Supporting information

Table of contents:

1. Synthesis and characterizations of SR$_9$.

1.1. Reactants and reagents:

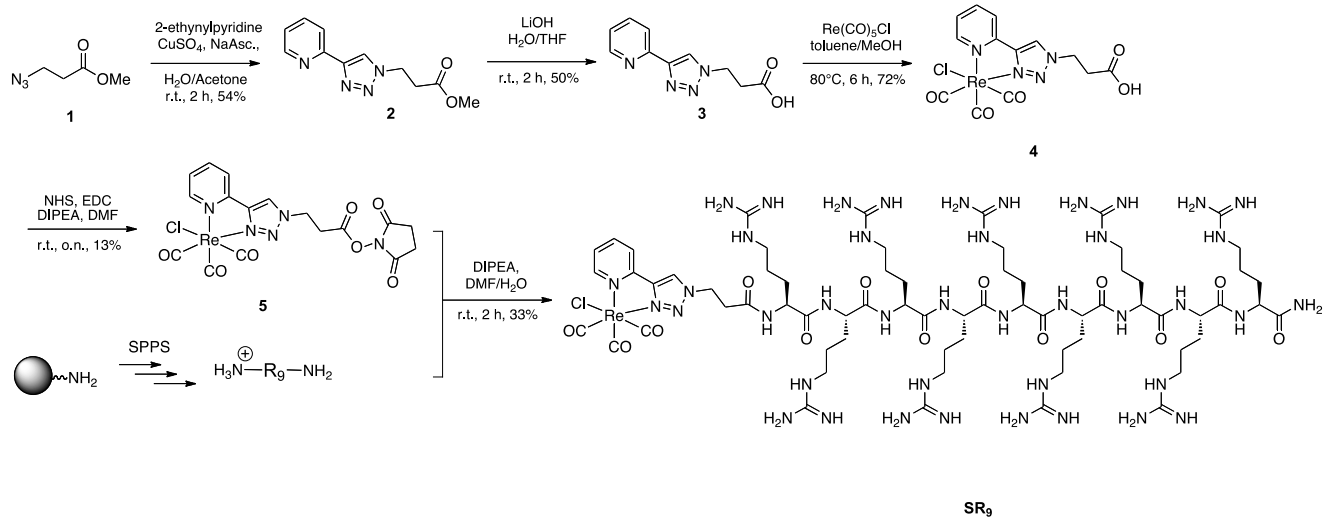
All chemicals and solvents were of synthesis grade and were used as received without further purification. Common solvents for solution synthesis were obtained from either Carlo Erba or VWR. Peptide synthesis: MBHA resin (0.54 mmol/g, Iris Biotech GmbH), Boc-L-Arg (Tos)-OH (Iris Biotech GmbH), HOBt (Molekula), DCC (Flamma), EDC•HCl (Novabiochem), DIPEA (Alfa Aesar), TFA (Carlo Erba), HF (Merck), Anisole (Acros), Me_2S (Acros).

1.2. Instrumentation

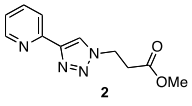
^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker DRX300 spectrometers. The chemical shifts (δ/ppm) were calibrated relative to residual solvent signals**.** High resolution mass spectra (HR-MS) were obtained on a Bruker hybride APEX spectrometer (ESI). Peptides and conjugates were characterized by MALDI-TOF-MS in the positive ion reflector mode on an ABI Voyager DE-Pro MALDI-TOF mass spectrometer (Applied Biosystems) using as matrix a saturated solution of CHCA in CH_3CN/H_2O/TFA (50:50:0.1 v:v:v).

The peptide was assembled by stepwise solid-phase synthesis with an ABI 433A synthesizer (Aplied Biosystems).

1.3. Procedures

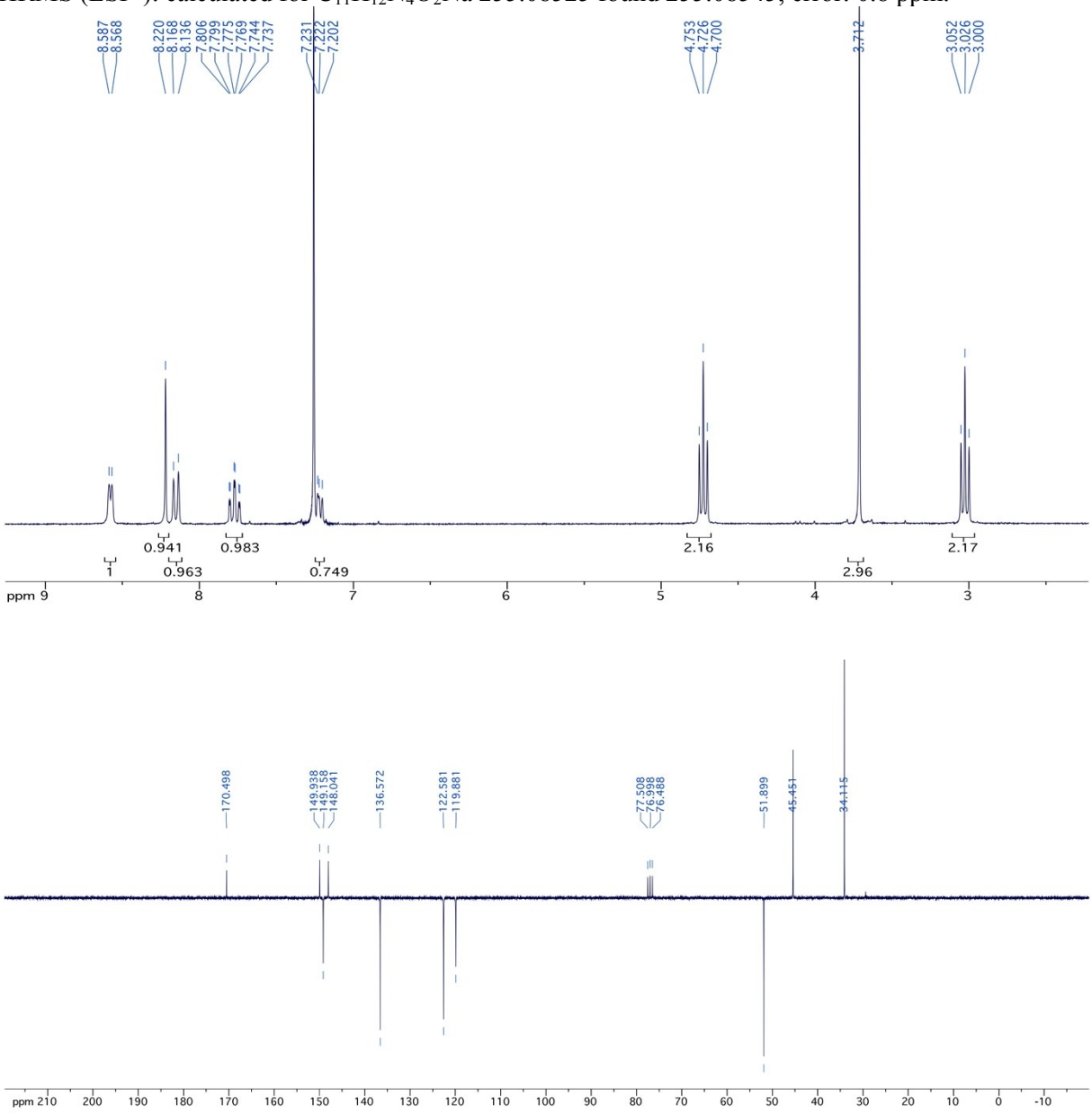


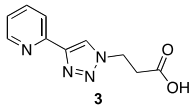
Scheme S1. Synthesis of the conjugated **SR$_9$**. Note that **SR$_9$** is purified by HPCL (preparative C18 column, flow: 14 mL/min, gradient: from 15% to 30% of CH_3CN in H_2O over 30 min both solvents containing 0.1% TFA) and an exchange Cl/TFA is possible (see below).



To a solution of **1**[1] (954 mg, 7.39 mmol, 1 equiv.) in a mixture acetone/H_2O (2/1 v:v, 44/88 mL) was added 2-ethynylpyridine (746 µL, 7.39 mmol, 1 equiv.). $CuSO_4$ (461 mg, 1.85 mmol, 0.25 equiv.) and sodium ascorbate (365 mg, 1.85 mmol, 0.25 equiv.) were then added and the reaction mixture was stirred for 2 hours at room temperature. The reaction mixture was diluted with DCM and an aqueous solution of NH_3 (28%) was added. The two layers were separated and the aqueous layer was extracted 2 times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated to afford **2** (brown solid, 926 mg, 54%), which was used without further purification. ^{1}H-NMR (250 MHz; $CDCl_3$): δ 8.58 (d, J = 4.7 Hz, 1H), 8.22 (s, 1H), 8.15 (d, J = 7.8 Hz, 1H), 7.77 (td, J = 7.8, 1.6 Hz, 1H), 7.22 (m, 1H), 4.73 (t, J = 6.6 Hz, 2H), 3.71 (s, 3H), 3.03 (t, J = 6.6

Hz, 2H). ^{13}C-NMR (63 MHz; $CDCl_3$): δ 170.5, 149.9, 149.2, 148.0, 136.6, 122.6, 119.9, 51.9, 45.5, 34.1. HRMS (ESI+): calculated for $C_{11}H_{12}N_4O_2Na$ 255.08525 found 255.08545, error: 0.8 ppm.





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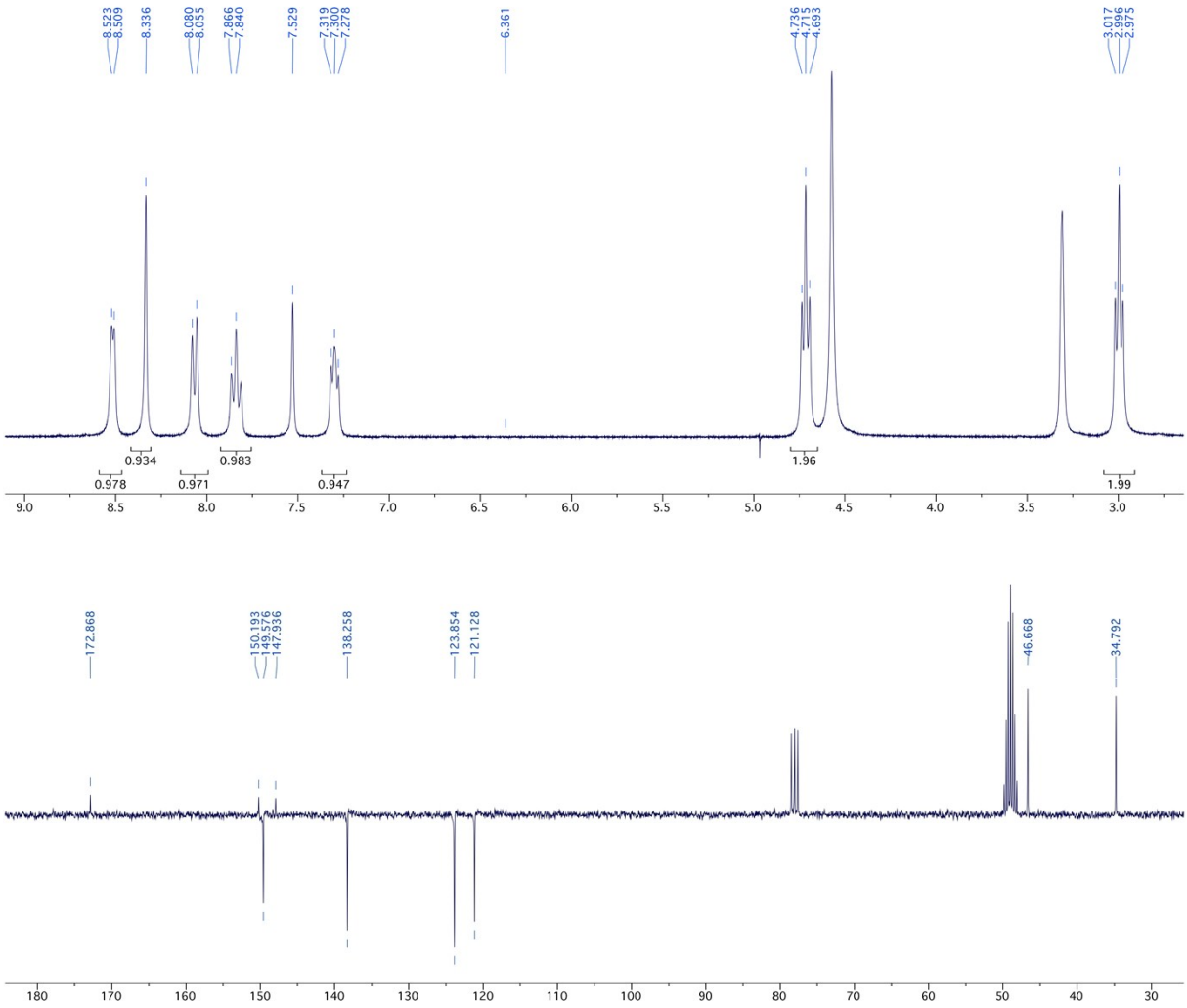
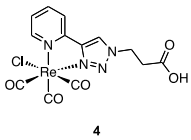
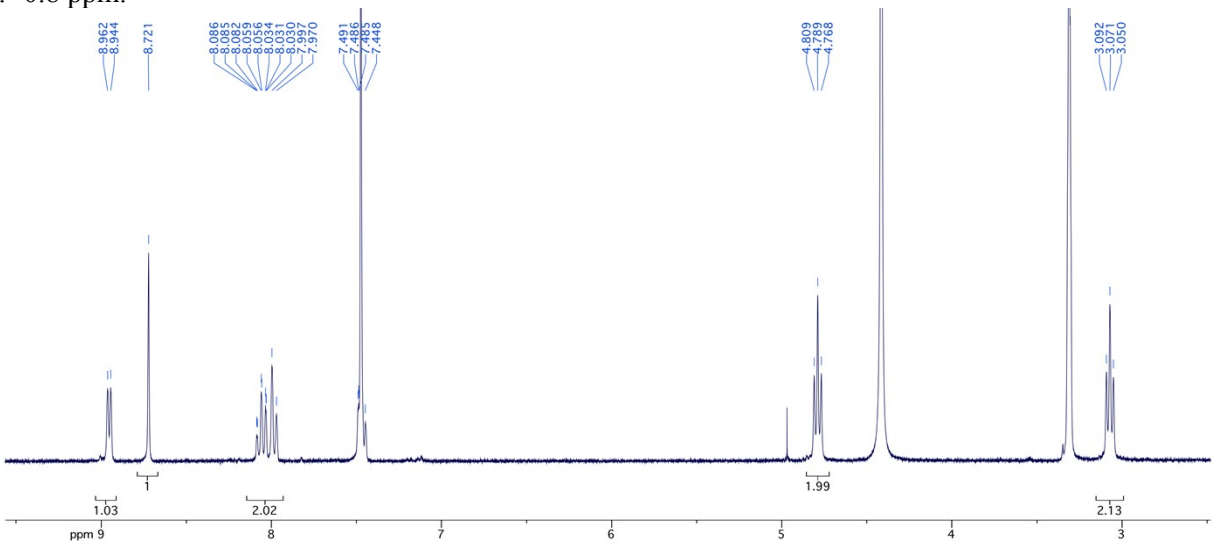
To a solution of **2** (450 mg, 1.94 mmol, 1 equiv.) in a mixture THF/H_2O (2/1 v:v, 18/9 mL) was added LiOH (162 mg, 3.88 mmol, 2 equiv.). The reaction mixture was stirred until completion (monitored by TLC, eluent: 100% EtOAc, approximately 2 hours) at room temperature. HCl 1M was added until the formation of a precipitate. THF was removed under vacuum, the solid was collected by filtration, washed with HCl 0.01 M and dried under vacuum to afford **3** (brown solid, 210 mg, 50%) which was used without further purification. ^{1}H-NMR (300 MHz; MeOD/$CDCl_3$ 3/1 v:v): δ 8.52 (d, J = 5.0 Hz, 1H), 8.34 (s, 1H), 8.07 (d, J = 7.7 Hz, 1H), 7.84 (d, J = 7.7 Hz, 1H), 7.30 (t, J = 5.0 Hz, 1H), 4.71 (t, J = 6.5 Hz, 2H), 3.00 (t, J = 6.5 Hz, 2H). ^{13}C-NMR (75 MHz; MeOD/$CDCl_3$ 3/1 v:v): δ 172.87, 150.19, 149.58, 147.94, 138.26, 123.85, 121.12, 46.67, 34.79. HRMS (ESI+): *m/z* calculated for $C_{10}H_{10}N_4O_2Na$ 241.06960 found 241.06955, error : -0.2 ppm.

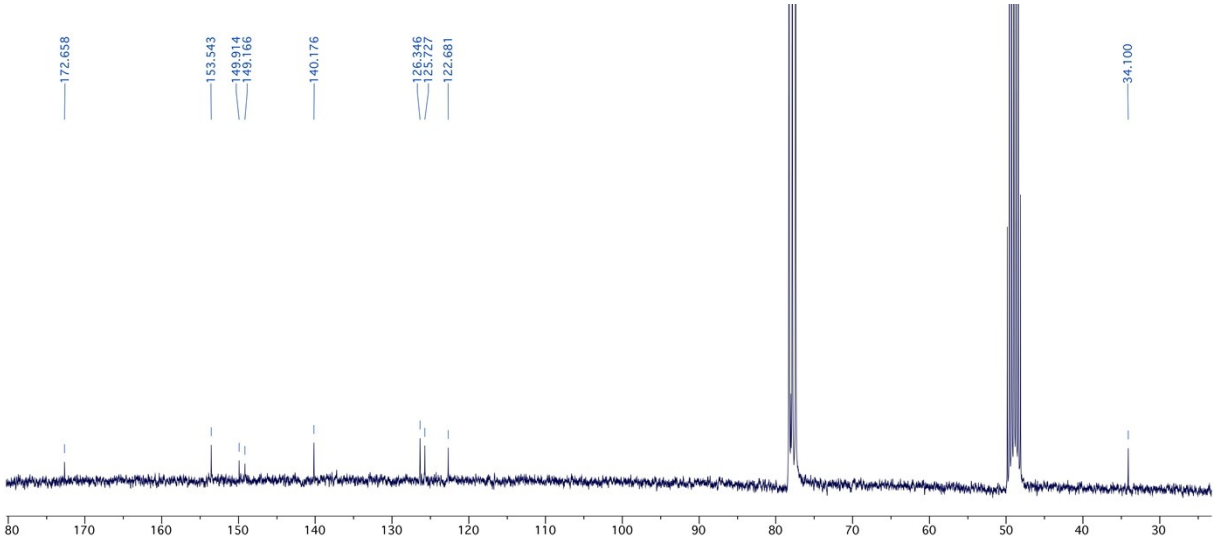


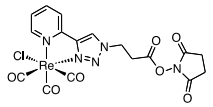


4

To a solution of **3** (178 mg, 0.82 mmol, 1 equiv.) in a mixture toluene/MeOH (4/1 v:v, 12/3 mL) was added $Re(CO)_5Cl$ (295 mg, 0.82 mmol, 1 equiv.). The reaction mixture was refluxed for 6 hours. The reaction mixture was cooled down to room temperature, concentrated to 1/3 of the volume, the precipitate was filtered off, washed once with toluene and dried under vacuum to afford **4** (light yellow solid, 308 mg, 72%) which was used without further purification. ^{1}H-NMR (300 MHz; MeOD/$CDCl_3$ 3/1 v:v): δ 8.95 (d, J = 5.5 Hz, 1H), 8.72 (s, 1H), 8.09-7.97 (m, 2H), 7.49-7.45 (m, 1H), 4.79 (t, J = 6.3 Hz, 2H), 3.07 (t, J = 6.3 Hz, 2H). ^{13}C-NMR (75 MHz; MeOD/$CDCl_3$ 3/1 v:v): δ 172.66, 153.54, 149.91, 149.17, 140.18, 126.35, 125.68, 122.68, 34.10. HRMS (ESI+): calculated for $C_{13}H_{10}ClN_4O_5ReNa$ 544.97615 found 544.97583, error : -0.8 ppm.

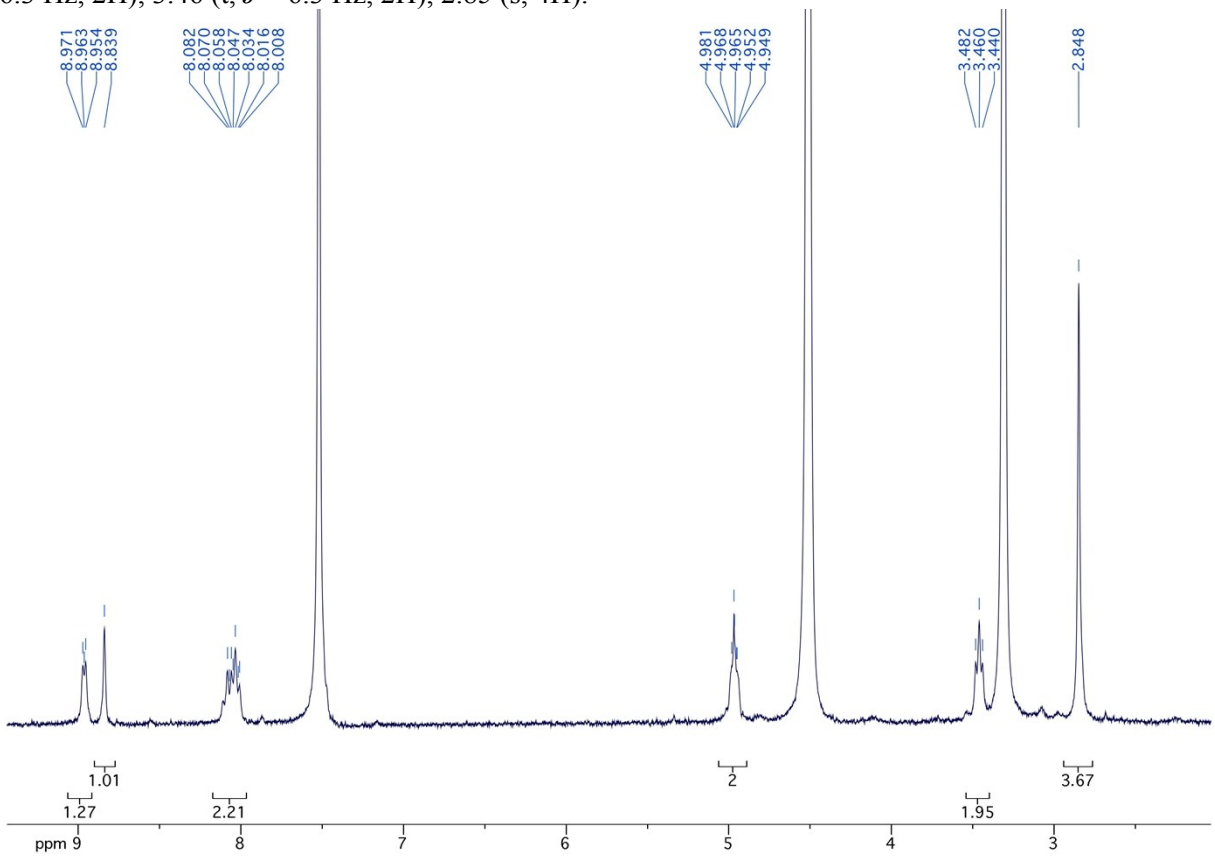


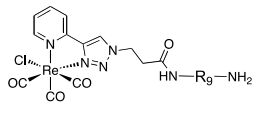




5

To a solution of **4** (250 mg, 0.48 mmol, 1 equiv.) in DMF (9 mL) was added EDC□HCl (124 mg, 0.63 mmol, 1.3 equiv.), DIPEA (372 µL, 2.15 mmol, 4.5 equiv.) and N-hydroxysuccinimide (55 mg, 0.48 mmol, 1 equiv.). The reaction mixture was stirred overnight at room temperature. The DMF was removed and the crude dissolved in EtOAc. The organic layer was washed twice with a saturated $NaHCO_3$ solution, once with brine, dried over Na_2SO_4, filtered and concentrated. The crude was triturated in Et_2O, filtered and dried under vacuum to afford **5** (yellow solid, 40 mg, 13%), which was used without further purification.^{1}H-NMR (300 MHz; MeOD/$CDCl_3$ 3/1 v:v): δ 8.96 (d, J = 4.2 Hz, 1H), 8.84 (s, 1H), 8.08-8.01 (m, 2H), 7.50 (m, 1H), 4.96 (t, J = 6.3 Hz, 2H), 3.46 (t, J = 6.3 Hz, 2H), 2.85 (s, 4H).





SR$_9$

The peptide was assembled by stepwise solid-phase synthesis using standard protocols for Boc chemistry : amino acid activation with dicyclohexylcarbodiimide/1-hydroxybenzotriazole, Boc deprotection with TFA, capping with Ac_2O in DCM. The peptide was cleaved from the resin by treatment with anhydrous HF in presence of anisole (1.5 mL/g of peptidyl-resin) and of dimethylsulfide (0.25 mg/mg of peptidyl-resin) for 2 hours at 0°C.

To a solution of crude $^{+}NH_3$-R_9-$CONH_2$ peptide (145 mg, 0.07 mmol, 1.2 equiv.) dissolved in a mixture DMF/H_2O (2/3 v:v, 0.4/0.6 mL) was added DIPEA (31 µL, 0.18 mmol, 3 equiv.). A solution of **5** (37 mg, 0.06 mmol, 1 equiv.) dissolved in a mixture DMF/H_2O (3 /1 v:v, 0.6/0.2 mL) was added to the previous solution and the reaction mixture was stirred for 2 hours at room temperature. The reaction was monitored by reversed phase HPLC. The solvents were removed and the crude purified by HPLC (preparative C18 column, flow: 14 mL/min) eluted with a gradient: from 15% to 30% of CH_3CN in H_2O over 30 min both solvents containing 0.1% TFA. The pure fractions of the expected peptide were combined and freeze-dried to afford 59 mg of **SR_9** (33%, purity: 93%). Retention time: 5.0 min. Maldi-TOF MS (CHCA matrix): 1890.7 $(M\text{-}Cl)^+$.

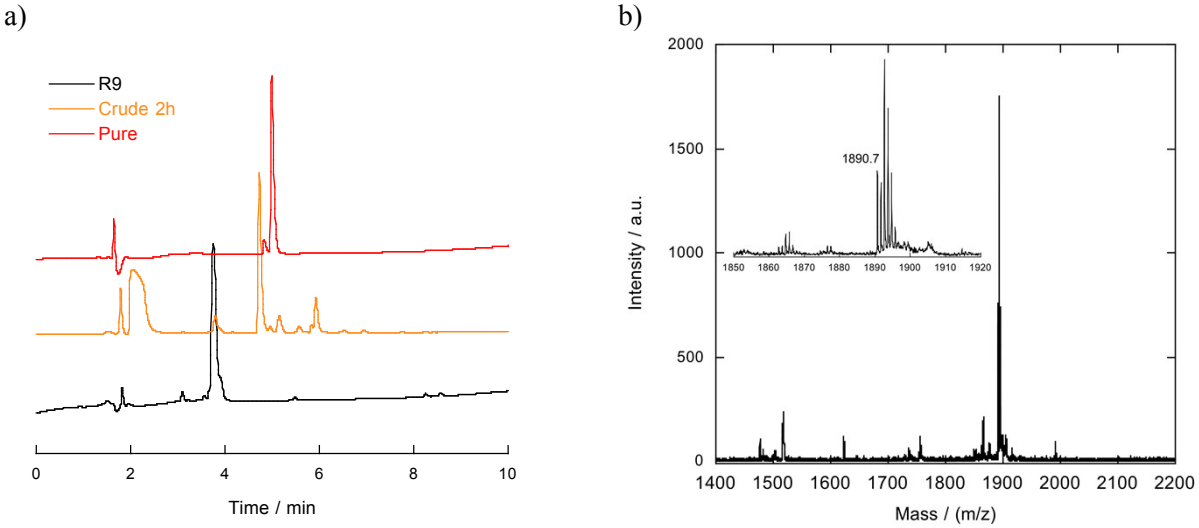


Figure S1. a) Monitoring of the reaction completion by HPLC. After 2 h, a main product was formed (retention time at 5.0 min). After purification the pure fraction possesses a different retention time due to an exchange of the chloride by a trifluoroacetate. b) Maldi TOF mass spectrum of the conjugated showing the expected mass with a loss of chloride anion.

2. Skin preparation

Skin biopsies were prepared as previously described[2] and mounted on Franz cells. 200 µL of a solution **SR_9** in water at 2 10^{-2} M were dropped onto the skin surface. During the experiment, Franz cells were not occluded but left open to the air. After 6 or 24 h, skin samples were collected. Each skin surface was washed with a cotton swab on which 200 µL of liquid receptor (distilled water containing 0.85% NaCl and 0.01% of bovine serum albumin) were dropped. The skin was then frozen at -20 °C. Biopsies were cryo-tomed at -20 °C with a thickness of 10 µm and mounted on CaF_2 windows a couple of days before the SR-FTIR measurements.

3. Synchrotron radiation FTIR spectromicroscopy (SR-FTIR-SM)

It was performed on the IR beamline SMIS (SOLEIL synchrotron, Saint-Aubin, France). Spectra were recorded in transmission mode on a Nicolet Continuum XL microscope (Thermo Fisher) equipped with a 50x50 µm^2 liquid nitrogen cooled MCT/A detector, a 32X/NA 0.65 Schwarzschild objective, a Prior XYZ motorised stage, and coupled to a Nicolet 5700 FTIR spectrometer (Thermo Fisher) equipped with a Michelson interferometer and a KBr beam splitter. The confocal aperture was set at 10x10 µm^2 and the microscope was operated in semi-confocal mode. Mappings were recorded by raster scanning the cells with steps of 10 µm in X and in Y with 16–128 scans at 6 cm^{-1} resolution in each square.

4. HaCat cells culture and luminescence microscopy

HaCat cells (human keratinocyte cell line) were routinely processed in DMEM GLUTAMAX high glucose (4.5 g/L) + 10% SVF. Both control and treated cells were processed in a similar way. They were seeded on glass coverslips in order to reach confluency after 48 h of incubation at 37 °C under an atmosphere of 95% air/ 5% CO_2. Medium was removed and fresh growth medium was added to each flask of control cells. In the case of treated cells, a solution of **SR_9** at 50 µM in fresh growth medium added. The cells were incubated at 37 °C under an atmosphere of 95% air/ 5% CO_2 for 1 h. The medium was then removed and the cells were washed twice with phosphate buffered saline (D-PBS, 1X, 2 mL). Cells were fixed by 4 % paraformaldehyde (1.5 mL) for 8 minutes at room temperature and washed once with D-PBS (1X, 2 mL) and once with pure water (2 mL). Slides were then mounted using Vectashield solution (H-1000, Vector Laboratories).

Luminescence microscopy was performed on a Zeiss LSM 710 confocal microscope using an X63 (NA 1.40) objective and 405 nm laser excitation. **SR$_9$** and DAPI-stained nuclei were located with [500-550 nm] and [450-470 nm] excitation ranges, respectively. The conditions of the luminescence detection of the SCoMPI unit were previously described.[3] Image analysis was performed using ImageJ software.

5. FTIR quantification

Calibration curves for the SCoMPI *fac*-Re(pyta)(CO)$_3$ core were generated using a liquid sealed cell (NaCl, pathlenght l: 1 mm) filled with solutions of **A** (previously described[4]) in different solvents, with concentrations ranging from 10^{-5} to 10^{-4} M (*ie* 10^{-2} to 10^{-1} mol m^3). Spectra were recorded on a Perkin Elmer Spectrum 100 in transmission mode (background on air) and analysed using Perkin Elmer Spectrum software. The height (absorbance) of the 2025 cm^{-1} peak (A_1-band) was measured and curves were obtained by plotting it against the corresponding concentration (Figure S2).

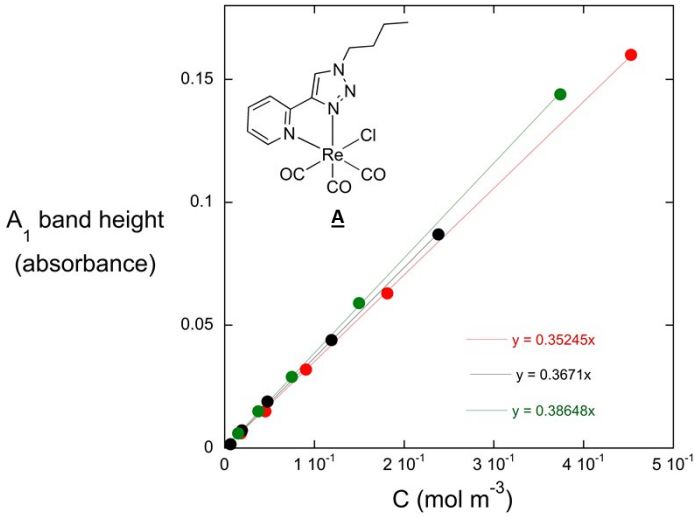


Figure S2. Structure of **A** and calibration curves in different solvents with corresponding linear regression: ethanol (black), benzylic alcohol (red), acetonitrile (green).

As it can be seen in the linear regressions, values for the absorption coefficient ε are very close and weakly influenced by the nature of the solvents. We hence assumed a mean value of **ε = 368 m^2.mol^{-1}** for the A_1-band absorption of the SCoMPI *fac*-Re(pyta)(CO)$_3$ core (since the pathlength of the liquid-cell is l = 10^{-3} m).

By considering the Beer-Lambert's law, a relationship between the number N of moles of irradiated molecules and the absorbance can be made:

$$A = \varepsilon \cdot l \cdot c = \varepsilon \cdot l \cdot \frac{N}{V} = \varepsilon \cdot l \cdot \frac{N}{S \cdot l} = \varepsilon \cdot \frac{N}{S} \qquad (1)$$

with A: absorbance, ε: absorption coefficient (m^2.mol^{-1}), l: pathlength (m), V: irradiated volume (m^3), S: irradiated area (m^2), N: number of moles of irradiated molecules (mol).

The irradiated area S corresponds to the aperture size of the IR-microscope and was set to 10x10 µm^2 = 10^{-10} m^2. Therefore the number N of irradiated molecules can be estimated for a given value of absorbance A.

Thanks to the DAPI staining of the nuclei of keratinocytes from the viable epidermis,[5] we were able to determine two groups of IR spectra recorded on SMIS beamline (see section 3): those from the Stratum Corneum (SC) (not DAPI-stained) and those from the viable epidermis (DAPI-stained).

These IR spectra were processed using TQ Analyst software (Thermoscientific software) to determine the height (absorbance) of the 2025 cm^{-1} peak (A_1-band, height 2040-2010 cm^{-1} with baseline, table S1). We have chosen hotspots in several skin sections and we have estimated in these regions the relative proportion found in the SC and in the VE. Values from Table S1 are given for a region of interest (ROI) corresponding to a surface of 10x10 µm^2 (beam size) and 10-µm thick skin slices.

SR_9 distribution	In the SC after 6 h	In the viable epidermis after 6 h	In the SC after 24 h	In the viable epidermis after 24 h
Number of spectra (number of skin sections, number of studied areas)	52 (5, 12)	94 (5, 12)	32 (2, 6)	28 (2, 6)
Height (absorbance) of the 2025 cm^{-1} peak (A_1-band)	0.030 ± 0.005	0.0021 ± 0.0003	0.005 ± 0.002	0.020 ± 0.002
N (mole) *per* ROI (using relation (1))	$8.2x10^{-15}$	$6.0x10^{-16}$	$1.4x10^{-15}$	$5.4x10^{-15}$

Table S1.

After 6 h, the quantity of **SR_9** in the viable epidermis is < 10 % of the quantity in the SC.
After 24 h, the quantity of **SR_9** in the viable epidermis is in the same range than the quantity in the SC.

6. Biological tests

LDH test

Cytotoxicity of compounds was tested by following the release of the cytosolic lactate dehydrogenase (LDH) into the supernatant, indicating membrane damages. The assay is based on the ability of LDH contained in cell lysates or supernatants to catalyze the reduction of pyruvate (0.6 mM) into lactate in the presence of NADH (0.18 mM), which is oxidized to form NAD^+, in PBS (1X, pH 7). 10 µL of lysate in 190 µL of PBS or 200 µL of supernatant was added to 800 µL of the mixture NADH/pyruvate. The level of LDH was proportional to amount of pyruvate consumed measured by monitoring the decrease in absorbance (340 nm) due to oxidation of NADH during 1 min. The percentage of LDH release in supernatants was calculated by dividing this LDH activity found in supernatant, by the sum of activity in supernatants and cell lysates. The experiment was led on tri-plicates. Cytotoxicity is usually considered when LDH release is more than 10%. Considering the error bars, we can infer that **SR_9** was not cytotoxic under the imaging conditions used. This is was also confirmed by the MTT test as shown later.

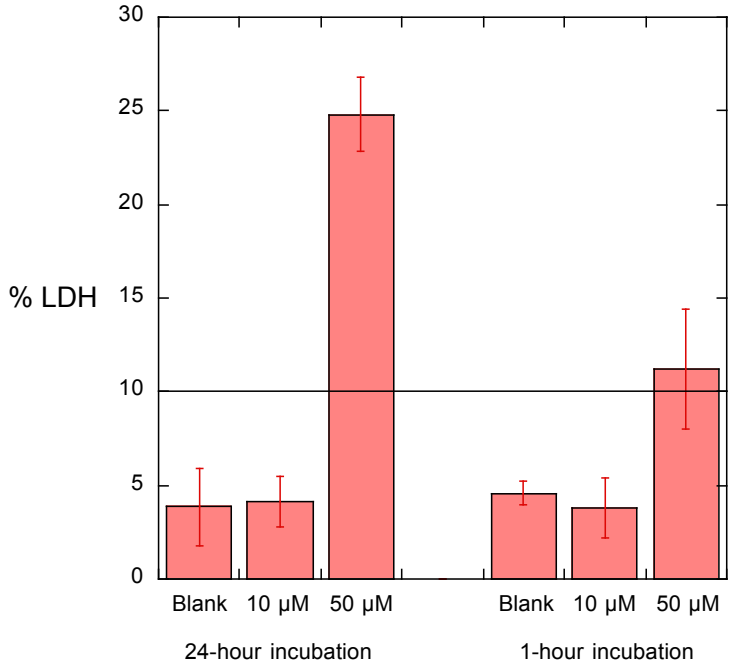


Figure S3. LDH test on HaCat cells incubated for 1 or 24 h at 10 or 50 µM with **SR_9**.

MTT test

Cell viability was assessed by the mitochondrial-dependent reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan. Cells were seeded in a six-well plate to reach 90% of confluency after 2 days. After 1 h of incubation without or with 10 or 50 µM **SR_9**, the supernatant was removed and HaCat cells were then incubated at 37°C with MTT (0.5 mg/mL) for 1 h. Culture medium was removed and cells were solubilized in 0.04 M HCl in absolute ethanol. The extent of reduction of MTT to formazan by the cells was quantified by the measurement of absorbance at 550 nm with a Cary 300 Bio Spectrophotometer. The experiments was led two times on four-plicates.

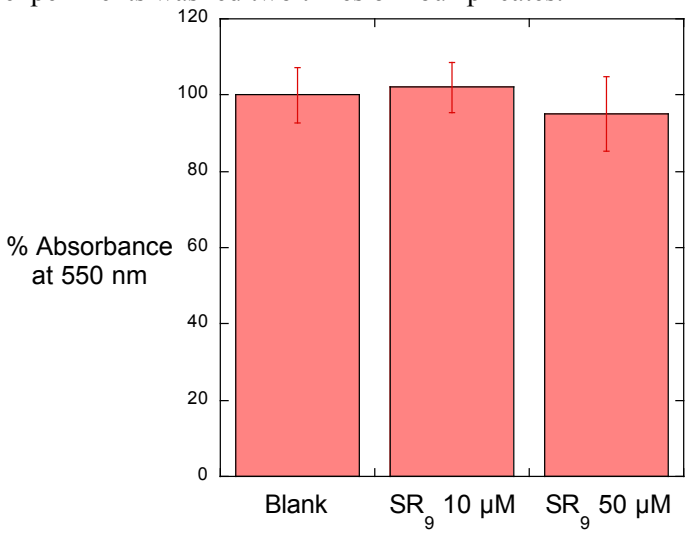


Figure S4. MTT test on HaCat cells incubated for 1 h at 10 or 50 µM with **SR_9**.

1. R. N. Murugan, J.-E. Park, D. Lim, M. Ahn, C. Cheong, T. Kwon, K.-Y. Nam, S. H. Choi, B. Y. Kim, D.-Y. Yoon, M. B. Yaffe, D.-Y. Yu, K. S. Lee and J. K. Bang, *Bioorg. Med. Chem.*, 2013, **21**, 2623.
2. E. Jungman, C. Laugel, D. N. Rutledge, P. Dumas and A. Baillet-Guffroy, *Int. J. Pharm.*, 2013, **441**, 628.
3. S. Clède, F. Lambert, C. Sandt, Z. Gueroui, M. Refregiers, M.-A. Plamont, P. Dumas, A. Vessieres and C. Policar, *Chem. Commun.*, 2012, **48**, 7729.
4. S. Clède, F. Lambert, R. Saint-Fort, M.-A. Plamont, H. Bertrand, A. Vessieres and C. Policar, *Chem. - Eur. J.*, 2014, **20**, 8714.
5. T. Subongkot, B. Pamornpathomkul, T. Rojanarata, P. Opanasopit and T. Ngawhirunpat, *Int J Nanomedicine*, 2014, **9**, 3539.