

The purity of the compound was analyzed by analytical RP HPLC using a Synergi Hydro-RP column, a solvent system composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), a flow of 1 mL/min and a gradient which was kept at 5% A for 4 min and was changed from 5% to 100% A in 11 min. The product **11** was eluted at 12.5 min and was detected at 440 nm.

Synthesis of amide derivative of vancomycin VancNH$_2$ (6)



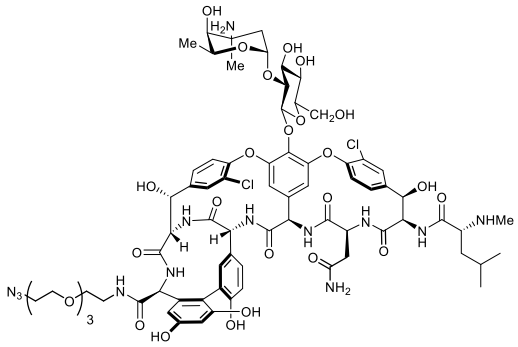
Vancomycin hydrochloride **7** (50 mg, 0.0337 mmol) was dissolved in dry DMF (0.7 mL), *N*,*N*-diisopropylethylamine (17ul, 0.101 mmol) was added following by HATU (38.4 mg, 0.101 mmol). The resulting mixture was stirred for 5 min at rt before bubbling $NH_{3,gas}$ for 10 min. Argon was bubbled to the reaction mixture and the solvent was evaporated. The reaction mixture was dissolved in MeCN:H_2O (1:1, with 0.1% HCO_2H) and filtered through a SPE column. The solvent was evaporated and the compound was purified by preparative RP-HPLC using a Synergi Hydro-RP column. The solvent system was composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), the flow was 20 mL/min and the gradient was kept at 0% A for 11 min, was changed from 0% A to 40% A in 31 min and from 40% A to 100% A in 7 min. The desired product, eluting at 23.0 min, was collected and lyophilized to afford VancNH$_2$ (**6**, 17 mg, 35%).

^{1}H NMR (500 MHz, DMSO-*d*$_6$) ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.59 (s, 1H), 8.38 (d, *J* = 5.6 Hz, 1H), 8.33 (s, 2H), 8.20 (s, 1H), 7.85 (d, *J* = 2.1 Hz, 1H), 7.77 – 7.70 (m, 1H), 7.50 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.45 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.38 – 7.22 (m, 6H), 7.21 (d, *J* = 2.2 Hz, 1H), 6.87 (s, 1H), 6.75 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.70 (d, *J* = 8.4 Hz, 1H), 6.67 – 6.60 (m, 1H), 6.37 – 6.32 (dd *J* = 2.1, 11.2, 2H), 5.90 – 5.80 (m, 1H), 5.74 (d, *J* = 8.1 Hz, 2H), 5.54 (s, 1H), 5.29 (d, *J* = 7.8 Hz, 1H), 5.25 (s, 1H), 5.21 (d, *J* = 2.2 Hz, 2H), 5.15 (d, *J* = 4.5 Hz, 1H), 4.90 – 4.84 (m, 1H), 4.67 (q, *J* = 6.5 Hz, 1H), 4.43 (d, *J* = 5.1 Hz, 1H), 4.40 – 4.34 (m, 1H), 4.30 (d, *J* = 5.5 Hz, 1H), 4.21 (d, *J* = 11.2 Hz, 1H), 4.04- 3.97 (m, 1H), 3.68 (d, *J* = 11.0 Hz, 1H), 3.58 – 3.49 (m, 2H), 3.45 (t, *J* = 7.2 Hz, 2H), 3.07 (s, 1H), 3.03 (t, *J* = 7.2 Hz, 1H), 2.30 (s, 3H), 2.14 (dd, *J* = 15.7, 6.2 Hz, 1H), 1.84 – 1.78 (m, 1H), 1.72 (p, *J* = 7.2 Hz, 1H), 1.64 (d, *J* = 13.2 Hz, 1H), 1.52 – 1.44 (m, 1H), 1.44 – 1.36 (m, 1H), 1.40 -1.20 (m, 6H), 1.06 (d, *J* = 6.4 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H).

ESI-HRMS: *m/z* 724.23031 Da ($C_{108}H_{120}O_{36}N_{16}Cl_2NaS^{2+}$; $[M+2H]^{2+}$; calc 724.23037 Da).

The purity of the compound was analyzed by analytical RP HPLC using a Synergi Hydro-RP column, a solvent system composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), a flow of 1 mL/min and a gradient which was kept at 0% A for 5 min and was changed from 0% to 100% A in 15 min. The product **6** was eluted at 14.8 min and was detected at 280 nm.

Synthesis of vancomycin derivative with PEG_3 linker $VancPEG_3N_3$ (2)

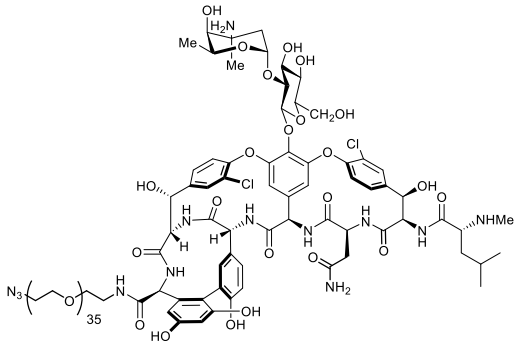


Vancomycin hydrochloride **7** (50 mg, 0.034 mmol) was dissolved in a mixture of dry DMF (0.68 mL) and dry DMSO (0.68 mL). To this mixture freshly distilled *N*,*N*-diisopropylethylamine (0.039 mL, 0.236 mmol) and propylphosphonic anhydride (0.030 mL, 0.101 mmol) were added following by 11-azido-3,6,9-trioxaundecan-1-amine (0.010 ml, 0.051 mmol). The reaction was stirred for 1 h at rt and PyBOP (17.5 mg, 0.037 mmol) was added. The reaction mixture was stirred 2 h at rt and the solvent was evaporated. The mixture was dissolved in MeCN:H_2O (1:1, with 0.1% HCO_2H) and filtered through a SPE column. The solvent was evaporated and the compound was purified by preparative RP-HPLC using a Synergi Hydro-RP column. The solvent system was composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), the flow was 20 mL/min and the gradient was kept at 5% A for 6 min, was changed from 5% A to 30% A in 44 min and from 30% A to 100% A in 12 min. The desired product, eluting at 11.8 min, was collected and lyophilized to afford $VancPEG_3N_3$ (**2**, 28.8 mg, 52%)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.41 (s, 1H), 8.21 (s, 1H), 7.98 – 7.78 (m, 2H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.40 – 7.28 (m, 2H), 7.26 – 7.17 (m, 1H), 6.95 - 6.82 (m, 1H), 6.79 – 6.61 (m, 2H), 6.47 – 6.35 (m, 1H), 6.23 (s, 1H), 5.74 (d, *J* = 7.7 Hz, 1H), 5.61 – 5.49 (m, 1H), 5.36 – 5.30 (m, 1H), 5.28 – 5.18 (m, 2H), 5.17 – 5.10 (m, 1H), 4.94 – 4.82 (m, 1H), 4.72 – 4.64 (m, 1H), 4.47 – 4.41 (m, 1H), 4.37 (d, *J* = 5.5 Hz, 1H), 4.22 (d, *J* = 11 Hz, 1H), 3.69 (d, *J* = 10.6 Hz, 2H), 3.60 (t, *J* = 5.1 Hz, 2H), 3.52 (s, 6H), 3.53 – 3.42 (m, 3H), 3.39 (t, *J* = 5.1 Hz, 2H), 3.35 – 3.11 (m, 4H), 2.44 – 2.25 (m, 2H), 2.21 – 2.08 (m, 1H), 1.77 – 1.63 (m, 1H), 1.50 – 1.36 (m, 4H), 1.34 – 1.21 (m, 2H), 1.05 (d, *J* = 5.3 Hz, 2H), 0.93 – 0.79 (m, 6H). **ESI-HRMS**: *m/z* 824.78583 Da ($C_{74}H_{93}O_{26}N_{13}Cl_2^{2+}$; $[M+2H]^{2+}$; calc 824.78604 Da).

The purity of the compound was analyzed by analytical RP HPLC using a Synergi Hydro-RP column, a solvent system composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), a flow of 1 mL/min and a gradient which was kept at 0% A for 5 min and was changed from 0% to 100% A in 15 min. The product **1** was eluted at 11.8 min and was detected at 280 nm.

Synthesis of vancomycin derivative with PEG_{35} linker $VancPEG_{35}N_3$ (3)

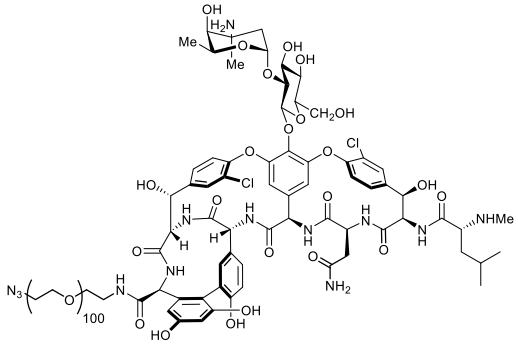


Vancomycin hydrochloride **7** (15.3 mg, 0.010 mmol) was dissolved in dry DMSO (0.2 mL). To this solution, freshly distilled *N*,*N* - diisopropylethylamine (8 µL, 0.05 mmol) and propylphosphonic anhydride (15 µL, 0.051 mmol) were added following by the azide-PEG_{35} amine (10 mg, 0.006 mmol). The reaction was stirred for 2h at rt. The solvent was evaporated and the reaction mixture was dissolved in MeCN:H_2O (1:1, with 0.1% HCO_2H) and filtered through a SPE column. The solvent was evaporated and the compound was purified by preparative RP-HPLC using a Synergi Hydro-RP column. The solvent system was composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), the flow was 20 mL/min and the gradient was kept at 10% A for 7 min, was changed from 10% to 50% A in 28 min and from 50% A to 100% A in 5 min. The desired product, eluting at 20.0 min, was collected and lyophilized to afford $VancPEG_{35}N_3$ (**3**, 7.7 mg, 24%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.33 (s, 1H), 8.60 (s, 1H), 8.42 (s, 3H), 7.95 – 7.83 (m, 2H), 7.78 – 7.65 (m, 1H), 7.46 (dd, J = 9.7 Hz, 23 Hz, 1H), 7.39 – 7.27 (m, 2H), 7.26 – 7.19 (m, 1H), 6.90 – 6.84 (m, 1H), 6.73 (dd, *J* = 8.6 Hz, *J* = 28.3 Hz, 1H), 6.68 – 6.62 (m, 2H), 6.36 (d, *J* = 2.2 Hz, 1H), 6.24 (d, *J* = 2.2 Hz, 1H), 5.90 – 5.83 (m, 1H), 5.76 – 5.70 (m, 1H), 5.56 – 5.49 (m, 1H), 5.34 – 5.28 (m, 1H), 5.24 – 5.17 (m, 2H), 5.16 – 5.04 (m, 2H), 4.90 – 4.83 (m, 1H), 4.70 – 4.62 (m, 1H), 4.43 (d, *J* = 4 Hz, 1H) 4.37 (d, *J* = 5.5 Hz, 1H), 4.22 (d, *J* = 10.7 Hz, 1H), 3.71 – 3.62 (m, 1H), 3.60 (t, *J* = 9.8, 2H), 3.57 – 3.53 (m, 6H), 3.53 – 3.48 (m, 140H), 3.41 – 3.37 (m, 3H), 3.05 – 2.95 (m, 2H), 2.30 (s, 3H), 2.19 – 2.10 (m, 1H), 2.03 – 1.95 (m, 1H), 1.76 – 1.69 (m, 1H), 1.60 – 1.53 (m, 1H), 1.51 – 1.45 (m, 2H), 1.44 – 1.36 (m, 1H),1.30 - 1.22 (m, 6H), 1.16 (d, *J* = 11.0 Hz, 3H), 1.04 (d, *J* = 6.3 Hz, 2H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.85 (d, *J* = 6.6 Hz, 3H). **ESI-HRMS**: *m/z* 1020.80375 Da ($C_{138}H_{219}O_{58}N_{13}Cl_2^{3+}$; $[M+3H]^{3+}$; calc 1020.80 Da).

The purity of the compound was analyzed by analytical RP HPLC using a Synergi Hydro-RP column, a solvent system composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), a flow of 1 mL/min and a gradient which was kept at 0% A for 5 min and was changed from 0% to 100% A in 15 min. The product **3** was eluted at 12.7 min and was detected at 280 nm.

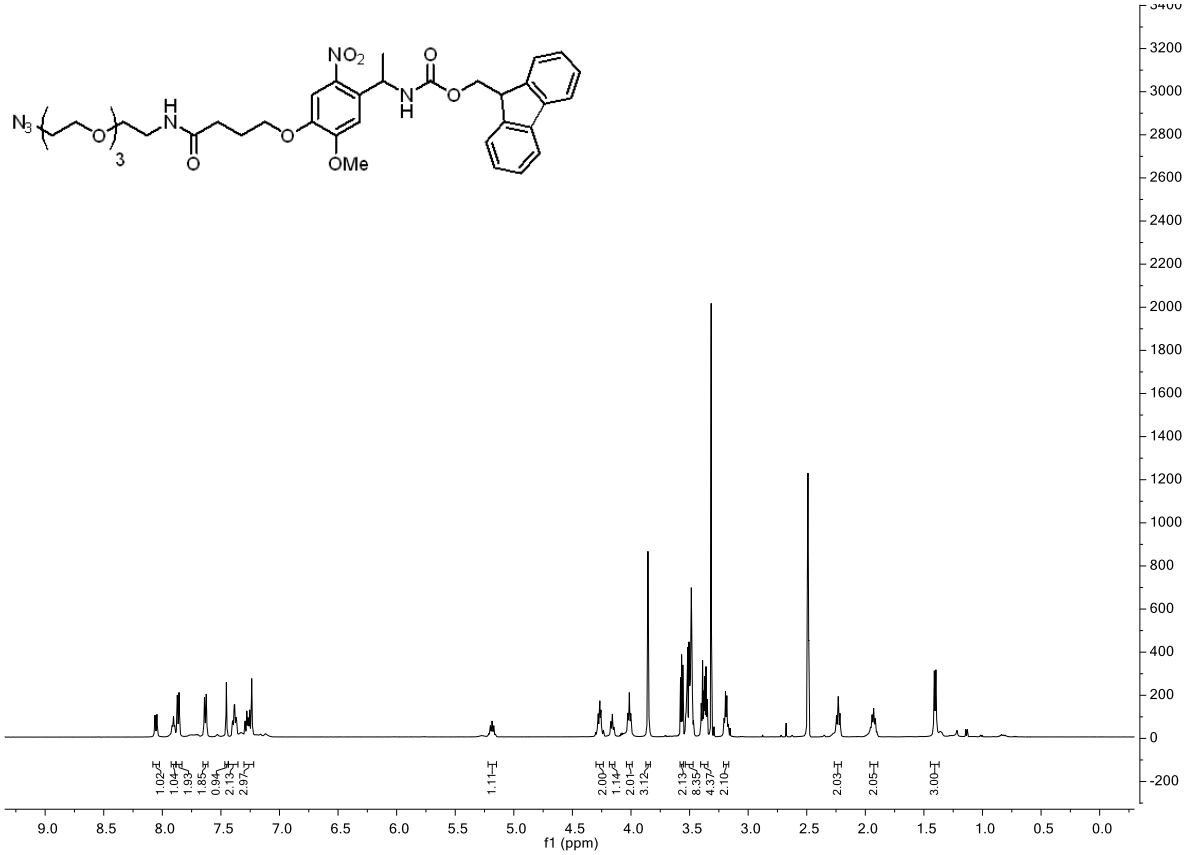
Synthesis of vancomycin derivative with PEG_{100} linker $VancPEG_{100}N_3$ (4)



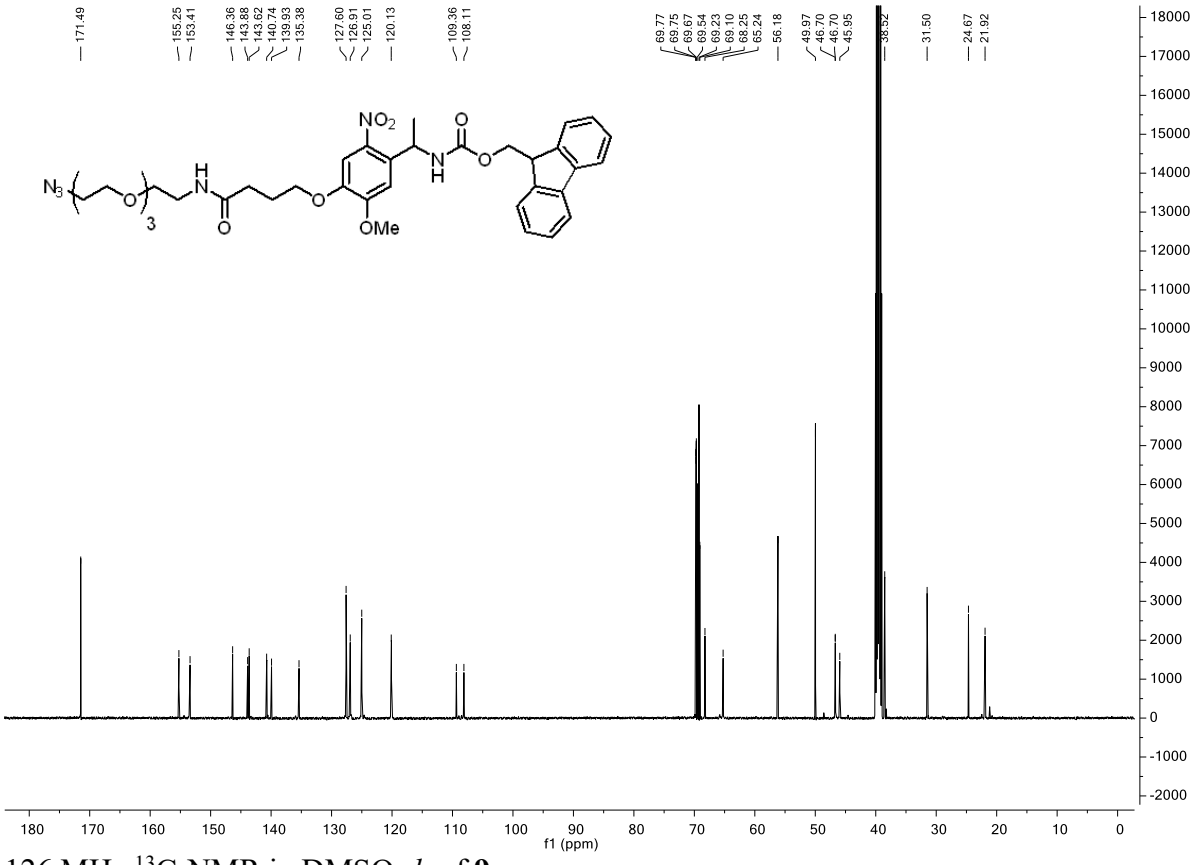
Vancomycin hydrochloride **7** (50 mg, 0.034 mmol) was dissolved in mixture of dry DMSO (0.600 mL) and dry toluene (0.1 mL). To the solution, freshly distilled *N*,*N*-diisopropylethylamine (8 µL, 0.05 mmol) and propylphosphonic anhydride (10 µL, 0.033 mmol) were added following by azide-PEG_{100} amine (MW of PEG average = 5000 Da, 86 mg, 0.0002 mmol). The reaction was stirred for 3h at rt and the solvent was evaporated. The purification was carried out by dialysis at rt. For this purpose, Biotech Grade Dialysis Membranes were used from regenerated cellulose (RC) and MWCO 3.5-5K. The reaction mixture was dissolved in H_2O (0.5 mL) and loaded into a dialysis tubing. A mixture of H_2O:MeCN (1:1, 500 mL, containing 0.1% HCO_2H) was used for the dialysis reservoir. The solvents mixture of the reservoir was renewed after 4 h and 13 h. The solution from the dialysis tubing was transferred into a flask and lyophilized to afford $VancPEG_{100}N_3$ (**4**, 0.730 mg, 65%) as a white solid.

MALDI-MS: *m/z* 5661.6–6718.4 Da.

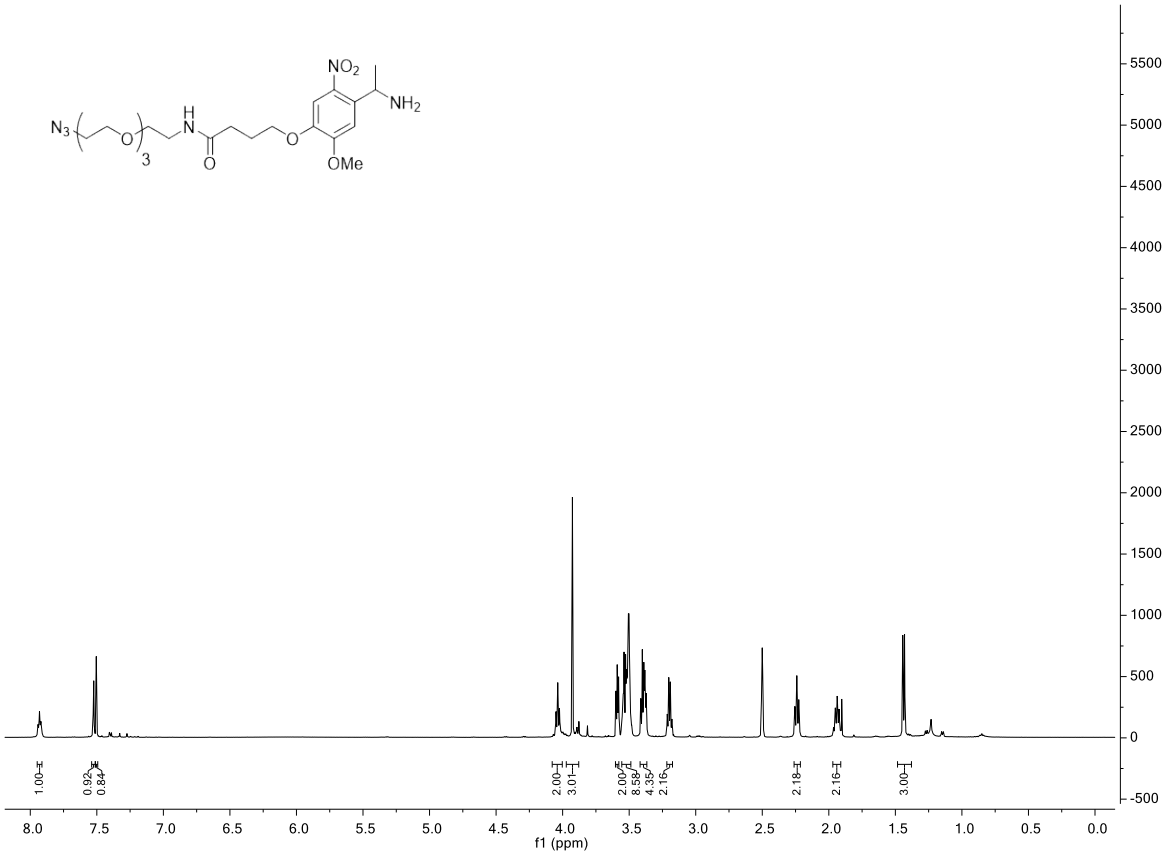
The purity of the compounds were analyzed by analytical RP HPLC using a Synergi Hydro-RP column, a solvent system composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1 HCO_2H), a flow of 1 mL/min and a gradient which was kept at 0% A for 5 min and was changed from 0% to 100% A in 15 min. $VancPEG_{100}N_3$ **4** were eluted between 16.4 to 17.4 min and were detected at 280 nm.



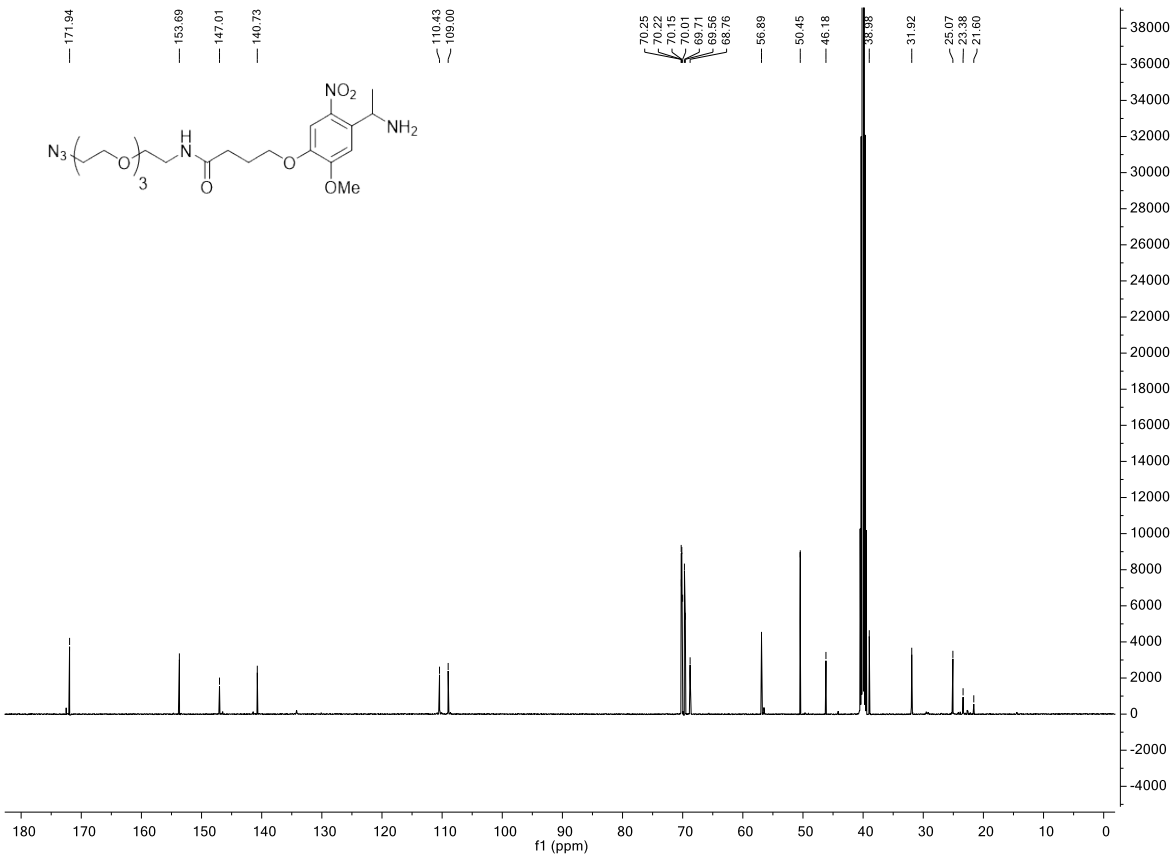
500 MHz ^{1}H-NMR in DMSO-d_6 of **9a**



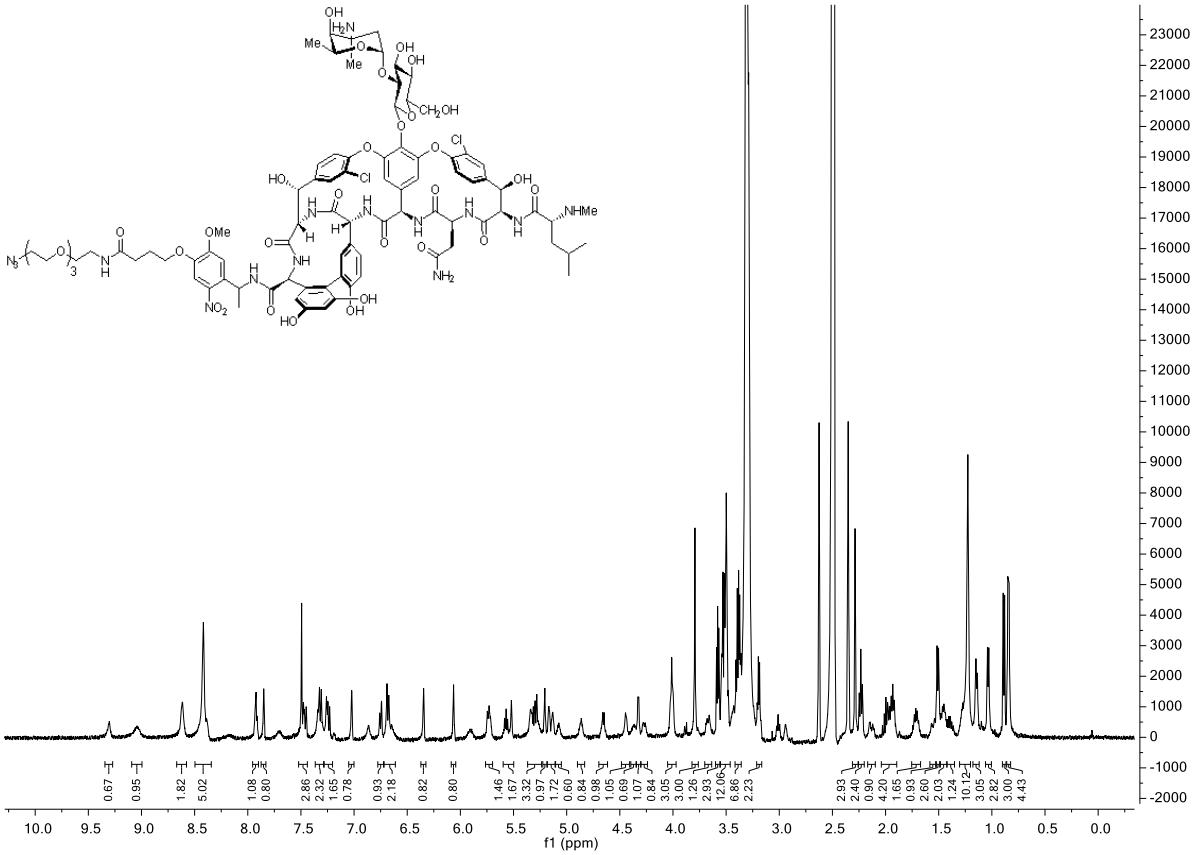
126 MHz ^{13}C-NMR in DMSO-d_6 of **9a**



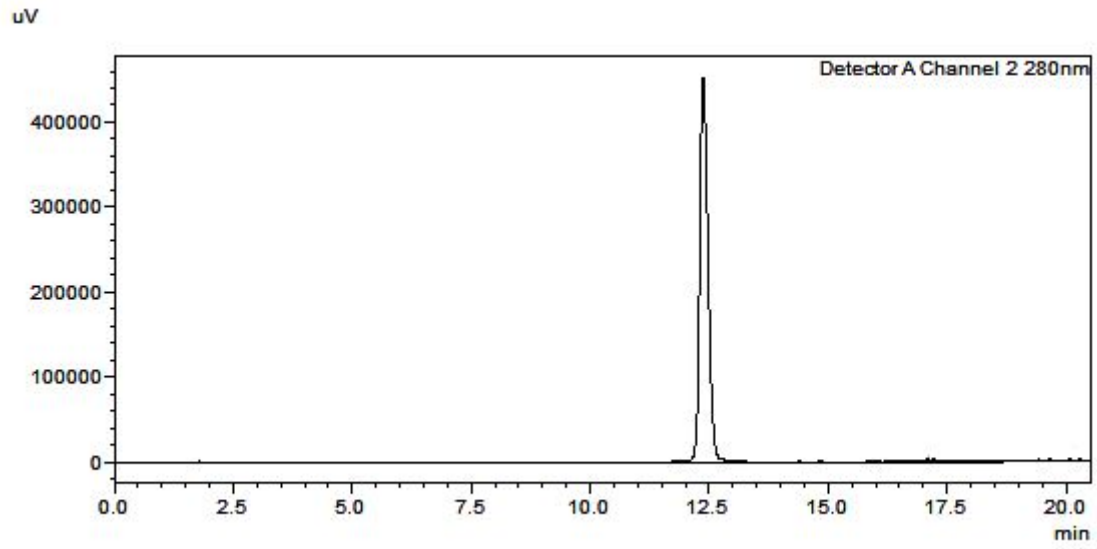
500 MHz ^{1}H-NMR in DMSO-d_6 of **9b**



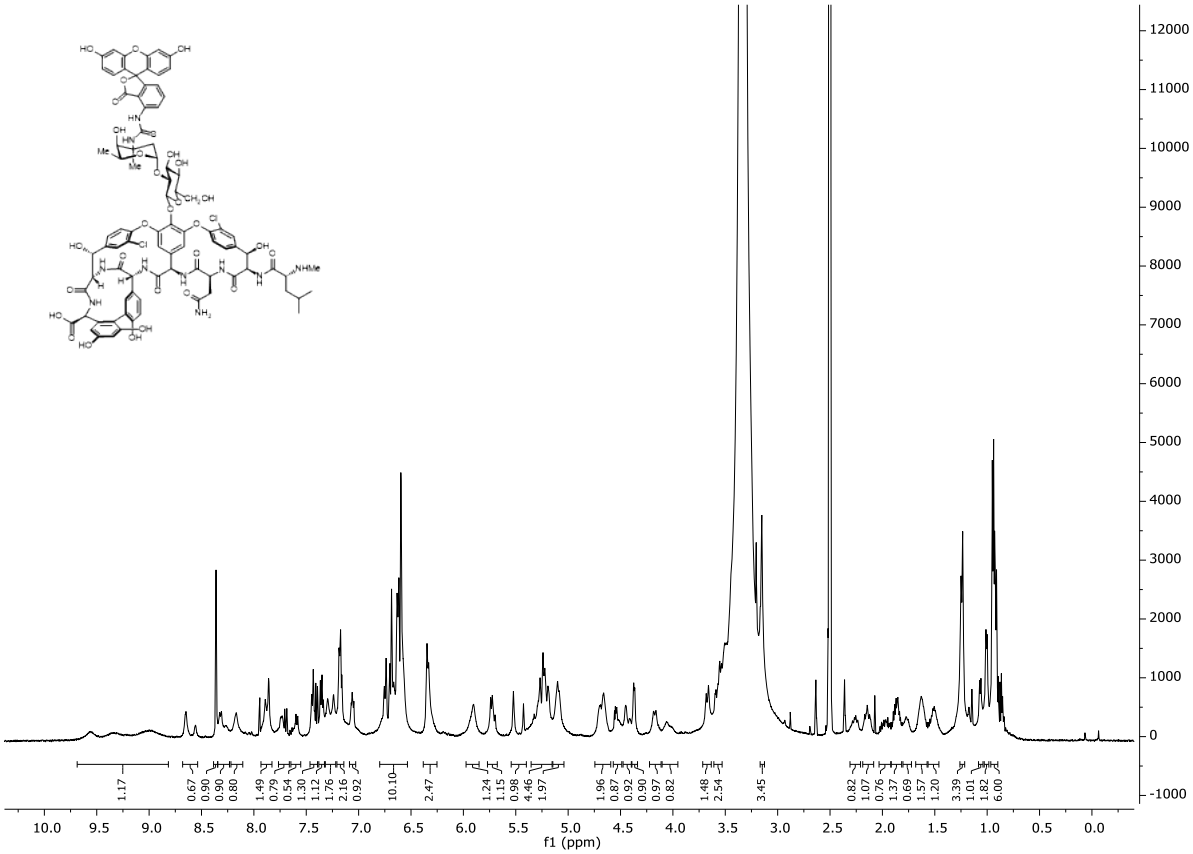
126 MHz ^{13}C-NMR in DMSO-d_6 of **9b**



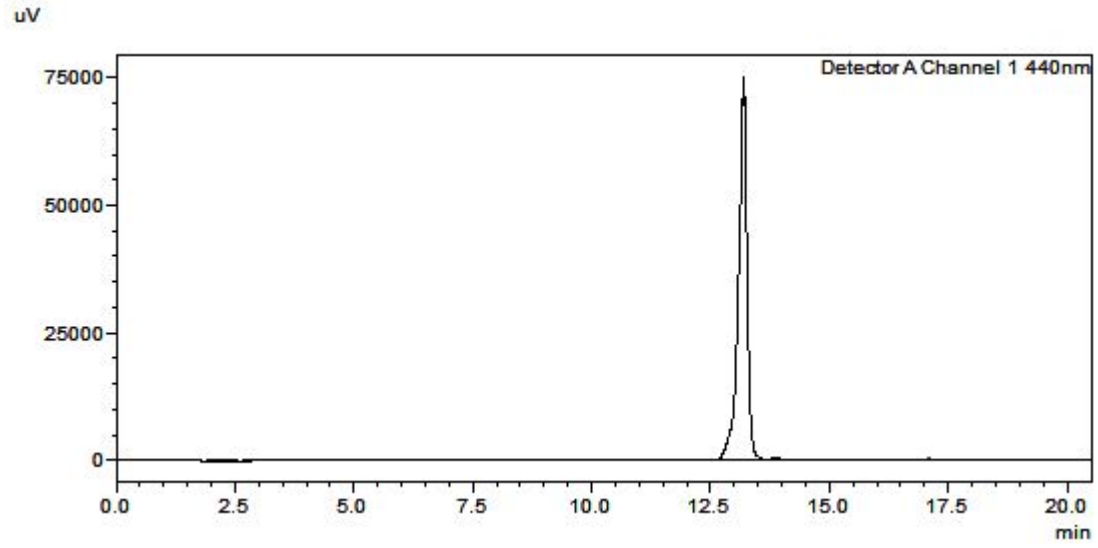
500 MHz ^{1}H-NMR in DMSO-d_6 of VancphotoN$_3$ (**5**)



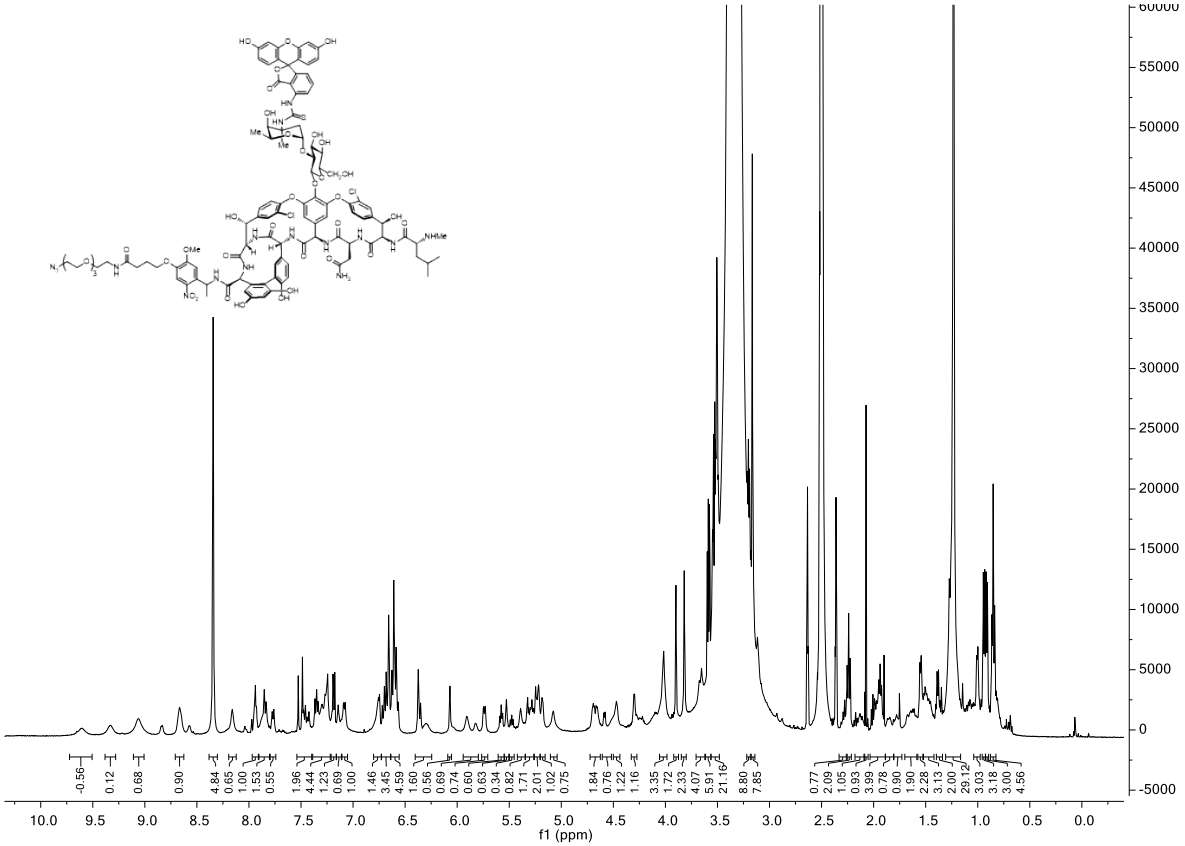
HPLC chromatogram of VancphotoN$_3$ (**5**)



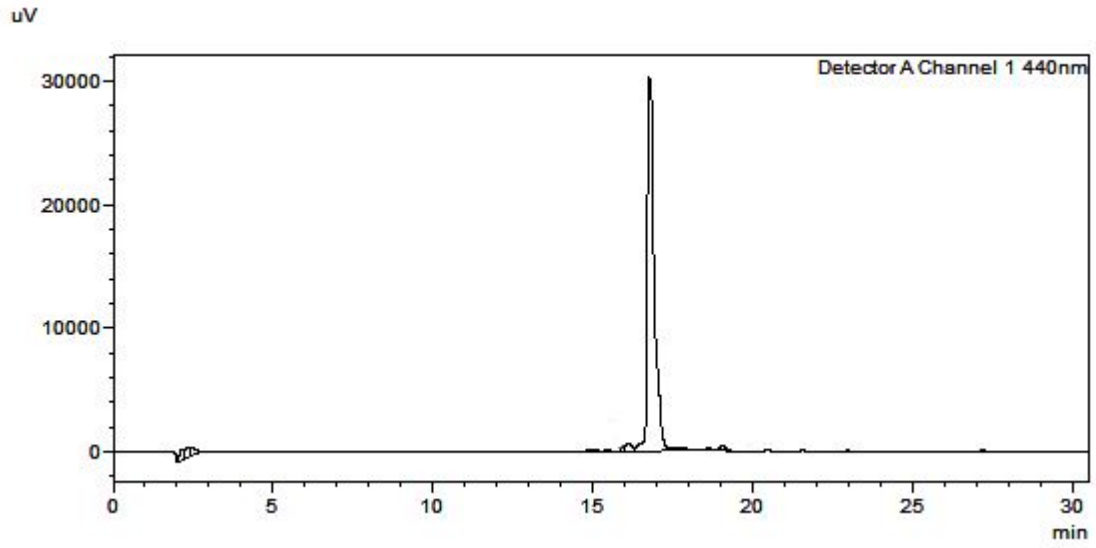
500 MHz ^{1}H-NMR in DMSO-d_6 of VancFITC (**10**)



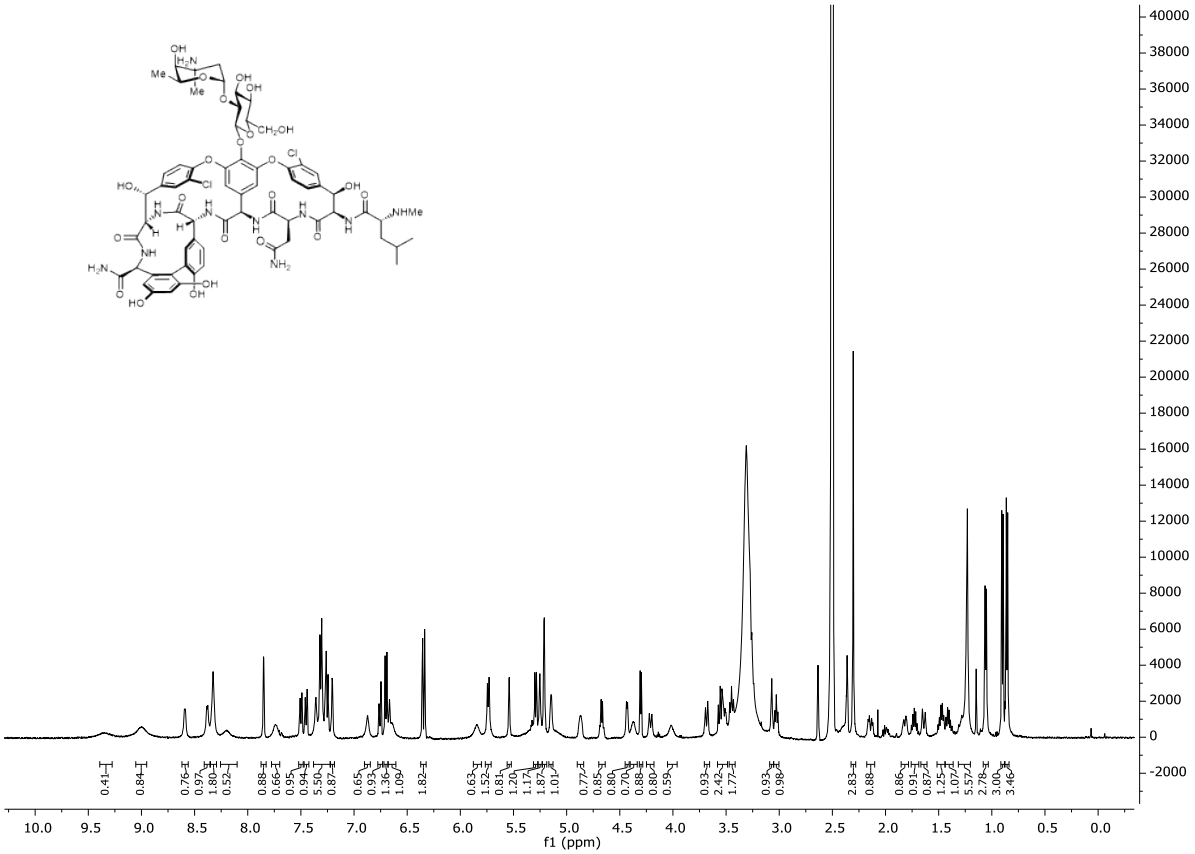
HPLC chromatogram of VancFITC (**10**)



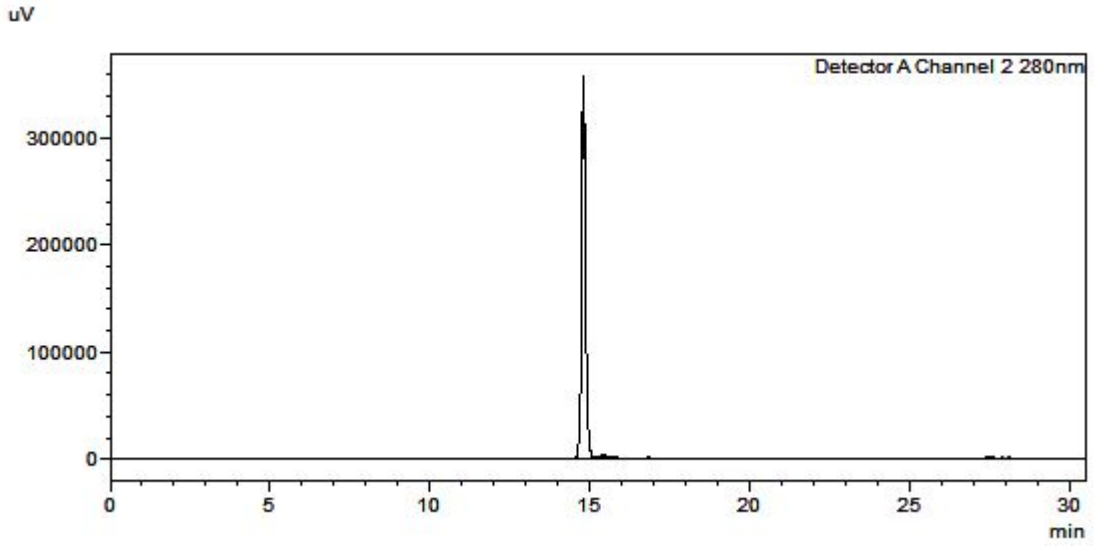
500 MHz ^{1}H-NMR in DMSO-d_6 of VancFITCphotoN$_3$ (**11**)



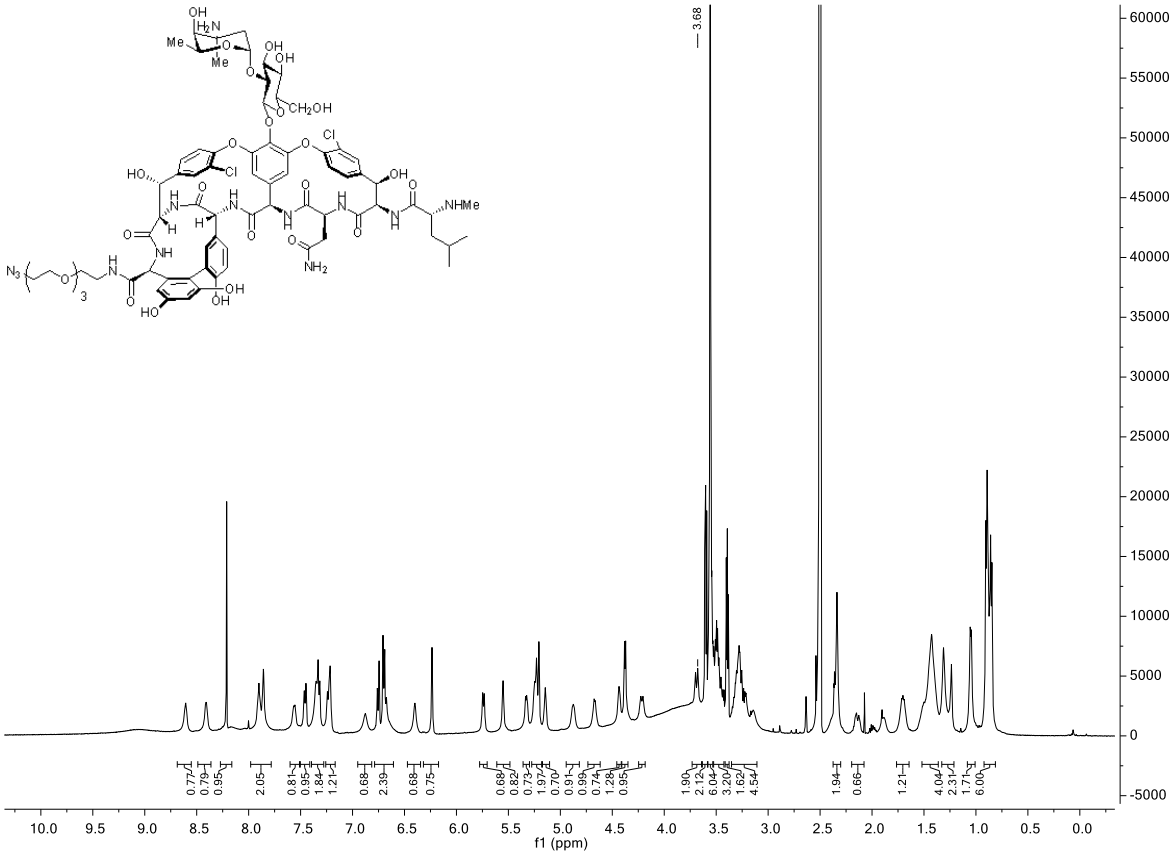
HPLC chromatogram of VancFITCphotoN$_3$ (**11**)



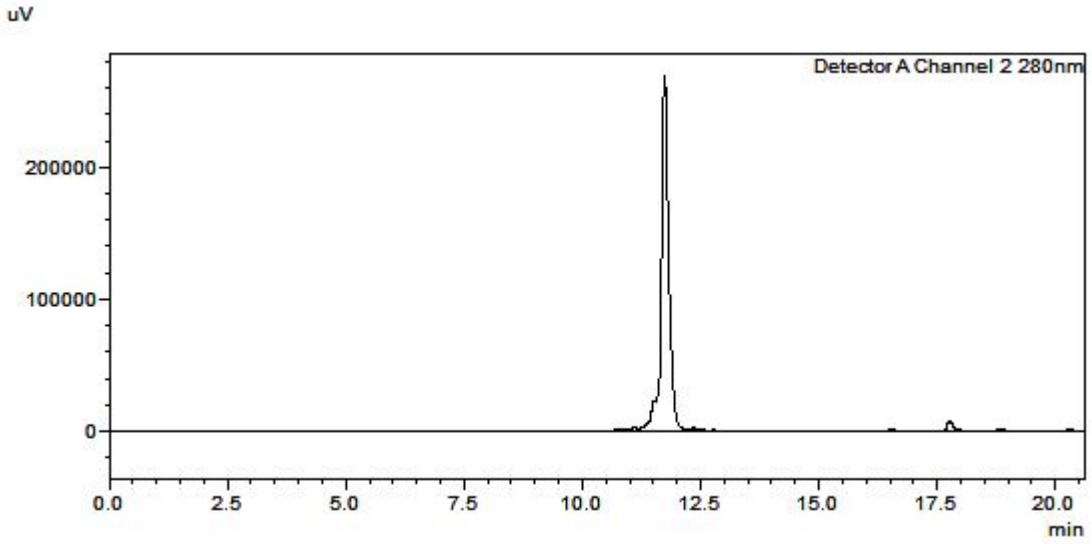
500 MHz ^{1}H-NMR in DMSO-d_6 of VancNH$_2$ (**6**)



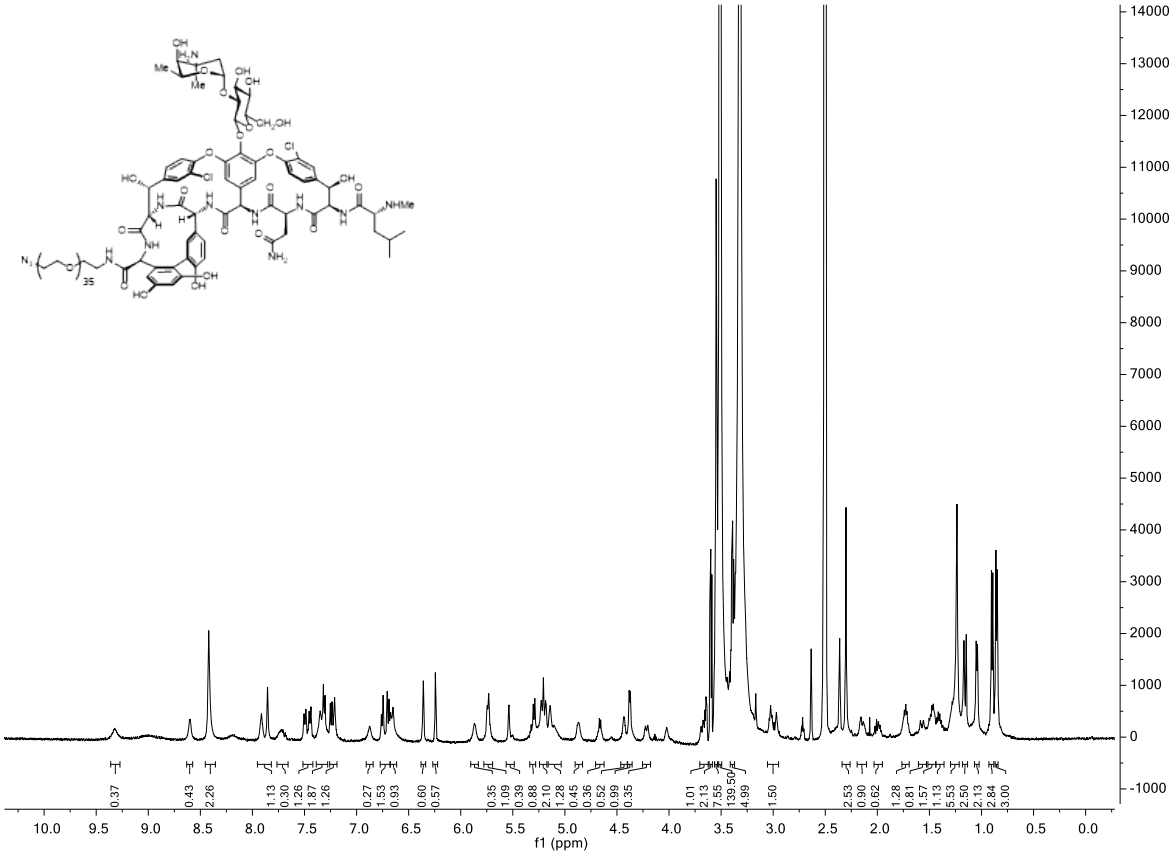
HPLC chromatogram of compound VancNH$_2$ (**6**)



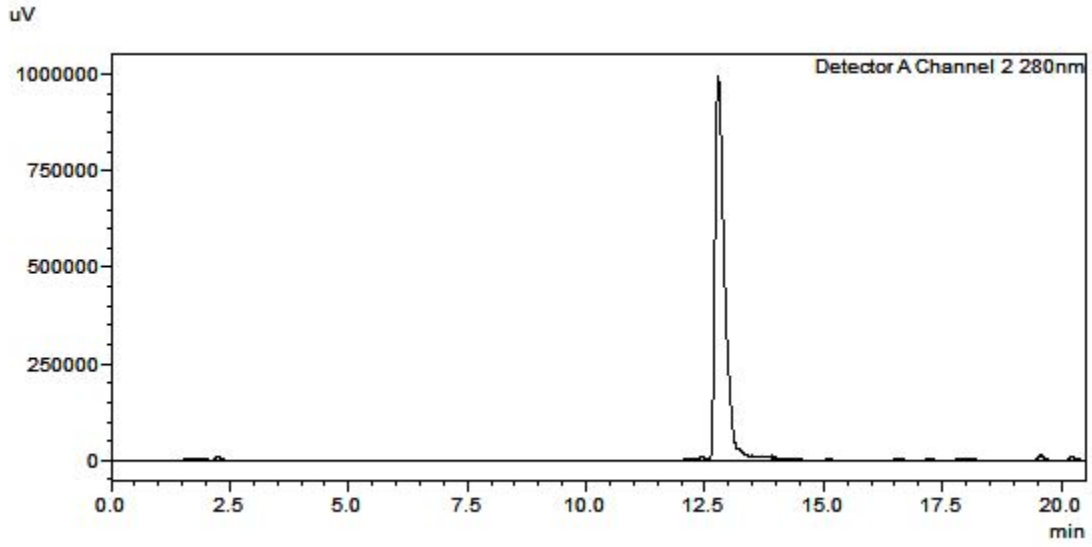
500 MHz ^{1}H-NMR in DMSO-d_6 of VancPEG$_3$N$_3$ (**2**)



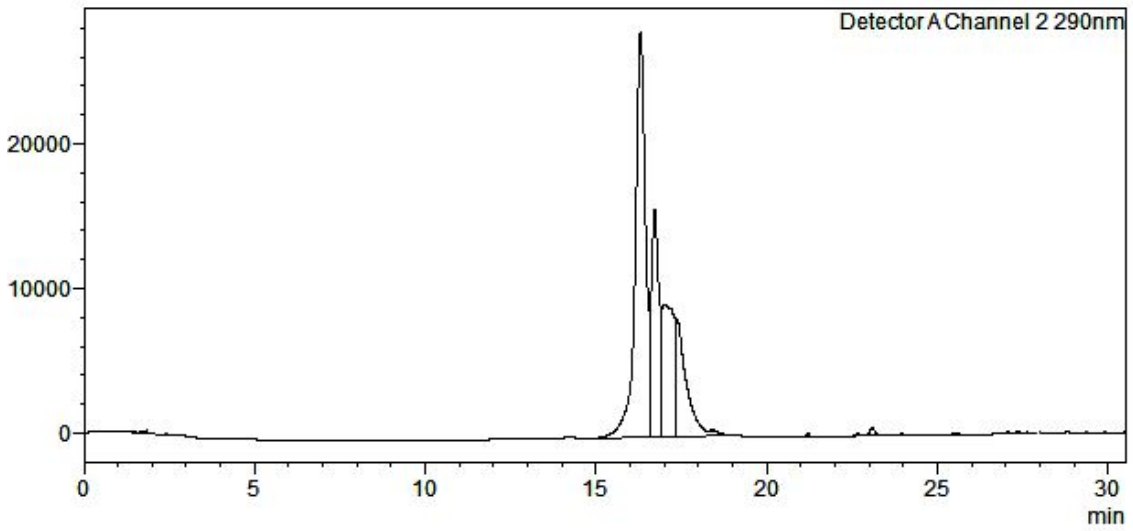
HPLC chromatogram of VancPEG$_3$N$_3$ (**2**)



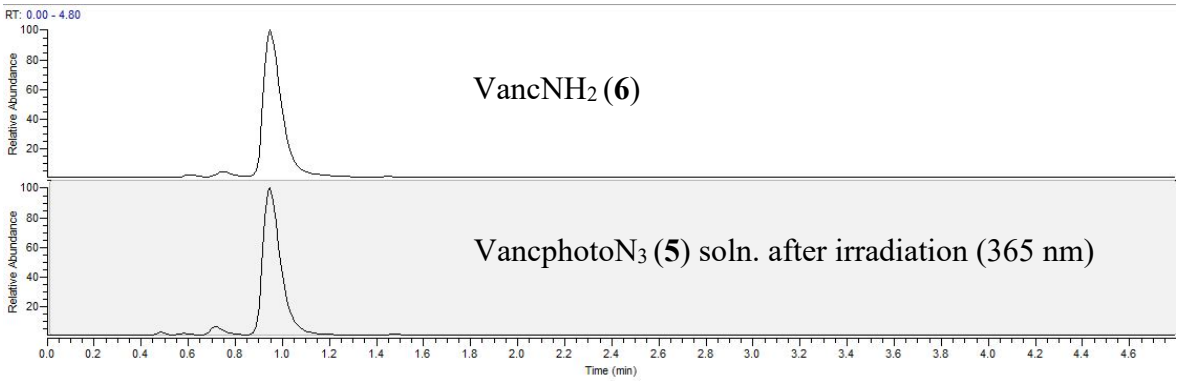
500 MHz ^{1}H-NMR in DMSO-d_6 of VancPEG$_{35}$N$_3$ (**3**)



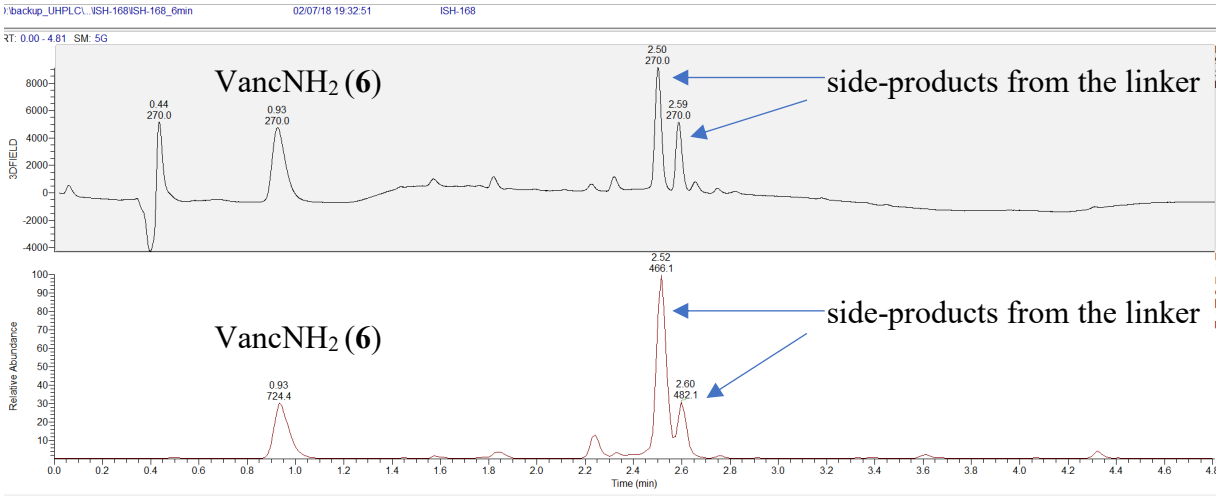
HPLC chromatogram of VancPEG$_{35}$N$_3$ (**3**)



HPLC chromatogram of compound VancPEG$_{100}$N$_3$ (**4**)



UHPLC-MS chromatograms comparison recorded in SIM mode of VancNH$_2$ (**6**). From top to bottom: synthetic VancNH$_2$ (**6**) and product after irradiation of the VancphotoN$_3$ (**5**) solution.



UHPLC-UV chromatogram (top) and UHPLC-MS chromatogram (bottom) of the reaction products of a VancphotoN$_3$ (0.01 mM) solution treated for 5 min at 365 nm.

3. Surface modification of *Chlamydomonas reinhardtii*

3.1 Cultivation of *Chlamydomonas reinhardtii*

Chlamydomonas reinhardtii (*C. reinhardtii*, 11-32b) was purchased from EPSAG (Experimentelle Phykologie und Sammlung von Algenkulturen der Universität Göttingen) and grown in Kuhl medium (1011.1 mg L^{-1} KNO_3, 621 mg L^{-1} $NaH_2PO_4*H_2O$, 89 mg L^{-1} $Na_2HPO_4*2H_2O$, 246.5 mg L^{-1} $MgSO_4*7H_2O$, 14.7 mg L^{-1} $CaCl_2*2H_2O$, 6.95 mg L^{-1} $FeSO_4*7H_2O$ (Fe-EDTA complex), 1 mL L^{-1} micronutrient solution (61.0 mg L^{-1} H_3BO_3, 169.0 mg L^{-1} $MnSO_4*H_2O$, 287.0 mg L^{-1} $ZnSO_4*7H_2O$ mg L^{-1}, 2.5 mg L^{-1} $CuSO_4*5H_2O$, 12.5 mg L^{-1} $(NH_4)_6Mo_7O_{24}*4H_2O$))[1] at 20°C under medium light.

3.2 General protocol for the surface modification of *C. reinhardtii*

The cell surface modification was carried out in two steps, the general protocol is described below.

Algae surface functionalization with dibenzocyclooctyne (1st step) :

An algae culture (1.2 mL) with a cell density $2.5x10^6$ cells/ml was centrifuged (5 min, 1500 rcf/min) and the supernatant was removed. The algae cells were washed with PBS (1 mL, pH = 7.4), centrifuged (5 min, 1000 rcf/min) and the supernatant was removed, this step was repeated 2 times. The cells were resuspended in a NHS-PEG_4-dibenzocyclooctyne (DBCO, **1**) solution (PBS, 0.250 mL). The mixture was shaken for 1h, the samples were centrifuged (5 min, 1000 rcf/min) and the supernatant was removed. The pellet was washed with PBS (1 mL, pH = 7.4), centrifuged (5 min, 1000 rcf/min) and the supernatant was removed, this step was repeated 2 times.

Attachment of the antibiotic derivatives (2nd step):

The modified algae were resuspended in a solution of a vancomycin azide derivatives (PBS, 0.250 mL, with 0.002% Tween 80). The mixture was incubated for 1 h, the samples were centrifuged (5 min, 1000 rcf/min), the supernatant was removed, the samples were washed (PBS, 1 mL, pH = 7.4), centrifuged (5 min, 1000 rcf/min) and the supernatant was removed. The washing step was repeated 5 times.

4. Confocal fluorescent microscopy

The functionalization steps of the algae surface were investigated using a fluorescent vancomycin derivative by confocal microscopy. The cells were modified by the procedure previously described above (**section 3.2**).
The conditions for the cells functionalization were:
DBCO **(1)** concentration: 0.30 mM
VancFITCphotoN$_3$ (**11**) concentration: 0.20 mM

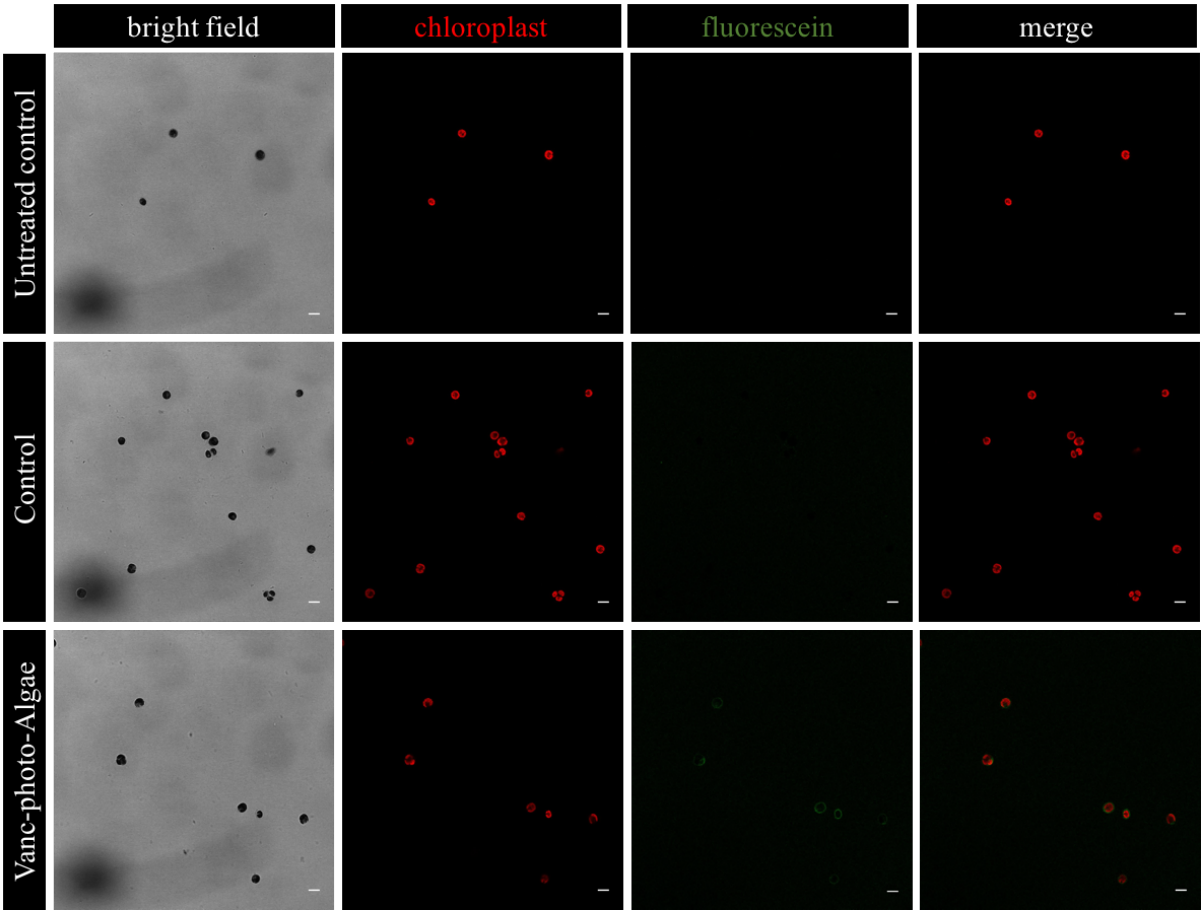


Figure S**1.** *C. reinhardtii* after modification with the VancFITCphotoN$_3$ (**11**). The scale bars are 10 µm.

5. Photolysis experiments

The products formation during the UV-irradiation step were investigated as well as the influence of algae on this process.

5.1. Quantification protocol of $VancNH_2$ (6) and $VancphotoN_3$ (5)

Stock solutions (1 mM) of vancomycin derivatives ($VancphotoN_3$ (**5**) and $VancNH_2$ (**6**)) were prepared in PBS (pH=7.4) and diluted to obtain several solutions (1 – 20 µM). These solutions were used for the experiments described below and for building the calibration curve. All stock solutions were freshly prepared.

Calibration curves were built by plotting linear regression of the mass intensity versus the concentration of the standard. From these curves the coefficients of correlation (R^2) and slope were calculated (Figures S**4**, S**6**, S**8**, S**9**).

5.2. Photolysis experiment of $VancphotoN_3$

$VancphotoN_3$ (**5**) was dissolved in PBS buffer (0.5 mL, 10 µM, pH = 7.4) and loaded onto a 24-wells plate. The solutions were irradiated with a wavelength at 365 nm. A sample (0.5 mL) was taken after different time of irradiation. The reaction was filtered and analyzed by UHPLC-MS.

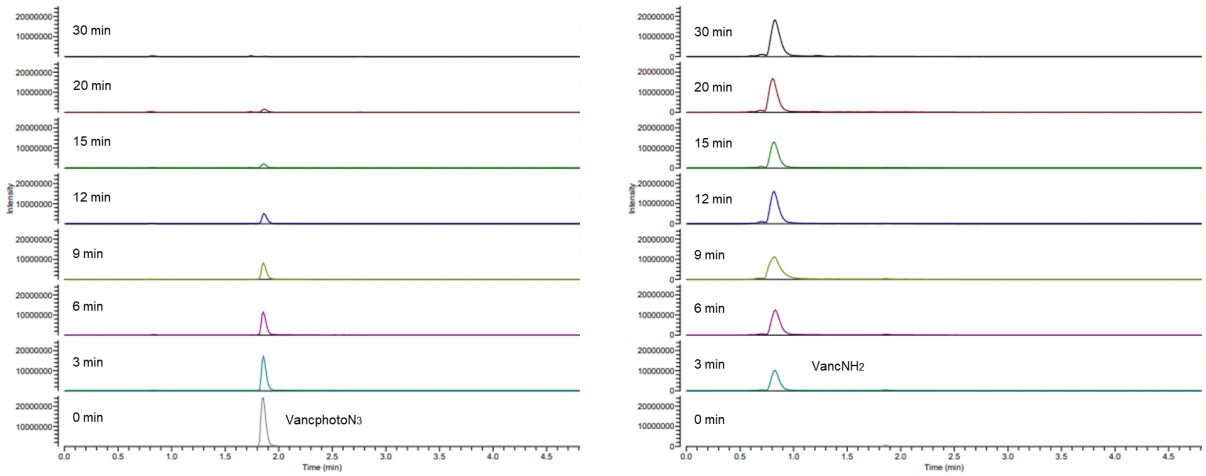


Figure 2. UHPLC-MS chromatograms overlay (left: SIM at 965.4 Da, *m/z* of **5**; right: SIM at 725.2, *m/z* of **6**) of the $VancphotoN_3$ (**5**) solutions (PBS, 0.5 mM) irradiated during different time (t = 0, 3, 6, 9, 12, 15, 20, 30min).

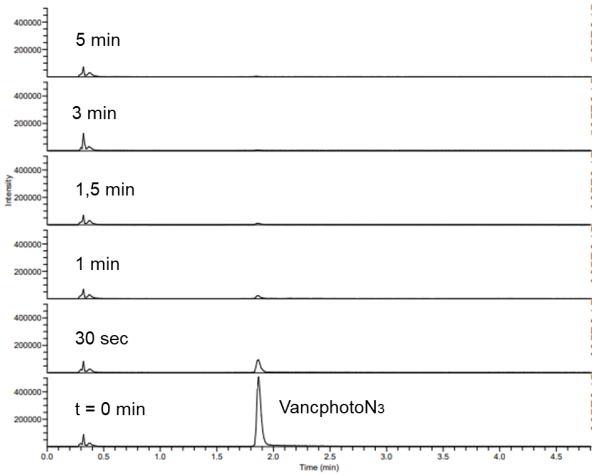


Figure S**3**. UHPLC-MS chromatograms overlay (SIM at 965.4 Da, *m/z* of **5**) of the VancphotoN$_3$ (**5**) solutions (PBS, 0.01 mM) irradiated during different time (t = 0, 0.5, 1, 1,5, 2, 3, 5, 7 min).

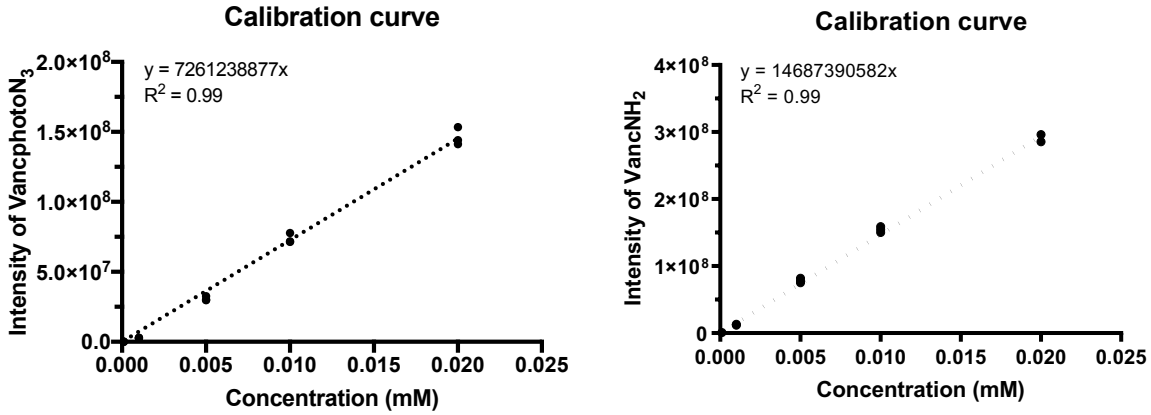


Figure S**4.** Calibration curves for VancphotoN$_3$ (**5**) on the left and for VancNH$_2$ **(6)** on the right. Data points represent mean value ± SD (n = 3).

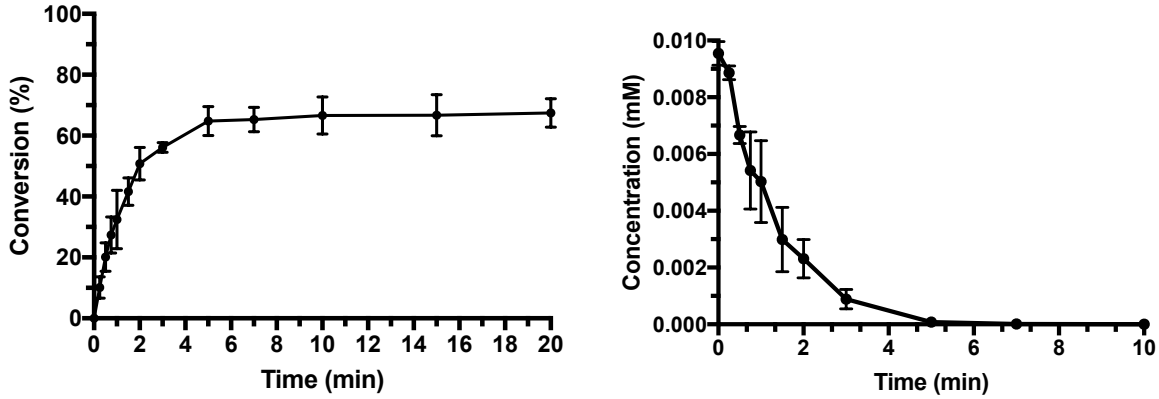


Figure S**5.** Left: Analysis of VancphotoN$_3$ (**5**) conversion into VancNH$_2$ (**6**) after the samples irradiation at a wavelength of 365 nm during different time. Right: Analysis of VancphotoN$_3$ (**5**) concentration after the samples irradiation at a wavelength of 365 nm during different time. Data points represent mean value ± SD (n = 3).

5.3. Photolysis experiment of VancphotoN$_3$ in presence of C. reinhardtii

An algae culture (1.2 mL) with a cell density of 2.5*10^6 cells/ml was centrifuged (5 min, 1500 rcf/min) and the supernatant was removed. The algae were washed with PBS (1 mL, pH = 7.4), centrifuged (5 min, 1000 rcf/min) and the supernatant was removed. This step was repeated 2 times. The algae were resuspended in a VancphotoN$_3$ (**5**) solution (0.5 mL, 10 µM, PBS, pH = 7.4). The mixtures were loaded onto a 24-wells plate and irradiated at a wavelength of 365 nm. The progress of the photolysis reaction was monitored by UHPLC-MS. Part of the sample (0.5 mL) was taken at a specific time point after 0; 0.25; 0.5; 0.75; 1; 1.5; 2; 3; 5; 7; 15; 20 min of irradiation. The samples were centrifuged, filtered and analysed.

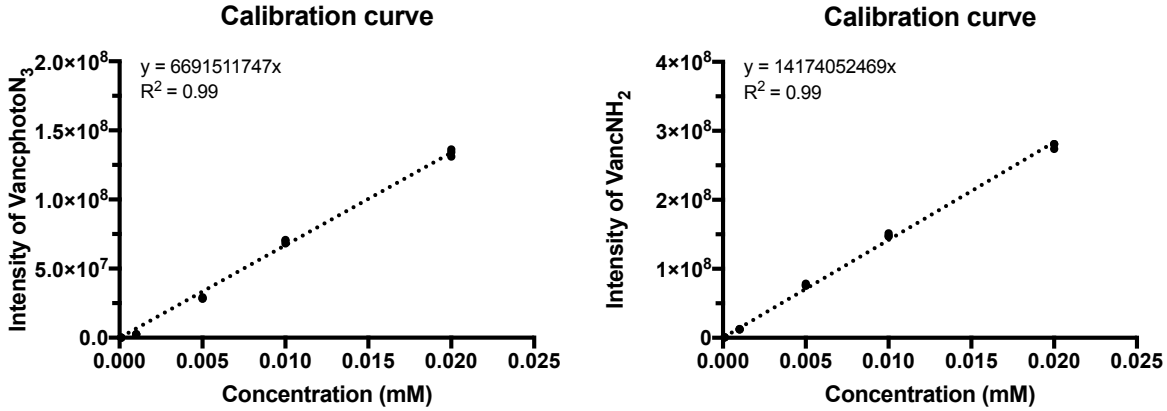


Figure S**6.** Calibration curves for VancphotoN$_3$ (**5**) on the left and for VancNH$_2$ (**6**) on the right. Data points represent mean value ± SD (n = 3).

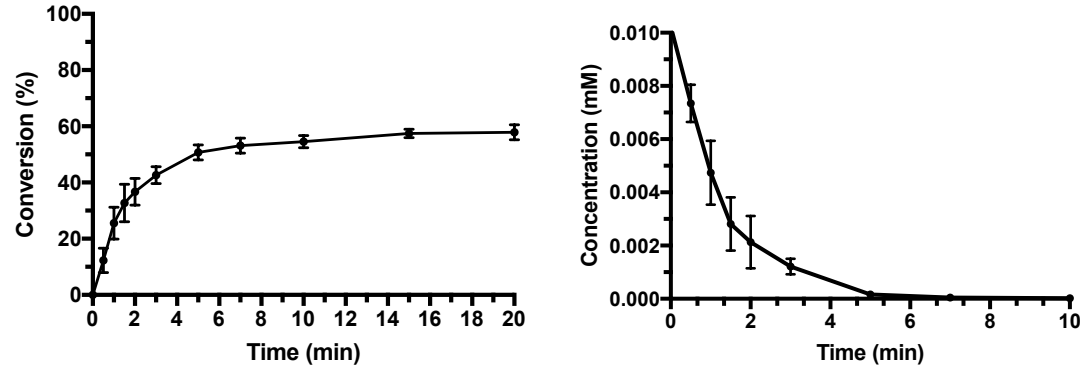


Figure S**7.** Analysis of VancphotoN$_3$ (**5**) conversion into VancNH$_2$ (**6**) after irradiated the samples at a wavelength of 365 nm during different time. Right: Analysis of VancphotoN$_3$ (**5**) concentration after irradiated the samples at a wavelength of 365 nm during different time. Data points represent mean value ± SD (n = 3).

5.4. Optimization of DBCO (1) and VancphotoN$_3$ (5) concentration

The concentration of DBCO (**1**) and VancphotoN$_3$ (**5**) were optimized by evaluating the percentage of unreactive VancphotoN$_3$ (**5**) after the 2nd step of *C. reinhardtii* surface functionalization. The algae were modified by the procedure previously described (**section 3.2**). After the 2nd modification step using VancphotoN$_3$ (**5**), the cells were centrifuged (5 min, 1000 rcf/min), the supernatant was removed and the cells were washed with PBS (1 mL, pH = 7.4), centrifuged (5 min, 1000 rcf/min) and the supernatant was removed. The washing step was repeated 5 times. The supernatants after the first centrifugation and after each washing step were collected, combined, filtered and analyzed by UHPLC-MS.

Table S**1**: Concentrations of DBCO **(1)** and VancphotoN$_3$ (**5**) used for *C. reinhardtii* surface functionalization

experiment	Incubation step	Concentration (mM)	
		DBCO (**1**)	VancphotoN$_3$ (**5**)
1	1st	0.05	
	2nd		0.01
2	1st	0.1	
	2nd		0.005
3	1st	0.2	
	2nd		0.01

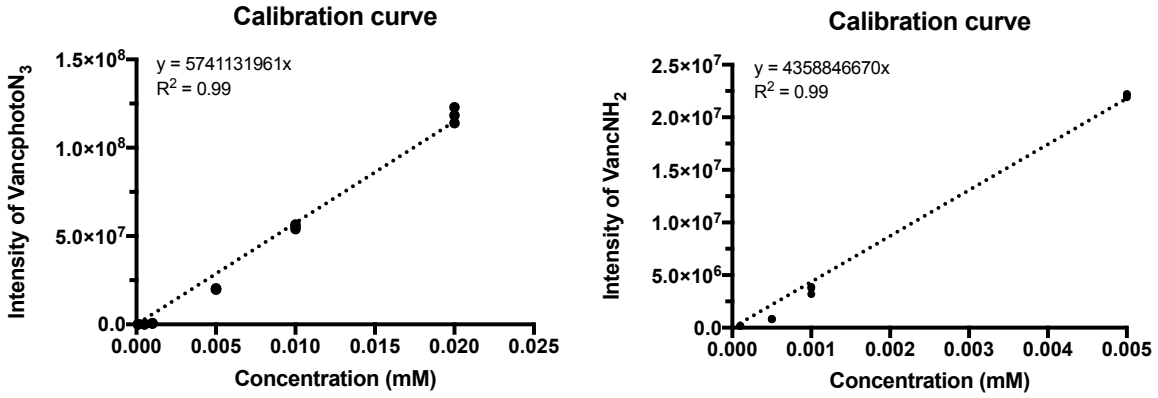


Figure S**8.** Calibration curves for VancphotoN$_3$ (**5**) on the left and for VancNH$_2$ (**6**) on the right for the first experiment (Table S**1**). Data points represent mean value ± SD (n = 3). Data points represent mean value ± SD (n = 3).

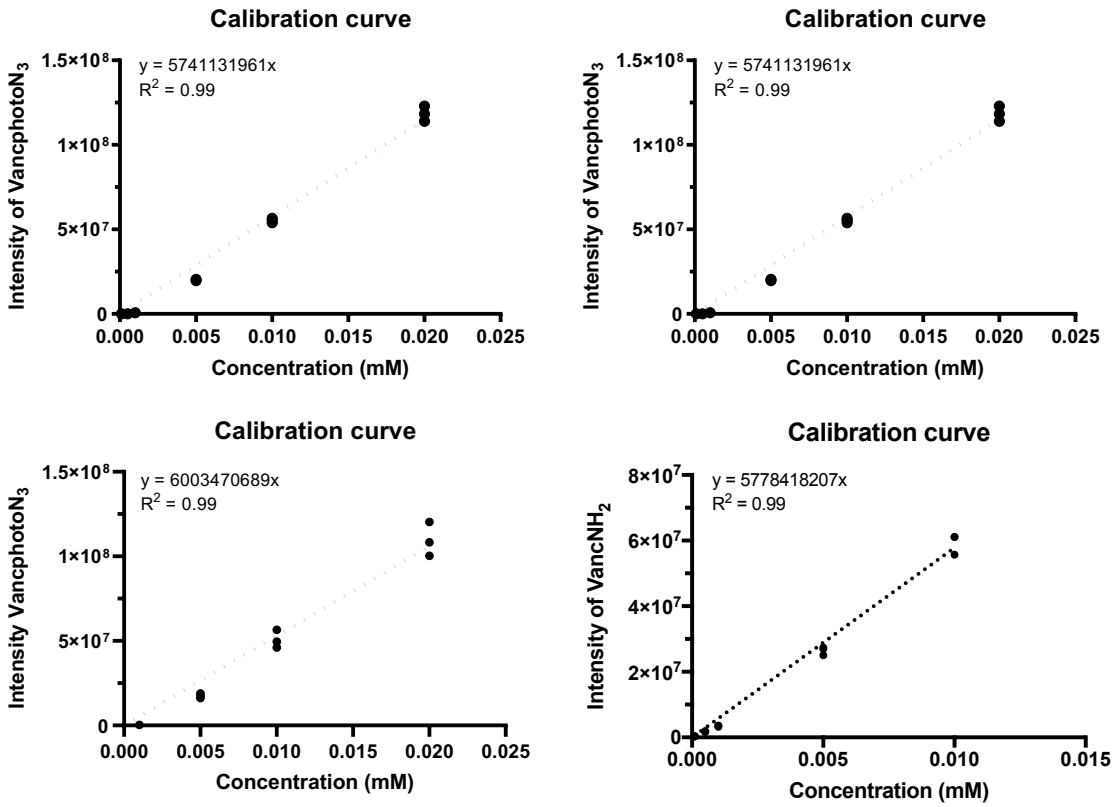


Figure S**9.** Calibration curves for VancphotoN$_3$ (**5**) on the left and for VancNH$_2$ (**6**) on the right for the second and third experiment (Table S**1**). Data points represent mean value ± SD (n = 3).. Data points represent mean value ± SD (n = 3)

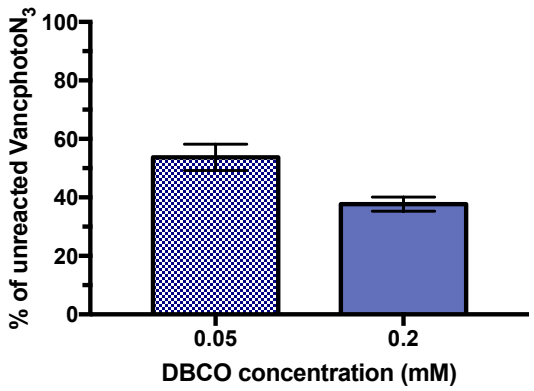


Figure S**10.** Percentage of unreacted VancphotoN$_3$ (**5**) using a DBCO (**1**) concentration of 0.05 mM and 0.2 mM. The initial concentration of VancphotoN$_3$ **(5)** was 0.01 mM. Data are represented mean value ± SD (n = 6).

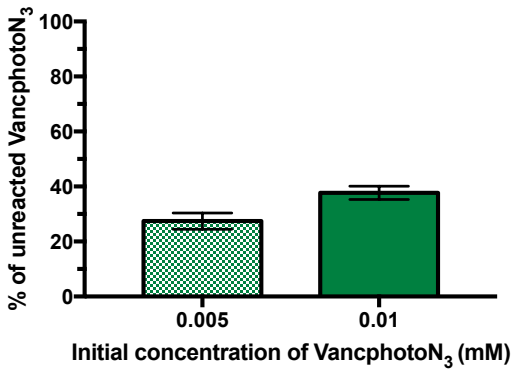


Figure S**11.** Percentage of unreacted VancphotoN$_3$ (**5**) using a VancphotoN$_3$ (**5**) concentration of 0.005 mM and 0.01 mM. The concentration of DBCO (**1**) was 0.1 mM with a concentration of **5** - 0.005 mM and 0.2 mM with a concentration of **5** - 0.01 mM . Data are represented mean value ± SD (n = 6).

5.5. Optimization of the reaction conditions for the release of $VancNH_2$ (6) from C. reinhardtii surface

The released concentration of $VancNH_2$ (**6**) from the functionalized surface of *C. reinhardtii* was determined by using the calibration curves described in the experimental part **5.4**. The amount of reacted $VancphotoN_3$ (**5**) was estimated by the procedure described in the experimental part **5.4**. The released $VancNH_2$ (**6**) was calculated according to the following equation:

$$Released\ VancNH_2\ (\%) = \frac{n_{VancNH_2}}{(n_{VancphotoN_3} - n'_{VancphotoN_3})} \times 100\%$$

Where n_{VancNH_2} is the released amount of $VancNH_2$ (**6**) after irradiation; $n_{VancphotoN_3}$ is the amount of $VancphotoN_3$ (**5**) used for the 2nd step of *C. reinhardtii* modification; $n'_{VancphotoN_3}$ is the amount of unreacted $VancphotoN_3$ (**5**) determined by the procedure in the part **5.4**.

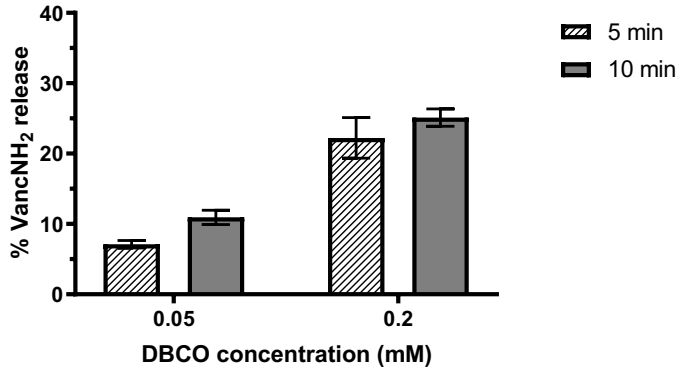


Figure S**12**. Percentage of $VancNH_2$ (**6**) released from the algae surface after 5 or 10 min of irradiation (365 nm). The concentration of $VancphotoN_3$ (**5**) was 0.01 mM and DBCO (**1**) were 0.05 mM (left columns) and 0.2 mM (right columns). Data are represented mean value ± SD (n = 3).

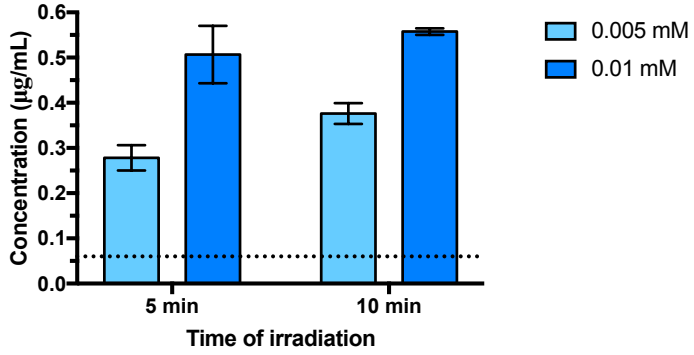


Figure S**13.** Concentration (µg/mL) of $VancNH_2$ (**6**) released from the algae surface after 5 min (left part of the graph) or 10 min (right part of the graph) of UV-irradiation at 365 nm. The concentrations of $VancphotoN_3$ (**5**) were 0.005 mM (light blue bar) and 0.01 mM (dark blue bar). The dot line corresponds to MIC results for $VancNH_2$ (**6**). Data are represented mean value ± SD (n = 3).

5.6. Effect of second irradiation step on the release of VancNH₂ (6)

The functionalized algae that have been already irradiated for 5 or 10 min (section **5.5**) were suspended in PBS (1 mL, pH= 7.4) and transferred into 12-wells plate. The samples were again irradiated for 5 or 10 min. The cells were transferred into Eppendorf tubes, centrifuged (5 min, 1000 rcf/min) and the supernatant was removed. The solutions were filtered and analysed by UHPLC-MS. The data after the first and second irradiation are represented in the graph below (figure S**14**).

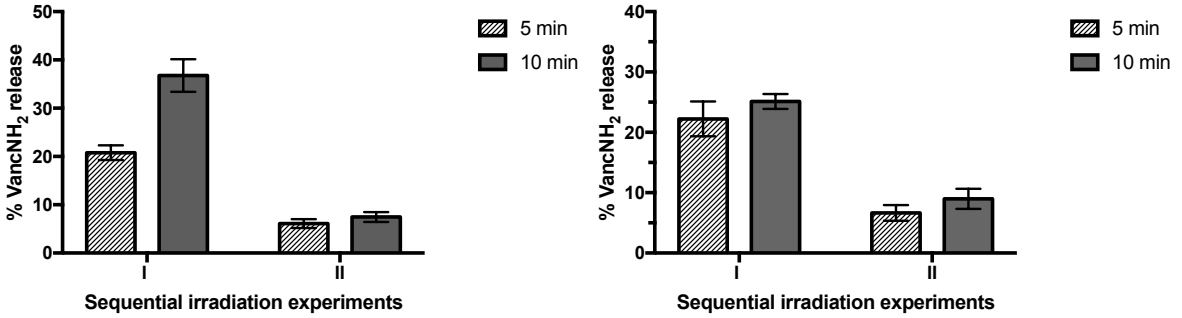


Figure S**14.** Percentage of $VancNH_2$ (**6**) released from the algae surface during the first irradiation step (I, left part of each graph using 5 or 10 min irradiation) and during the second irradiation step (II, right part of each graph using 5 or 10 min irradiation). The concentration of $VancphotoN_3$ (**5**) - 0.005 mM, DBCO **(1)** - 0.01 mM for the left graph and (**5**) - 0.01 mM, (**1**) – 0.2 mM for the right graph. Data are represented mean value ± SD (n = 3).

6. *C. reinhardtii* viability

The effect on *C. reinhardtii* cell viability was determined for the irradiation step and the surface functionalization procedure (section **3.2**) by flow cytometry. The cell pellet was suspended in Kuhl medium (0.5 mL) and strained through a 40 µm mesh to remove clustered cells. Freshly prepared solution of propidium iodide in DMSO (1.5 µL, 2.5 µM) was added to the sample prior to analysis.

Preparation of controls:

Positive control: An algae culture (2.5 mL, 2.5×10^6 cells/ml) was centrifuged (5 min, 1500 rcf/min) and the cells were subjected to the same general procedure **(section 3.2)**, however, neither DBCO **(1)** nor vancomycin derivatives were added during the incubation steps. The cells were washed the same way as the samples.

Negative control: The algae culture (2.5 mL, 2.5×10^6 cells/ml) was heated to 90°C for 15 min. In the gated autofluorescence, the reduction of autofluorescence compared to the living cells was observed.

6.1. Influence of the duration of the irradiation step on C. reinhardtii viability

C. reinhardtii culture (2.5 mL, 2.5×10^6 cells/ml) was centrifuged (5 min, 1500 rcf/min) and the supernatant was removed. The algae cells were washed with Kuhl Medium (1 mL), centrifuged (5 min, 1000 rcf/min) and the supernatant was removed. The washing step was repeated 2 times. The cells were resuspended in Kuhl Medium (1 mL), replaced onto the 12-wells plate, and irradiated at a wavelength of 365 nm for 5 min, 10 min or 15 min. The mixture was divided into two halves and prepared for the analysis according the flow cytometry protocol described above. First half was analysed immediately after irradiation and second one after 24 h.

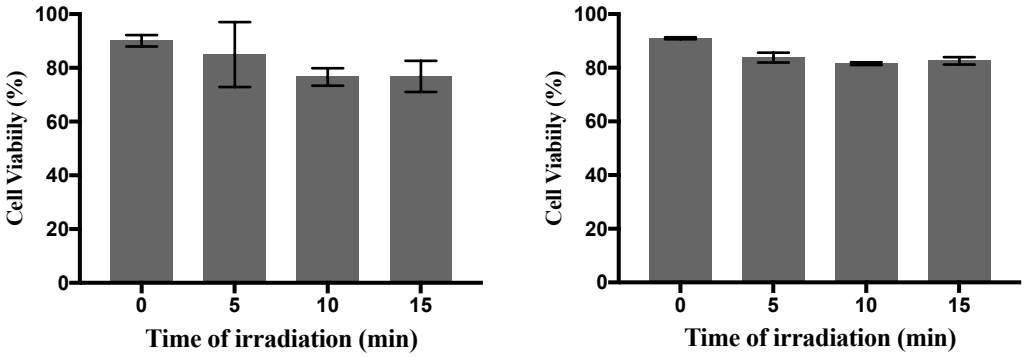


Figure S**15**. *C. reinhardtii* cells viability was analyzed after 0, 5, 10 and 15 min irradiation at a wavelength of 365 nm. The analyses were carried out just after the irradiation step (left graph) and after 24 h (right graph). Data are represented mean value ± SD (n = 3).

6.2. Influence of the algae modification procedure on C. reinhardtii viability

C. reinhardtii cells viability was determined by propidium iodide incubation and flow-cytometry experiments after the two steps functionalization procedure described in the section **3.2**. and after the 5 min irradiation step described in the section **5**.

The conditions used during the two steps functionalization are described below:
DBCO (**1**) concentration: 0.1 mM, V= 0.250 mL
VancphotoN$_3$ (**5**) concentration: 0.005 mM, V= 0.250 mL

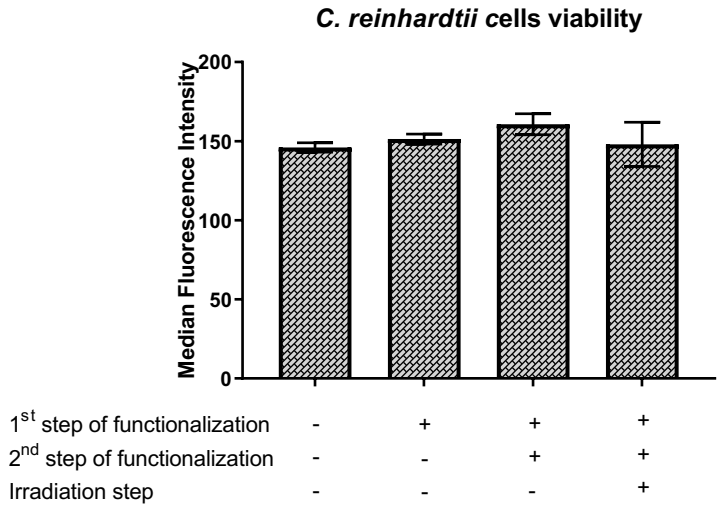


Figure S**16.** The graph represents the *C. reinhardtii* cell viability results. First bar from left side: the cell viability was assessed without any modification; Second bar from left side: the cell viability was assessed after the 1st modification step using DBCO (**1**, 0.1 mM); Third bar from left side: the cell viability was assessed after the 2nd modification step using DBCO (**1**, 0.1 mM) and VancphotoN$_3$ (**5**, 0.005 mM); fourth bar from left side: the cell viability was assessed after the 2nd modification step using DBCO (**1**, 0.1 mM) and VancphotoN$_3$ (**5**, 0.005 mM) and after the irradiation step of 5 min with a wavelength of 365 nm. Data are represented mean value ± SD (n = 3).

7. Antimicrobial activity

7.1. Bacterial strains and growth conditions

Bacillus subtilis (*B. subtilis*, ATCC 6633) was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). The bacteria culture was stored at –80°C, and new cultures were prepared by streaking on Mueller Hinton Agar plates. The overnight culture was prepared by inoculating a single colony into an Erlenmeyer flask (100 mL) containing the bacteria medium (15 mL, 5g 1^{-1} peptone, 3g 1^{-1} meat extract, 10 mg 1^{-1} $MnSO_4*7H_2O$)[1] and the cultures were shaken (200 rcf/min) overnight at 37°C.

7.2. MIC value determination

A single colony of *B. subtilis* (ATCC 6633) was inoculated from a fresh agar plate into a 0.9% NaCl aqueous solution. The turbidity of the suspension was adjusted to McFarland standard 0.5, and then diluted 1 to 200 in cation-adjusted Mueller-Hinton-II broth. The compounds were dissolved in water at a concentration of 1 mg/ml. In 96-wells microtiter plates, two-fold serial dilutions of the compounds (ranging from 64 µg/ml to 0.5 ng/ml) were prepared in Mueller-Hinton-II broth with 0.002% Tween 80 to a final volume of 50 µl. The bacterial suspension (50µl) was added into each well on the microtiter plate for inoculation, corresponding to approximately $5x10^5$ CFU/ml. The microplates were shaken (200 rcf/min) overnight at 37 °C. The MIC was determined by visual inspection of bacterial growth.

Table S**2**: MIC value determination of **7**, **5**, **10** and **6**

Bacteria strain	MIC (µg/ml)			
	Vancomycin (**7**)	VancphotoN_3 (**5**)	VancFITC (**10**)	VancNH_2 (**6**)
B. subtilis ATCC 6633	0.125-0.25	0.25-0.5	2-16	0.06

7.3. Effect of several parameters on bacterial growth

7.3.1. General protocol

An incubated culture was diluted to an optical density (600 nm) of 0.1 by adding a mixture of Kuhl (0.250 ml) and bacteria medium (0.250 ml), both containing Tween 20 (0.05%). The mixture was irradiated at a wavelength of 365 nm for 5 min (unless stated otherwise) and grown in a 24-wells plate at 37°C. The cell density (600 nm) was measured every 15 min for 12 h (with shaking between measurements) in a microplate reader. All experiments were performed in triplicates.

7.3.2. Influence of irradiation time

To determine the influence of the irradiation time on the viability of *B. subtilis* , bacterial solutions (OD600 = 0.1) were irradiated for 0, 5, 10, 15 or 20 min at a wavelength of 365 nm and the culture growths were recorded.

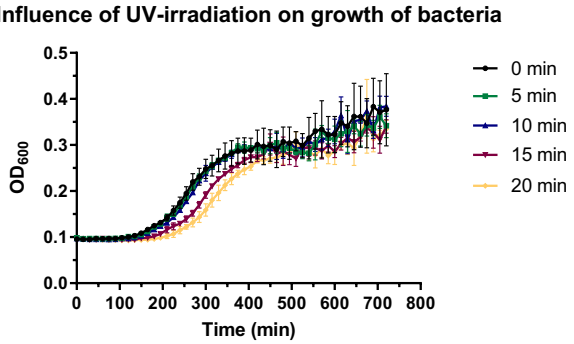


Figure S**17.** Growth of *B. subtilis* after the irradiation step (365 nm) during 0, 5, 10, 15 and 20 min.) at different time points. Data points represent mean value ± SD (n=3).

7.3.3. Antibacterial activity of C. reinhardtii unfunctionalized and functionalized with DBCO (1)

The algae were exposed to the same incubation and washing steps as was described before (section **3.2**), DBCO (**1**) and VancphotoN$_3$ (**5**) were not added during the functionalization steps. The antibacterial activity of the system was recorded by replacing the Kuhl medium (0.250 mL) from the general protocol **7.3.1**. with the algae resuspended in fresh Kuhl Medium (0.250 mL).

The algae were exposed to the same incubation and washing steps as was described before (section **3.2**), using DBCO (**1**, 0.1 mM) for the 1st step and VancphotoN$_3$ (**5**) was not added during the 2nd step. The antibacterial activity of the system was recorded by replacing the Kuhl medium (0.250 mL) from the general protocol **7.3.1.** with the algae resuspended in fresh Kuhl Medium (0.250 mL).

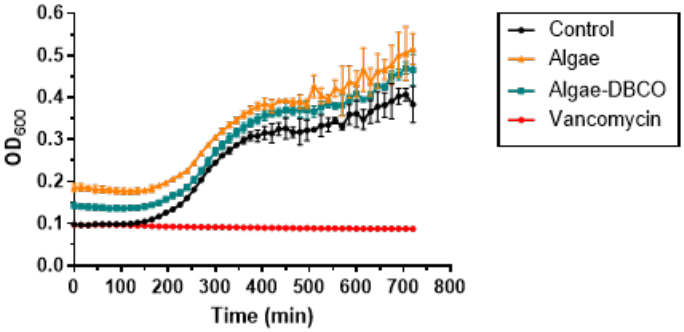


Figure S**18.** Growth of *B. subtilis* incubated with vancomycin (1 µg/mL, red line), with unfunctionalized algae (orange line), and with algae functionalized with DBCO (green line). The black line is the negative control (*B. subtilis* growth). Data points represent mean value ± SD (n=3)

7.3.4. Antibacterial activity of C. reinhardtii only treated with VancphotoN$_3$ (5)

The algae were exposed to the same incubation and washing steps as was described before (section **3.2**), DBCO (**1**) was not added during the 1st step and VancphotoN$_3$ (**5**, 0.005 mM) were added during the 2nd step. The antibacterial activity of the system was recorded by replacing the Kuhl medium (0.250 mL) from the general protocol **7.3.1**. with the algae resuspended in fresh Kuhl Medium (0.250 mL). The bacterial growth was recorded with or without the irradiation step.

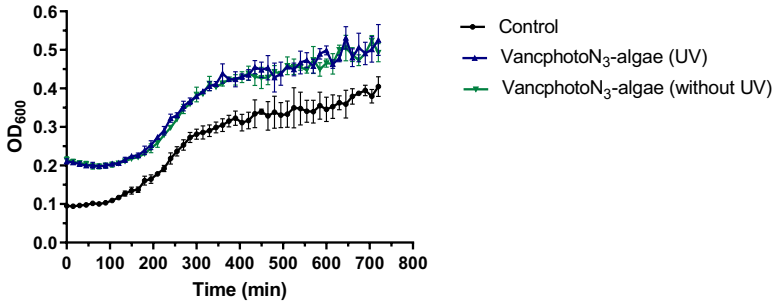


Figure S**19.** Growth of *B. subtilis* incubated with algae treated with VancphotoN$_3$ (**5**, 0.005 mM). The samples were also analysed by omitting the 5 min irradiation step (365 nm). Data points represent mean value ± SD (n=3)

7.3.5. Antibacterial activity of the functionalized C. reinhardtii after the irradiation step

The algae were exposed to the same incubation and washing steps as was described before (section **3.2**). The functionalised *C. reinhardtii* cells were resuspended in PBS (0.5 mL), transferred into the 24-wells plate and irradiated at a wavelength of 365 nm for 5 min. Released $VancNH_2$ was washed out with PBS (1 mL), the sample was centrifuged (5 min, 1000 rcf/min), the supernatant was removed and the washing step was repeated 2 times. The antibacterial activity of the system was recorded by replacing the Kuhl medium (0.250 mL) from the general protocol **7.3.1**. with the algae resuspended in fresh Kuhl Medium (0.250 mL).

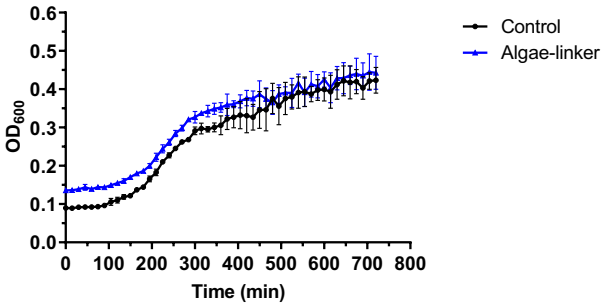


Figure S**20.** Growth of *B. subtilis* incubated with the functionalized algae after the irradiation step (365 nm). Data points represent mean value ± SD (n=3).

7.3.6. Antibacterial activity of the functionalized C. reinhardtii without a photocleavable linker

The algae were exposed to the same incubation and washing steps as was described before (section **3.2**), DBCO (**1**, 0.65 mM) was added during the 1st step and $VancPEG_3N_3$ (**2**, 1 mM), $VancPEG_{35}N_3$ (**3**, 1 mM) or $VancPEG_{100}N_3$ (**4**, 1 mM) were added during the 2nd step. The functionalized algae were mixed with bacteria suspension (OD600 = 0.1) in conic tube (5 mL). The tubes were incubated (6h, 37°C, 200 rcf/min). The suspension (0.5 mL) was taken every 60 min mixed with bacteria medium (0.5 mL) and optical density was determined.

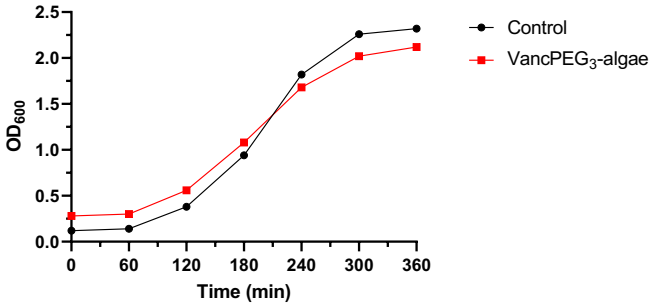


Figure S**21.** Growth of *B. subtilis* incubated with the algae functionalized with DBCO (**1**, 0.65 mM) and $VancPEG_3N_3$ (**2**, 1 mM). Data points represent mean value ± SD (n=1).

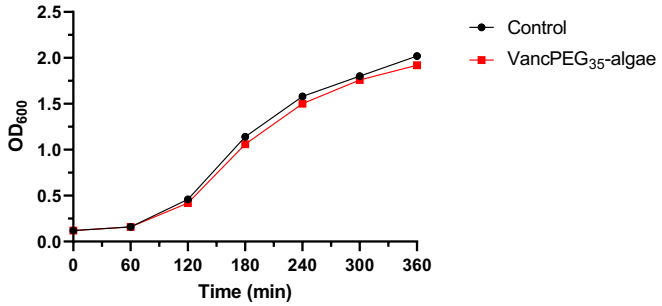


Figure S**22.** Growth of *B. subtilis* incubated with the algae functionalized with DBCO (**1**, 0.65 mM) and $VancPEG_{35}N_3$ (**3**, 1 mM). Data points represent mean value ± SD (n=1).

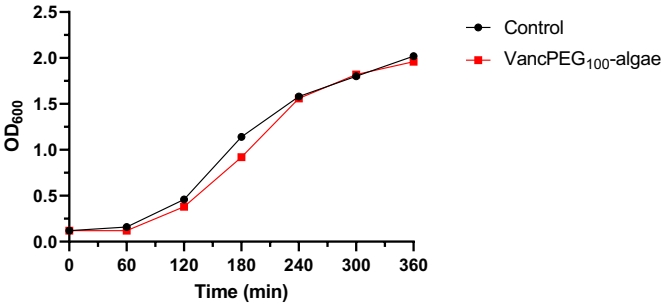


Figure S**23.** Growth of *B. subtilis* incubated with the algae functionalized with DBCO (0.65 mM) and $VancPEG_{100}N_3$ (**4**, 1 mM). Data points represent mean value ± SD (n=1).

7.4. Antibacterial activity of the system with optimized parameters

7.4.1. Low concentration protocol

The algae were exposed to the same incubation and washing steps as was described before (section **3.2**), DBCO (**1**, 0.05 mM) was added during the 1st step and VancphotoN$_3$ (**5**, 0.0025 mM) was added during the 2nd step. The antibacterial activity of the system was recorded by replacing the Kuhl medium (0.250 mL) from the general protocol **7.3.1**. with the algae resuspended in fresh Kuhl Medium (0.250 mL). The bacterial growth was recorded with or without the irradiation step.

functionalized cells algae cells

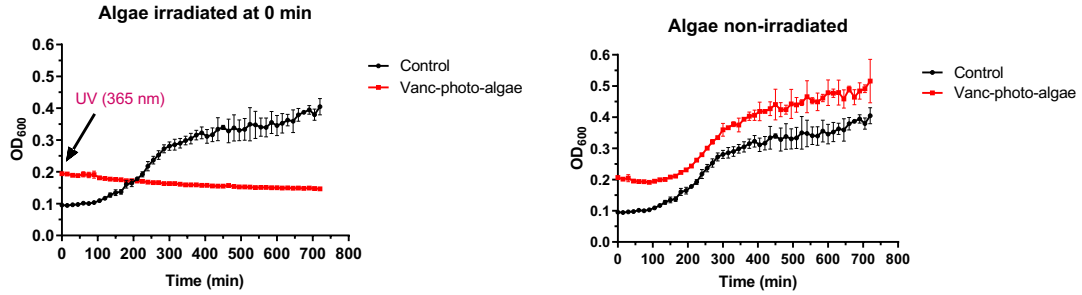


Figure S**24** Growth of *B. subtilis* incubated with algae functionalized with DBCO (**1**, 0.05 mM) and VancphotoN$_3$ (**5**, 0.0025 mM) after the irradiation step (5 min, 365 nm). The samples were also analysed by omitting the 5 min irradiation step (365 nm). Data points represent mean value ± SD (n=3)

7.4.2. High concentration protocol

The algae were exposed to the same incubation and washing steps as was described before (section **3.2**), DBCO (0.1 mM) was added during the 1st step and VancphotoN$_3$ (**5**, 0.005 mM) was added during the 2nd step. The antibacterial activity of the system was recorded by replacing the Kuhl medium (0.250 mL) from the general protocol **7.3.1**. with the algae resuspended in fresh Kuhl Medium (0.250 mL). The bacterial growth was recorded with or without the irradiation step.

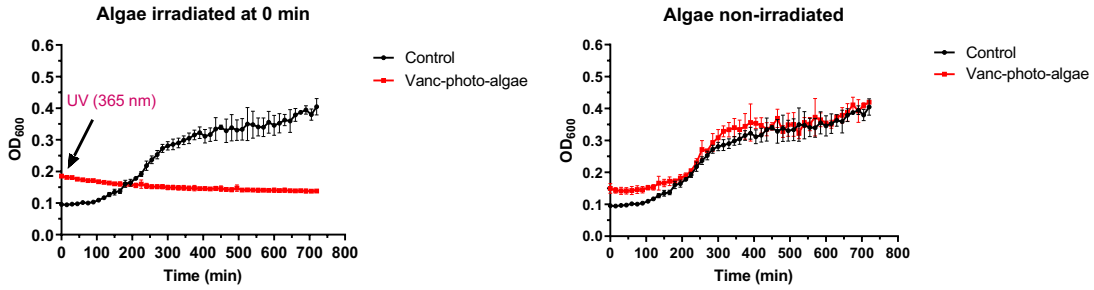


Figure S**25.** Growth of *B. subtilis* incubated with algae functionalized with DBCO (**1**, 0.1 mM) and VancphotoN$_3$ (**5**, 0.005 mM) after the irradiation step (5 min, 365 nm). The samples were also analysed by omitting the 5 min irradiation step (365 nm). Data points represent mean value ± SD (n=3)

7.5.Antibacterial activity of the system at exponential phase of bacterial growth

The algae were exposed to the same incubation and washing steps as was described before (section **3.2**), DBCO (**1**) was added during the 1st step and VancphotoN$_3$ (**5**) was added during the 2nd step according to low concentration **7.4.1** and high concentration **7.4.2** protocols. The antibacterial activity of the system was recorded by replacing the Kuhl medium (0.250 mL) from the general protocol **7.3.1**. with the algae resuspended in fresh Kuhl Medium (0.250 mL). The bacterial growth was recorded with the irradiation step after 5h of bacterial growth.

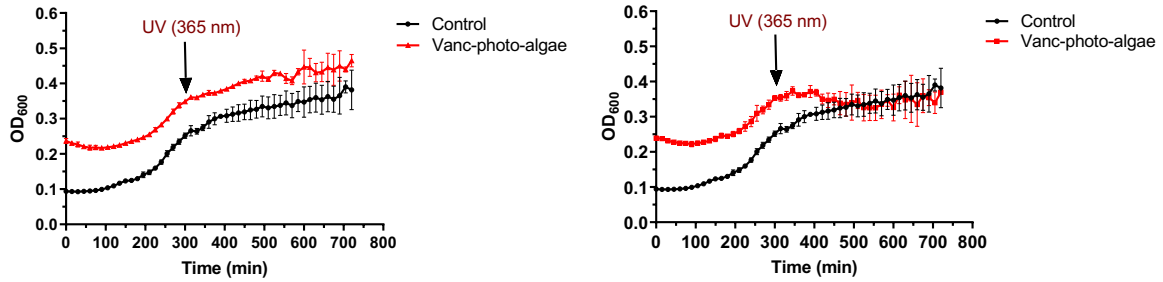


Figure S**26.** Growth of *B. subtilis* incubated with algae functionalized with (left graph) low concentration protocol (DBCO (**1**, 0.05 mM) and VancphotoN$_3$ (**5**, 0.0025 mM)) or (right graph) high concentration protocol (DBCO (**1**, 0.1 mM) and VancphotoN$_3$ (**5**, 0.005 mM)). The irradiation step (5 min, 365 nm) was applied after 5h of bacterial growth. Data points represent mean value ± SD (n=3)

8. References

[1] J.-Y. Wach, S. Bonazzi, K. Gademann, *Angew. Chem. Int. Ed*. **2008**, *47*, 7123–7126