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Electronic Supplementary Information (ESI)

Active quinine-based films able to release antimicrobial compounds *via* melt quaternization at low temperature

Alejandro Latorre-Sánchez, Mats Johansson, Yuning Zhang, Michael Malkoch and José A. Pomposo**

**E-mail: josetxo.pomposo@ehu.eus*

matskq@kth.se

Table of contents

Materials

Quinine (suitable for fluorescence, anhydrous, ≥ 98.0 %, ≤ 5 % dihydroquinine), Trimethylolpropane tris(3-mercaptopropionate)(TMPMP) (≥ 95 %), α,α'-Dichloro-*p*-xylene (98 %), α,α'-Dibromo-*p*-xylene (97 %) were purchased from Sigma-Aldrich. THIOCURE ETTMP 1300 - ethoxilated trimethylolpropan tris(3-mercaptopropionate) was purchased from Bruno Bock, photoinitiator IRGACURE 651 - 2,2-dimethoxy-1,2-diphenylethan-1-one was purchased from BASF, deuterated dimethyl sulfoxide (99.8 atom % D) was purchased from Thermo Fisher scientific. Purified water was obtained from a Thermo Scientific apparatus (Barnstead TII Pure Water System). Tetrahydrofuran (≥ 99.9 %, stabilized with 250 ppm of BHT) and dimethyl sulfoxide (DMSO) (99.5 %) were purchased from Scharlab.

Human Dermal Fibroblasts (hDF) and mouse macrophage cell line Raw 264.7 were purchased from ATCC (American Tissue Culture Collection) and were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10 % fetal bovine serum (FBS) and 100 units per ml of penicillin, as well as 100 mg ml^{-1} streptomycin under 5 % CO_2 at 37 °C.

E. coli was purchased from ATCC and maintained in LB broth (Lennox) from Alfa Aesar.

DMEM, PBS, penicillin/streptomycin and tripsin-EDTA were purchased from Gibco®.

Instrumentation

^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded at room temperature on Bruker spectrometers operating at 400 MHz or 500 MHz, using DMSO-d_6 as solvent. NMR spectra of compound **6** were recorded at 60 °C.

Elemental Analysis (EA) measurements were performed in a Euro EA3000 Elemental Analyzer (CHNS).

Matrix-Assisted Laser Desorption / Ionization-Time of Flight (MALDI-TOF) measurements were carried out in a Bruker AutoFlex Speed instrument working with samples dissolved in water at 2 mg ml^{-1}.

Fourier Transform Infra-Red (FTIR) spectroscopy spectra were recorded at room temperature on a Perkin-Elmer Spectrum 2000 FT-IR instrument.

Thermal Gravimetric Analysis (TGA) measurements were performed in a Q500-TA Instruments apparatus at a heating rate of 10 °C/min under nitrogen atmosphere from room temperature to 700 °C.

Differential Scanning Calorimetry (DSC) measurements were carried out in a DSC-Q2000 apparatus from TA-Instruments. Modulated program was selected using the amplitude at ± 0.5 °C every 60 seconds at a heating rate of 3 °C/min under Helium atmosphere.

Ultraviolet / Visible (UV/Vis) spectroscopy measurements were performed at 25 °C in an Agilent 8453A apparatus with Peltier thermostatic cell holder, T-controller 89090A.

UV-Light source used for crosslinking of the films **F1a** and **F1b** and synthesis of compound **6** was a Black Ray B-100AP (100 W, λ = 365 nm).

Atomic Force Microscopy (AFM) measurements were carried out using a Bruker Multimode 8 microscope, equipped with a Nanoscope V controller. In order to characterize both the topography and the mechanical properties simultaneously, we have used the PeakForce Quantitative Nanomechanical Mapping (PF-QNM) method, with a constant peak-force of 25 nN. The measurements were performed over randomly selected surface areas, at a resolution of 256 x 256 pixels, using Tap300Al-G probes by Budgetsensors. The actual cantilever spring constant (k) was determined using the Sader Method.[1,2] A value of k = 20 N/m was found for every probe. The tip radius was calibrated against a polystyrene standard provided by Bruker, and resulted in a typical value close to 30 nm. To obtain the mechanical modulus, the force curves was fitted using the Derjaguin–Muller–Toporov (DMT) model:[3,4]

$$F - F_{\mathrm{adh}} = \frac{4}{3} E^* \sqrt{R(d - d_0)^3} \quad \text{(S1)}$$

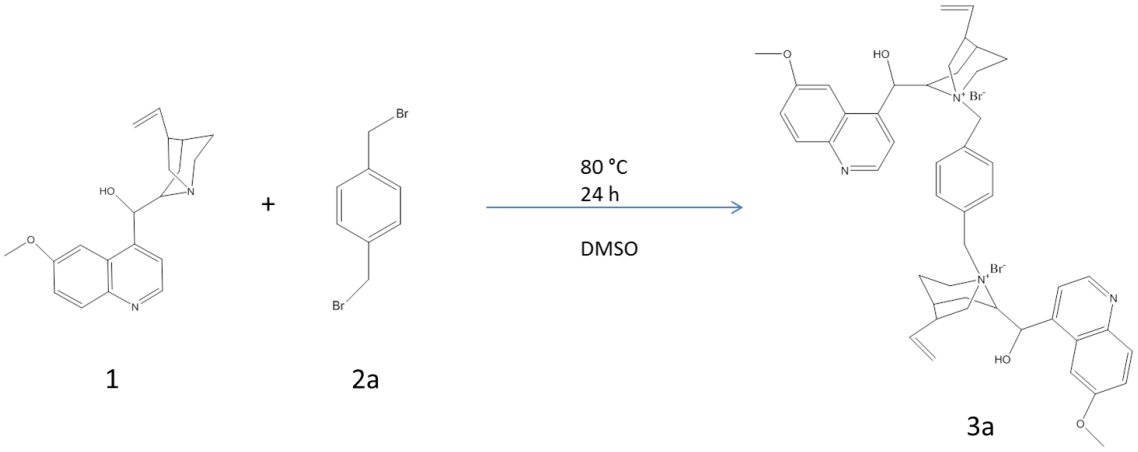
Where $F–F_{adh}$ is the force on the cantilever (F) relative to the adhesion force (F_{adh}), R is the tip end radius, and $d - d_0$ is the deformation of the sample, *i.e.* the penetration of the tip in the sample. The result of this fit is the reduced modulus E^*. Then, the Young's Modulus (E) of the sample was calculated with the following equation:

$$E^* = \left[\frac{1 - {\nu_{\mathrm{s}}}^2}{E} + \frac{1 - {\nu_{\mathrm{tip}}}^2}{E_{\mathrm{tip}}} \right]^{-1} \quad \text{(S2)}$$

Where ν_s is the Poisson's ratio of the sample, ν_{tip} is the Poisson's ratio of the tip, and E_{tip} is the mechanical modulus of the tip. It is safe to assume that the modulus of the tip is very high in comparison to that of the sample and then the second term in the right hand side of Eq. (S2) can be neglected to a first approximation. In this case, the Young's Modulus only depends on the ν_s value. In this work, the reduced modulus data were transformed into Young's modulus following Eq. (S2) and taking a value of $\nu_s = 0.3$ for our samples.

Synthesis of 3a

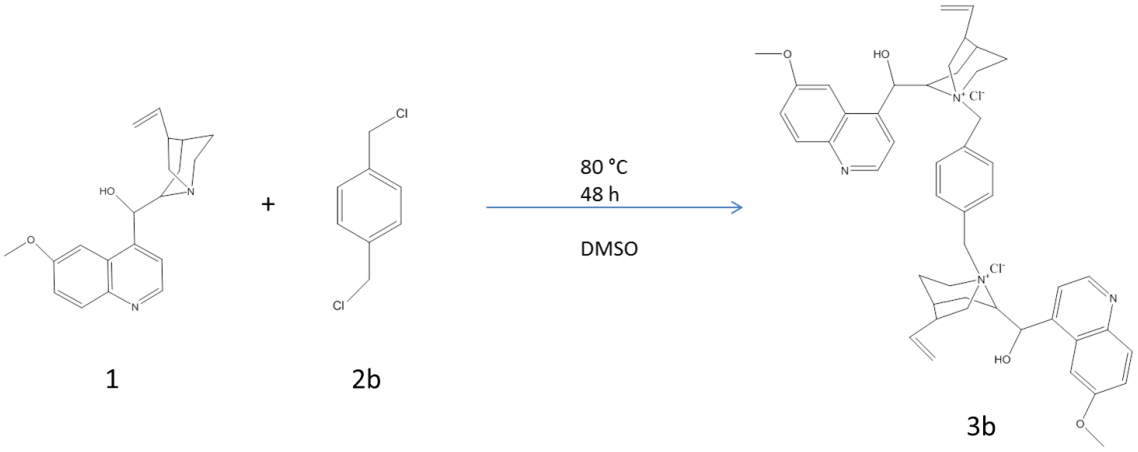
α,α'-Dibromo-p-xylene (**2a**) (0.42 g, 1.6 mmol) and quinine (**1**) (1.5 g, 4.6 mmol) were dissolved and mixed in dimethyl sulfoxide (DMSO) (60 ml). The quaternization reaction was carried out at 80 °C for 24 h. The resulting compound **3a** was isolated by precipitation in tetrahydrofuran and further drying under dynamic vacuum (yield = 58 %). An alternative route for the synthesis of compound **3a** is provided in ref. 5.



1 2a 3a

Synthesis of 3b

α,α'-Dichloro-p-xylene (**2b**) (0.28 g, 1.6 mmol) and **1** (1.5 g, 4.6 mmol) were dissolved and mixed in dimethyl sulfoxide (DMSO) (60 ml). The quaternization reaction was carried out at 80 °C for 48 h. The resulting compound **3b** was isolated by precipitation in tetrahydrofuran and further drying under dynamic vacuum (yield = 65 %).



1 2b 3b

Characterization of 3a by NMR spectroscopy

The ^{1}H COSY and ^{1}H-^{13}C HSQC NMR spectra of **3a** in DMSO-d_6 are shown below in Figures S1 and S2.

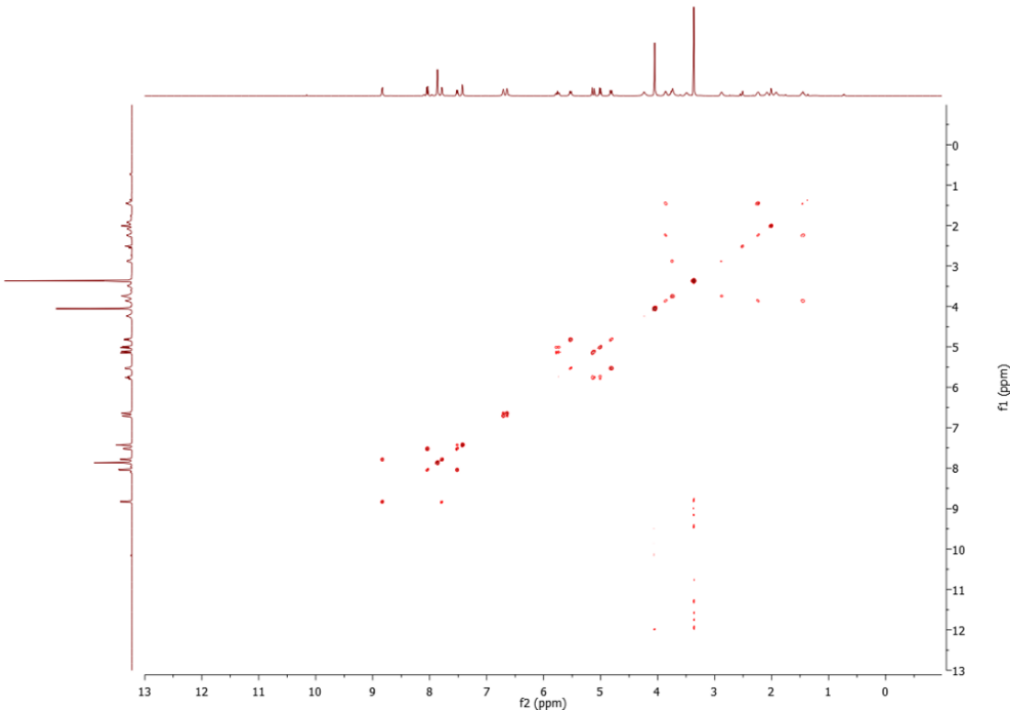


Figure S1: ^{1}H COSY spectrum of 3a.

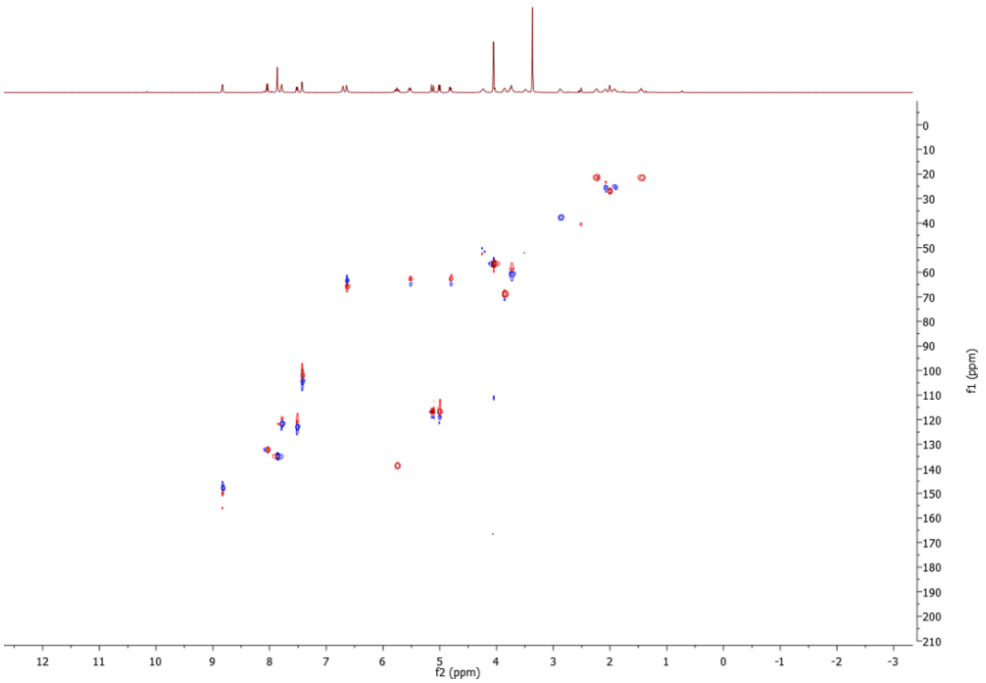


Figure S2: ^{1}H-^{13}C HSQC spectrum of 3a.

Characterization of 3b by NMR spectroscopy

The NMR spectra of **3b** in DMSO-d_6 are shown below (Figures S3, S4 and S5) with the corresponding proton assignments.

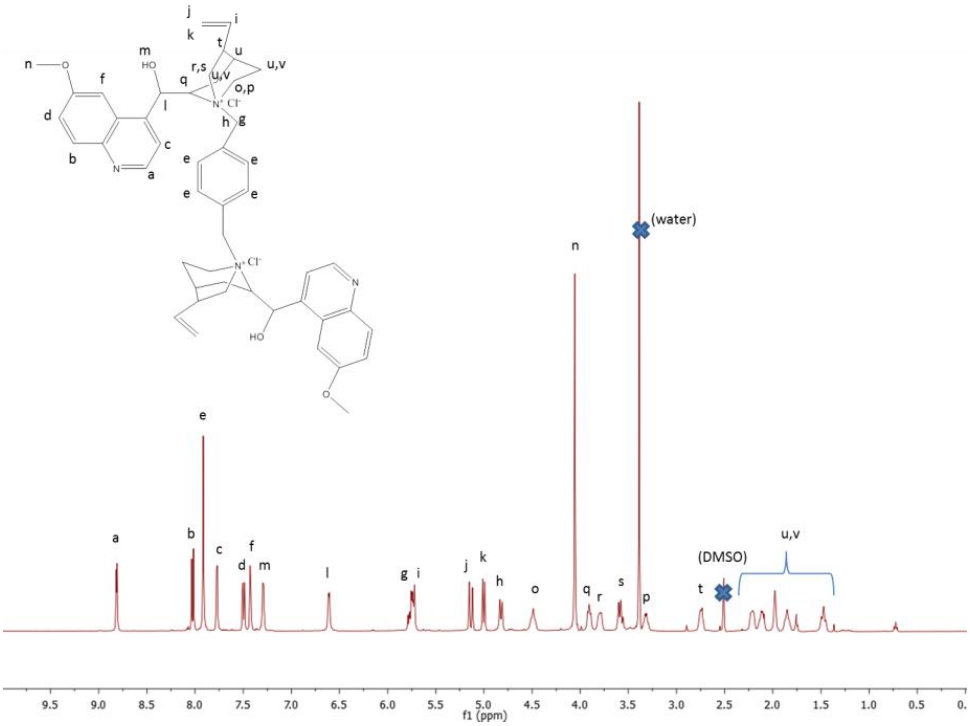


Figure S3: ^{1}H NMR spectrum of 3b.

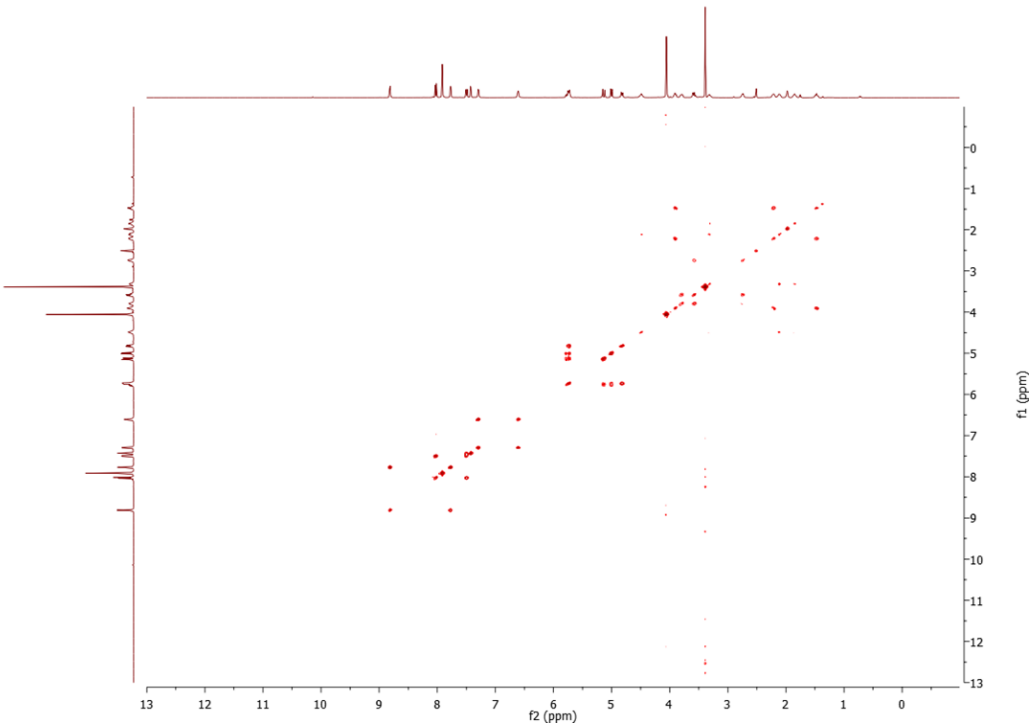


Figure S4: ^{1}H COSY spectrum of 3b.

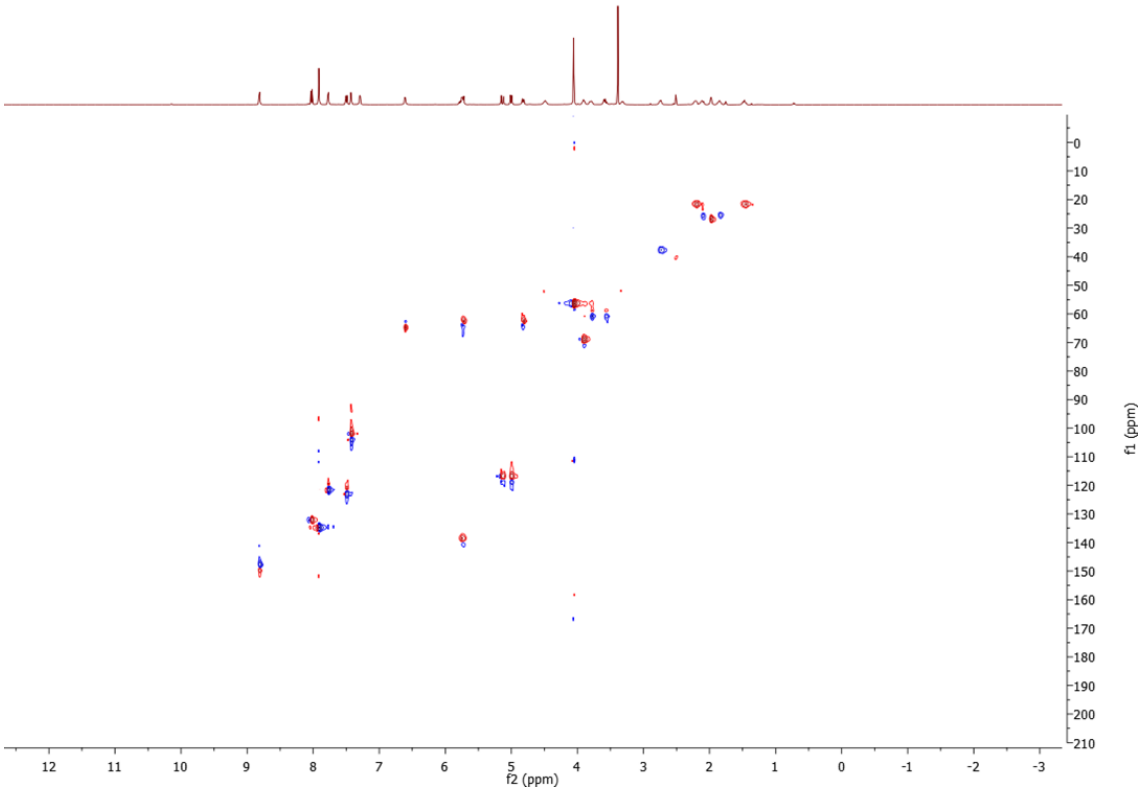


Figure S5: ^{1}H-^{13}C HSQC spectrum of 3b.

Characterization of 3a and 3b by elemental analysis

Table S1 shows the elemental analysis results of **3a** and **3b**, providing a good agreement between the obtained results and the expected ones.

Table S1: Elemental analysis of 3a and 3b.

Sample	% C real	% H real	% N real	% C calculated	% H calculated	% N calculated
3a	63.16	6.07	5.53	63.17	6.14	6.14
3b	69.98	6.73	6.12	70.0	6.81	6.81

Characterization of 3a and 3b by FTIR spectroscopy

As illustrated in Figure S6, changes in the IR band corresponding to the C-N vibration (around 1100 cm^{-1}) were observed upon quaternization.[6,7]

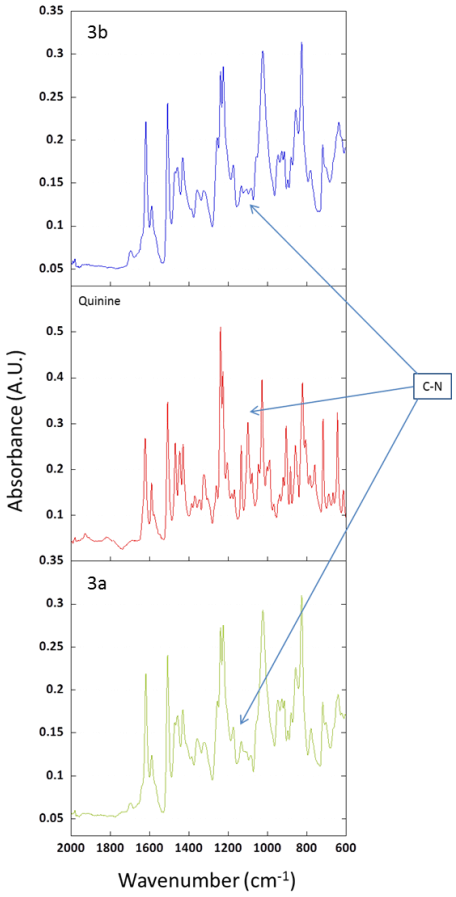
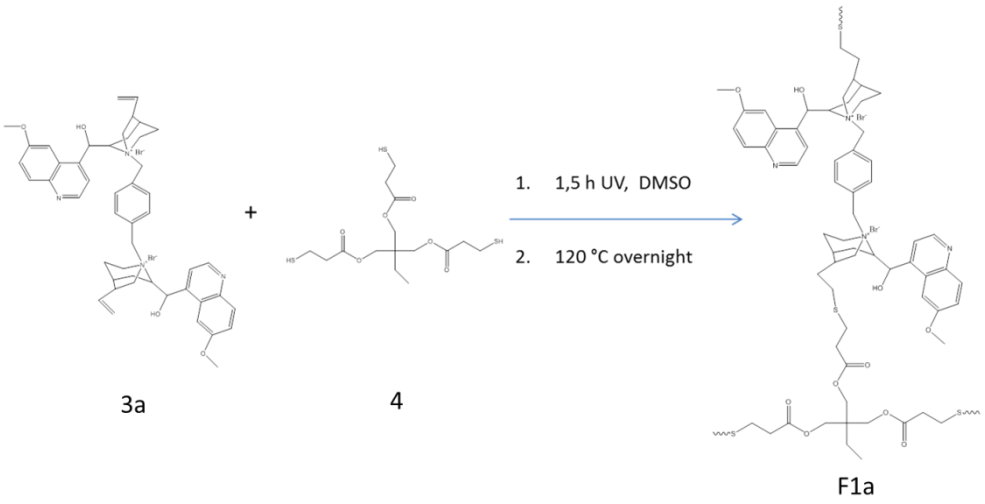


Figure S6: FTIR spectra of 3b (blue) and 3a (green) in comparison to 1 (red).

Synthesis of films with trimethylolpropane tris(3-mercaptopropionate)

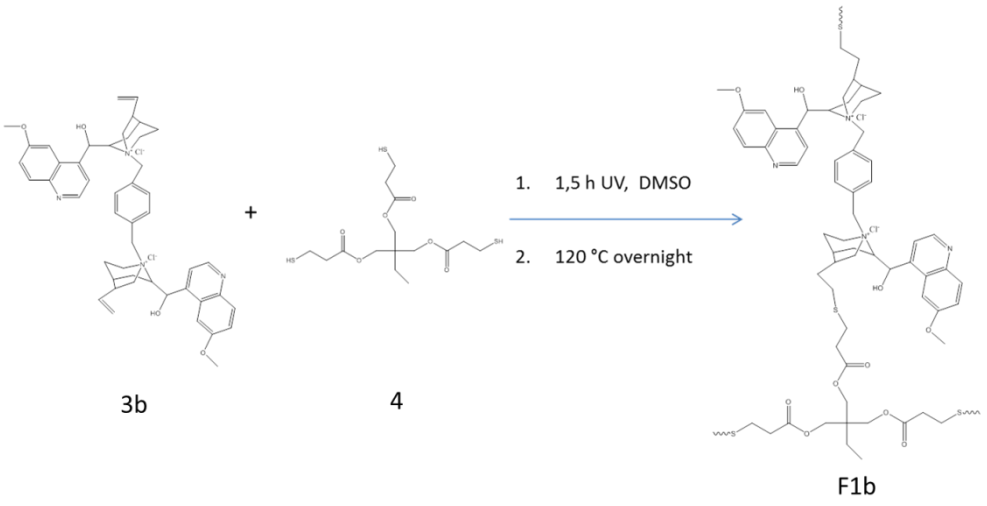
- **Synthesis of F1a**

3a (65.5 mg, 0.14 mmol of quinine) was mixed with trimethylolpropane tris(3-mercaptopropionate) (**4**) (20.2 mg, 0.14 mmol of –SH) and dissolved in DMSO (188 µl) at room temperature. Then, 2,2-dimethoxy-1,2-diphenylethan-1-one (4.0 mg, 0.016 mmol) was added and the mixture was distributed in different small glasses with 10 µl of the final solution in each one. After that, the samples were maintained 1.5 h under UV light and subsequently, were kept into an oven at 120 °C overnight to remove the DMSO solvent.



- **Synthesis of F1b**

3b (59.7 mg, 0.15 mmol of quinine) was mixed with **4** (20.4 mg, 0.15 mmol of –SH) and dissolved in DMSO (193 µl) at room temperature. Then, 2,2-dimethoxy-1,2-diphenylethan-1-one (3.3 mg, 0.013 mmol) was added and the mixture was distributed in different small glasses with 10 µl of the final solution in each one. After that, the samples were maintained 1.5 h under UV light and subsequently, were kept into the oven at 120 °C overnight to remove the DMSO solvent.



As expected, after the cross-linking reaction the film becomes insoluble and for this reason the main technique used to analyze films **F1a** and **F1b** is FTIR.

Characterization of F1a and F1b by FTIR spectroscopy

Figures S7 and S8 show the FTIR spectra of **F1a** and **F1b** in comparison to **4**. When the film is formed, changes in the bands corresponding to C=C stretching and bending vibrations can be observed due to thiol-ene reaction. Although the reaction is not completed, the intensity of these bands decreases. In the cross-linked films, the appearance of other bands (e.g. C=O stretching) supports the presence of trimethylolpropane tris(3-mercaptopropionate) in the insoluble sample.[6-8]

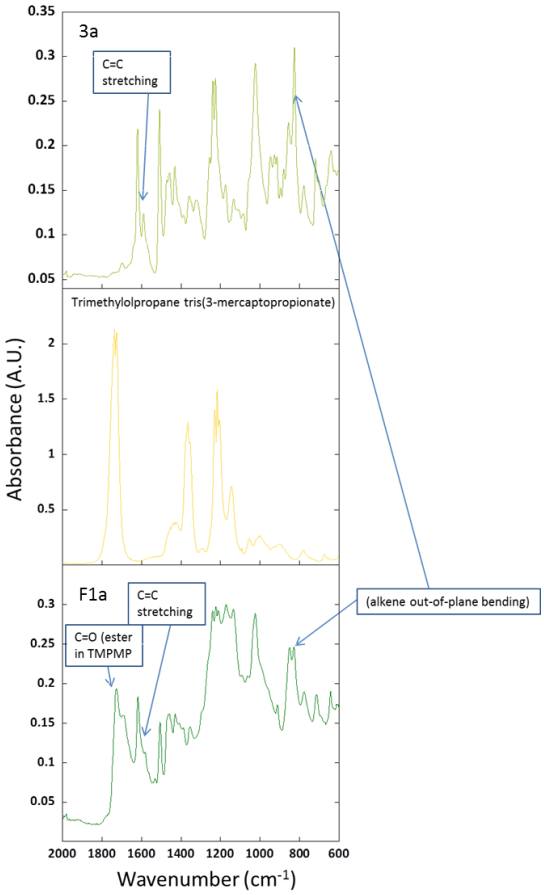


Figure S7: FTIR spectra of 3a (light green) and F1a (dark green) in comparison to 4 (yellow).

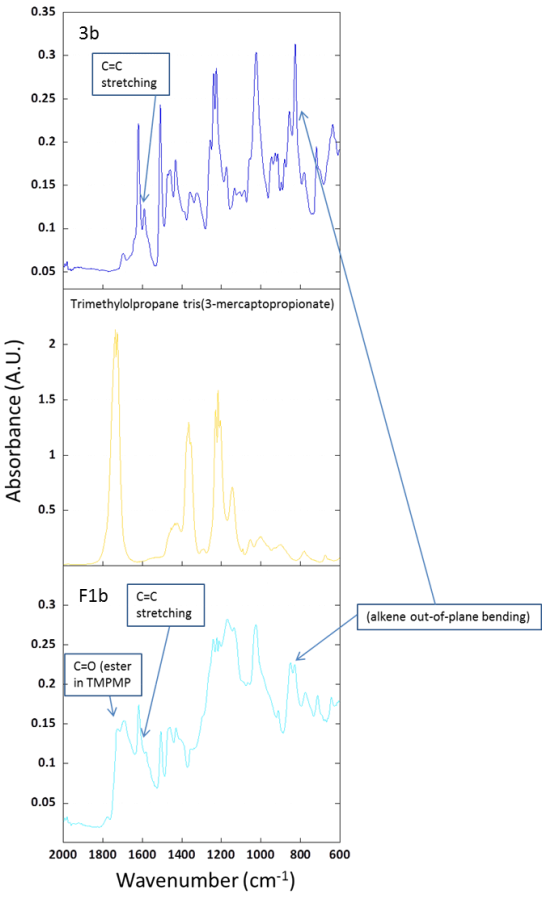


Figure S8: FTIR spectra of 3b (dark blue) and F1b (light blue) in comparison to 4 (yellow).

Characterization of 5 by NMR spectroscopy

The NMR spectra of **5** in DMSO-d_6 are shown below (Figures S9, S10 and S11) with the corresponding proton assignments.

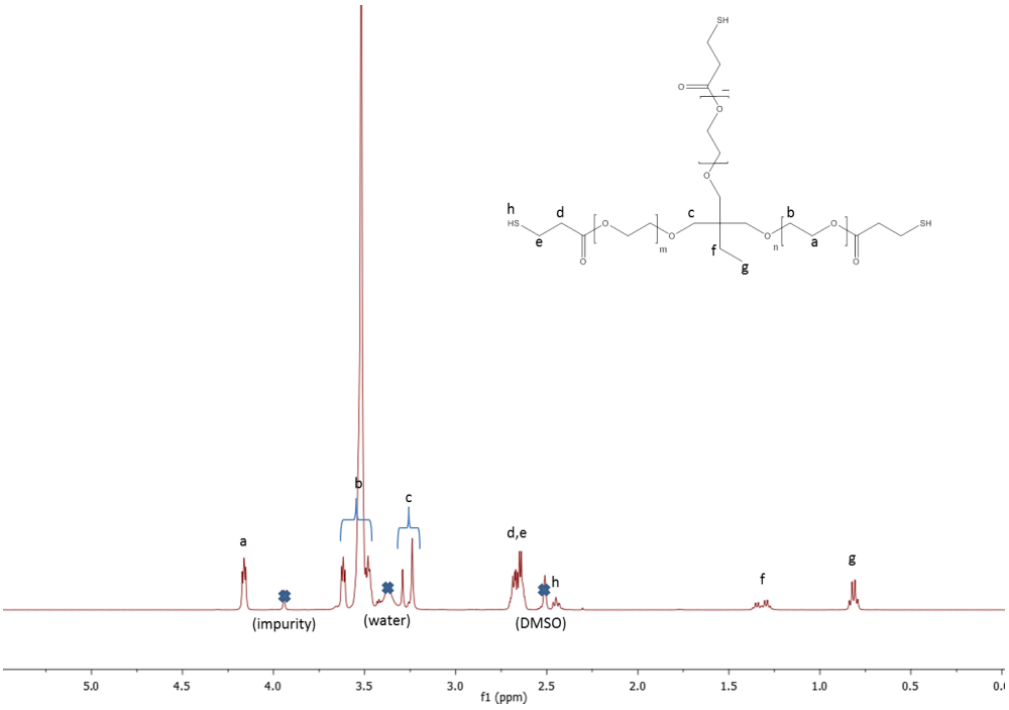


Figure S9: ^{1}H NMR spectrum of 5.

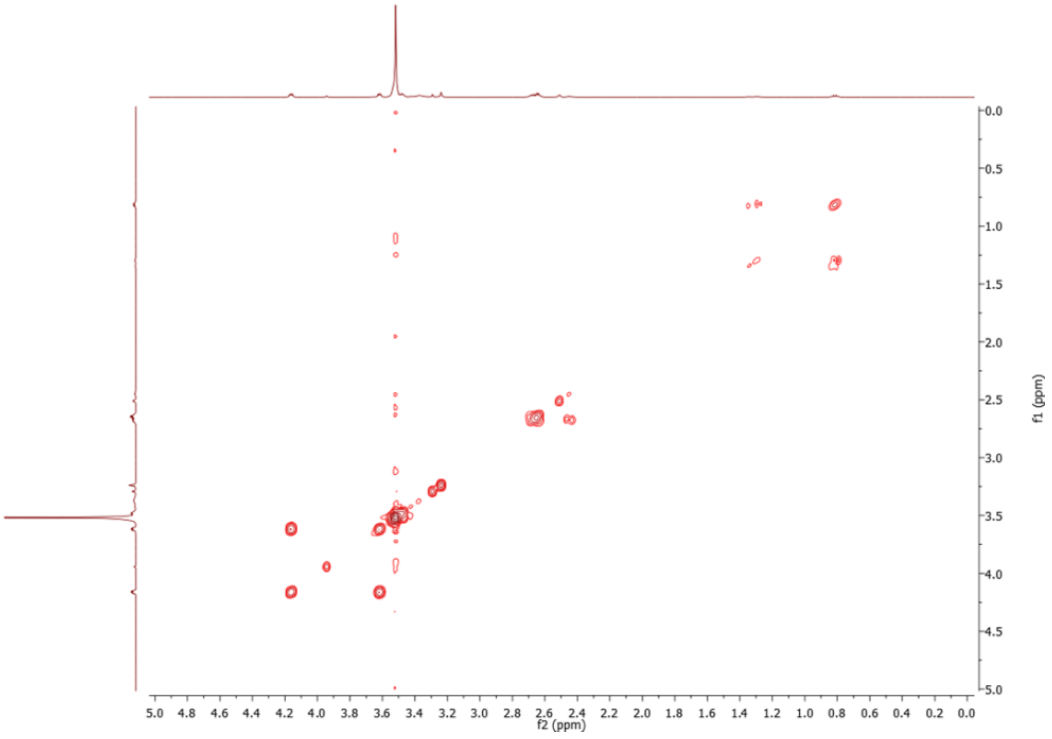


Figure S10: ^{1}H COSY spectrum of 5.

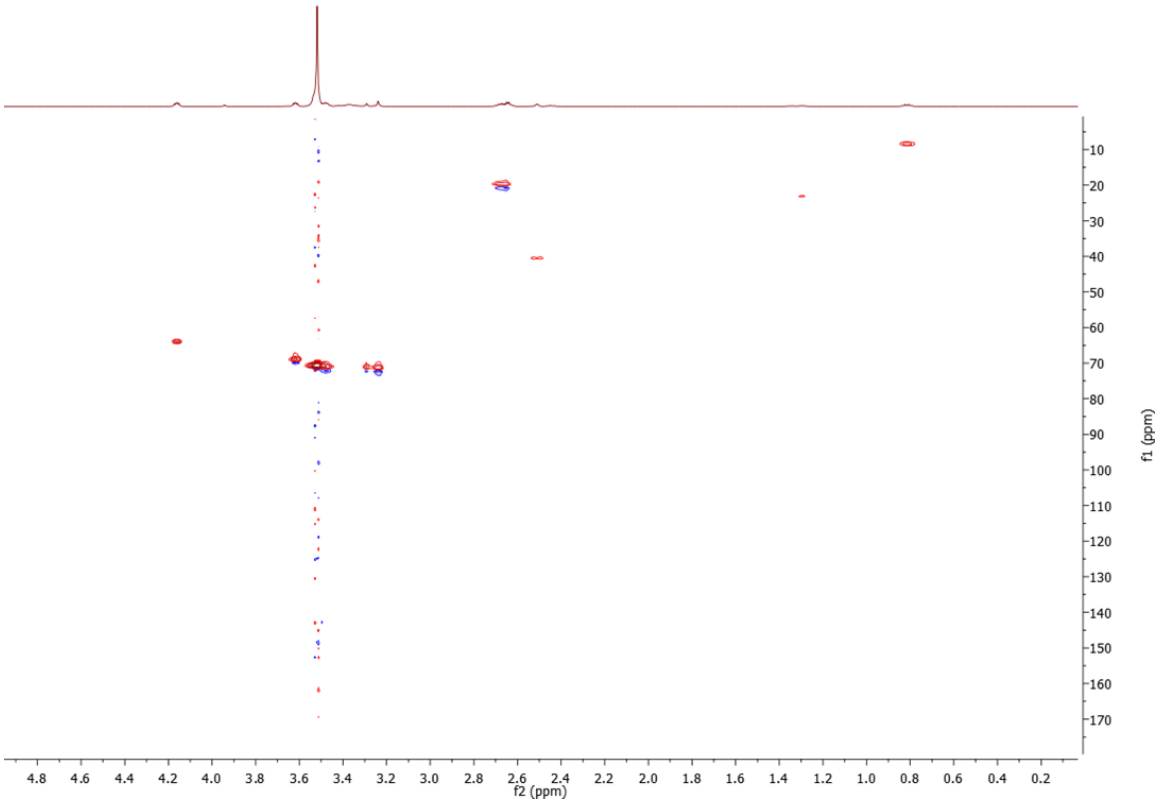
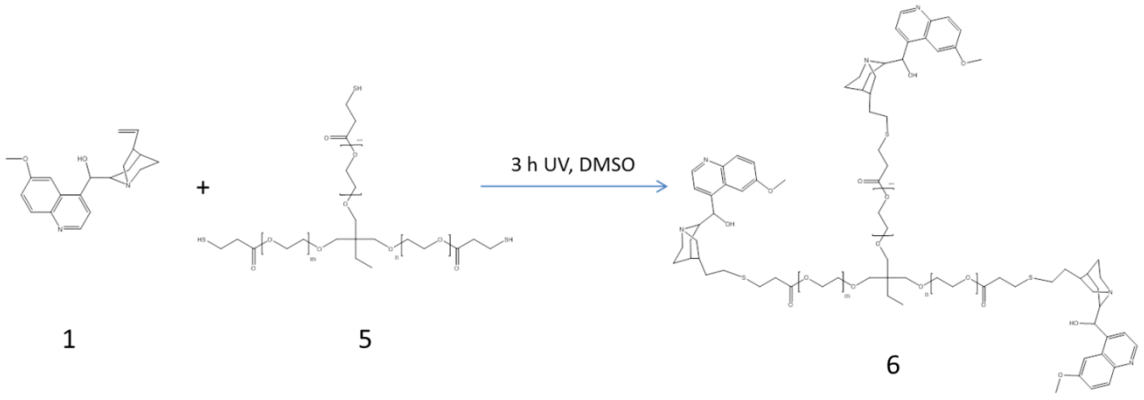


Figure S11: ^{1}H-^{13}C HSQC spectrum of 5.

Synthesis of 6

THIOCURE ETTMP 1300 - ethoxilated trimethylolpropan tris(3-mercaptopropionate) (**5**) (2.0 g, 1.5 mmol) and **1** (3.5 g, 10.8 mmol) were dissolved and mixed in DMSO (17 ml) at room temperature. Then, 2,2-dimethoxy-1,2-diphenylethan-1-one (248 mg, 0.96 mmol) was added and the mixture was maintained 3 h under UV light. The resulting compound **6** was isolated by precipitation in toluene and further drying under dynamic vacuum (Yield = 20 %).



Characterization of 6 by FTIR spectroscopy

Figure S12 shows the FTIR spectra of **6** in comparison to **1**. When **6** is formed, changes in the bands corresponding to the C=C stretching and bending vibrations can be observed upon the reaction of the thiol with the quinine double bond. Furthermore, the appearance of other bands (e.g. C=O stretching) evince the presence of ethoxilated trimethylolpropan tris(3-mercaptopropionate) in the sample.[6-8]

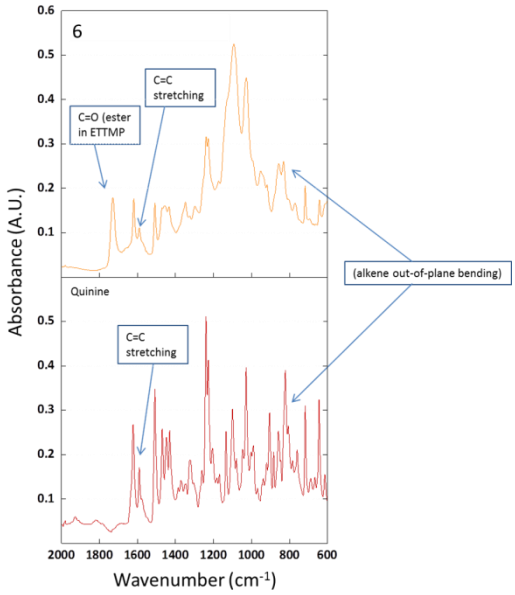


Figure S12: FTIR spectra of 6 (orange) in comparison to quinine (1) (red).

Synthesis of films with ethoxilated trimethylolpropan tris(3-mercaptopropionate)

- **Synthesis of F2b**

In a typical procedure, **6** was mixed in melt at 120 °C with α,α'-dichloro-*p*-xylene **(2b)**. Subsequently, the mixture was spread on a thin glass forming a film and was maintained at 120 °C for 48 h. Different amounts of the crosslinker **2b** were used, as summarized in Table S2.

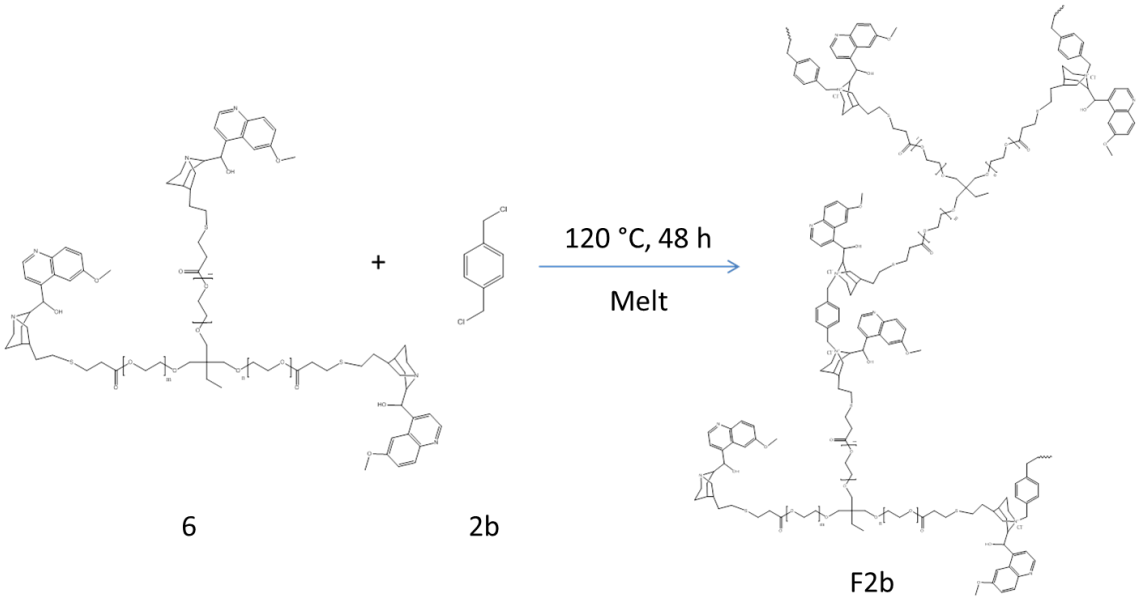


Table S2: Different F2b films prepared in this work

F2b film	6	2b	[2b] / [quinine] ratio
Film **A**	33.0 mg (0.044 mmol quinine)	0.96 mg (0.0055 mmol)	1/8
Film **B**	31.2 mg (0.041 mmol quinine)	1.80 mg (0.0103 mmol)	1/4
Film **C**	30.5 mg (0.040 mmol quinine)	2.33 mg (0.0133 mmol)	1/3
Film **D**	31.4 mg (0.041 mmol quinine)	3.59 mg (0.0205 mmol)	1/2

Characterization of F2b by FTIR spectroscopy

FTIR spectra showed in Figure S13 do not provide enough resolution to observe quaternization bands, due to the superposition of the characteristic quaternization bands with the bands of the ethoxilated trimethylolpropan tris(3-mercaptopropionate) compound which is incorporated to the cross-linked film via thiol-ene reaction. Evidence that the quaternization reaction was produced comes from the decrease in the intensity of the C=C vibration bands upon increasing the amount of **2b**. Moreover, a solubility test confirmed the cross-linked nature of the film due to its insolubility.

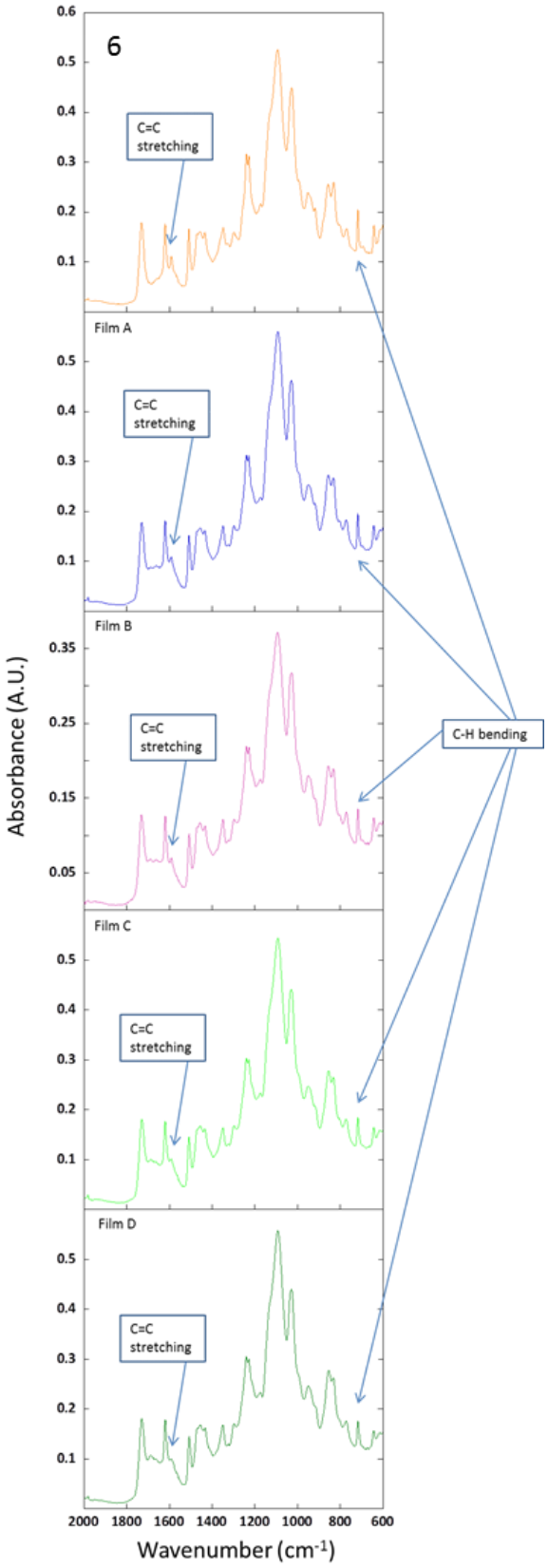


Figure S13: FTIR spectra of film A (blue), film B (pink), film C (light green) and film D (dark green) in comparison to 6.

Characterization of F2b by TGA

Thermal decomposition of the samples was investigated by TGA illustrated in Figure S14. Film **D** ([2b] /[quinine] ratio = 1/2) shows a slight increase in thermal stability in comparison to compound **6** which is not cross-linked and the final residue is slightly higher in the case of film **D**.

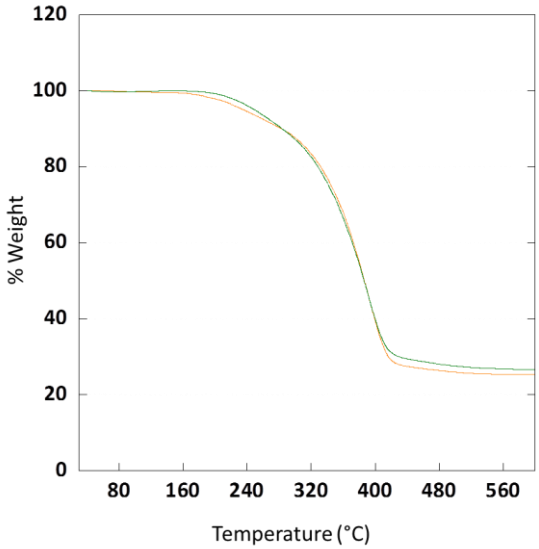


Figure S14: TGA curves for 6 (orange) and film D (green).

Characterization of F2b by DSC

Figure S15 shows the DSC curves for **5**, **6** and film **D**. As can be observed, the DSC curves of **5** and **6** are very different (as a consequence of the incorporation of quinine moieties to **5**) which increases the glass transition temperature, T_g. On the contrary, DSC curves for **6** and film **D** are very similar due to the amporhous structure of **6** that provides high flexibility to the film even after cross-linking. The values of T_g corresponding to **5**, **6** and film **D** are shown in Table S3.

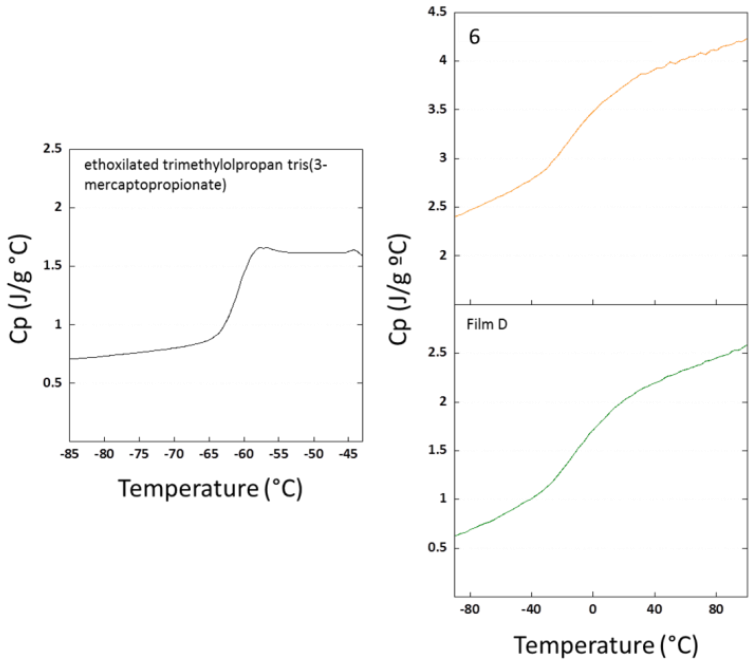


Figure S15: DSC curves for 5 (black), 6 (orange) and film D (green).

Table S3: glass transition temperature for ethoxilated trimethylolpropan tris(3-mercaptopropionate), quinine-based dendrimer and film D (100 % quaternization).

Sample	Tg (°C)
ethoxilated trimethylolpropan tris(3-mercaptopropionate) (5)	-60
(6)	-18
Film D (2b /Quinine ratio = 1/2)	-14

Photographs of F2b

Figure S16 shows photographs of the films. As can be observed, these films present a dark brown color and a flexible appearance. Moreover, the films spread on a thin glass can be easily detached from the surface.

Figure S16: Photographs of the F2b.

Figure S17 shows a water drop under UV light immediately after being deposited on top of film **D**. The drop turns fluorescent rapidly due to the fast delivery of unreacted compound **6** from the film.

Figure S17: A water drop deposited on top of Film D under UV light.

Characterization of F2b by UV-Vis spectroscopy

Upon immersion of F2b in water, fluorescence was observed. Figure S18 shows the absorption band arising upon immersion of film D (green) and film A (blue) in water. Both films present the same UV absorption band located at around 330 nm which can be assigned to the quinine absorption band.[9-12]

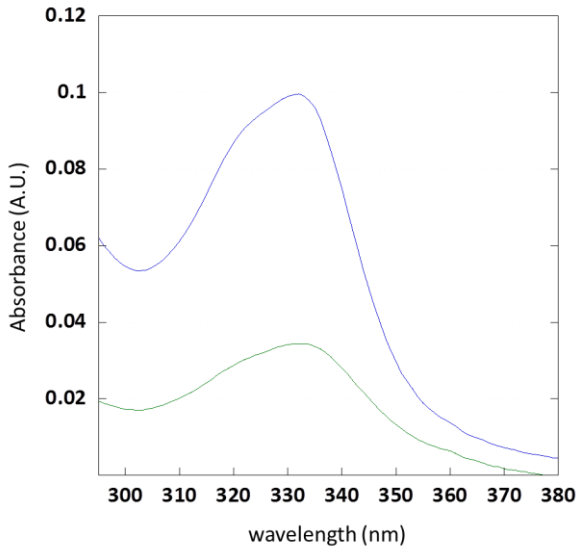


Figure S18: UV-Vis spectra of the delivered compound from Film A (blue) and Film D (green) at time t = 0 h.

Biological tests of F2b

Cell and bacterial tests were carried out to analyze some biological properties of the ethoxilated trimethylolpropan tris(3-mercaptopropionate) films. The methodology for each test is described below.

- Cell test

Cells were washed with PBS and harvested by using trypsin for hDF and scraping for Raw 264.7. Then the cells were suspended in DMEM and counted with a hemocytometer.

0.5 ml DMEM containing 1×10^6 cells were transferred into each well of a 48 well plate. Cells were incubated for 24 h before exposure to the testing compounds. For the elution test, cells were incubated with 300 µL of elution medium (films incubated in 1.5 ml DMEM for 24 h to prepare the elution medium), 4 replicates were prepared for each film, and 4 wells were set for each individual film. Cells without any treatment were used as control. DMEM medium without cells were used as blanks. Cells were incubated at 37 °C for 72 h, then, 30 µL

AlamarBlue agent were added. 4 h later, the plate was read by a fluorescent plate reader (Infinite 200 PRO) at ex/em 560/590 nm.

- Bacterial Test

Films were sterilized under UV light. 2 mL of LB medium were added to each film and incubated 24 h. For the elution test, 300 µL of elution medium were transferred into 48 well plate. 10^6 bacteria in 10 µL broth were inoculated to each well and incubated at 37 °C for 4 h. Then, 30 µL AlamarBlue agent were added for another 2 h. LB broth with E.coli. was used as control and sample with pure LB broth was used as blank, 4 replicates were prepared for each type of film and 4 parallel wells were set for each individual film. Fluorescent intensity was determined by a fluorescent plate reader with ex/em 560/590 nm.

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