Supporting information

Keto-Polymethines: a Versatile Class of Dyes with Outstanding

Spectroscopic Properties for In Cellulo and In Vivo Two-Photon

Microscopy Imaging

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Synthesis. NMR spectra (¹H, ¹³C) were recorded on a BRUKER® Advance operating at 500.10 MHZ and 125.75 MHz for ¹H and ¹³C, respectively. ¹³C NMR spectra were recorded using the uDEFT experiment.¹ Data are listed in parts per million (ppm) and are reported relative to residual solvent peaks being used as internal standard (¹H (CDCl₃): 7.26 ppm, ¹³C (CDCl₃): 77.2 ppm; ¹H (DMSO-d6): 2.50 ppm, ¹³C (DMSO-d6): 39.5 ppm; ¹H (CD₃CN): 1.94 ppm). High resolution mass spectrometry measurements were performed at Centre Commun de Spectrometrie de Masse (Villeurbanne, France) by ESI-TOF: a Bruker Daltonics ® Micro TOF-Q II was used with a resolution of 8000, in positive mode with a capillary tension of 4500V, a source temperature of 180 °C, and a cone tension of 60V. The internal reference used for calibration was sodium formate. GPC/SEC measurement was performed at Centre for the Characterization of Polymers by Liquid Chromatography of the Institut de Chimie de Lyon using a Viscotek apparatus with the following configuration: Rhéodyne 7725i injector, three Styragel HR 4E columns (WAT0044241) at 70 °C and RI-55 °C (Viskotek VE3580) and viscosimeter (Viskotek T60A) detectors. Starting materials were purchased from Sigma Aldrich®, Acros Organics® or Alfa Aesar® with the best available quality grade. All reactions were routinely performed under argon atmosphere in anhydrous solvents. Column chromatography were performed using Acros Organics® (0.035-0.070 mm) silicagel or neutral aluminium oxide (50-200 µm, 60 A). All reagents were purchased from commercial sources and used without further purification, otherwise noted. 1, 2, D¹, D³A², D⁵, ³D³, ⁴D⁴, ⁵ and AA^6 were prepared according to published procedures.

DOSY characterization for the polymer. 1D-1H and 2D-DOSY experiments were carried out at 298 K on a 500 MHz BRUKER® Avance NMR spectrometer employing a 5 mm TXI probe equipped with z-gradients. 2D-DOSY experiments were acquired using a LED experiment with bipolar pulses. Gradients were linearly sampled in 50 points. 16 scans were acquired on 8192 data points. The gradient pulse length was d/2 = 1.5 ms and the D diffusion delay were adapted to the sample for values in the 150-250 ms range. The DOSY spectra were obtained by applying an Inverse Laplace Transform (ILT) along the diffusion axis, using the Gifa algorithm embedded into the commercial software NMR notebook (NMRTEC, Illkirch). Careful spline polynomial correction was applied along the F2 dimension before the ILT processing. PDI was then calculated from the ratio of diffusion coefficients D_w/D_n measured from DOSY signals, D_w corresponding to the average diffusion coefficient of the polymer main chain, while D_n corresponds to the average diffusion coefficient of the chromophoric extremities.

Titrations. Plot of the spectroscopic data obtained upon titration of each keto-chromophore in solution with methanol aliquots was done using the following general methodology: to a known volume (3 mL) of a solution of the keto-heptamethine in dichloromethane ($D^1D^1=O$ and $D^2A=O$) or acetonitrile (AA=O) of identical concentrations (2.7 10⁻⁶ M) were progressively added aliquots of methanol. At each point of the titration the total concentration of keto-chromophore, C₀ (mol.L⁻¹) is given by

$$C_0 = C_{in} \times \frac{V_0}{(V_0 + V_a)}$$

With C_i : initial concentration of the keto-chromophore's solution (mol.L⁻¹), V_0 : initial volume of the solution (L), V_a : added methanol volume (L)

Similarly it can be shown that the total concentration in methanol_S (mol.L⁻¹)_s given by

$$S = \frac{\rho_{MeOH}}{M_{MeOH}} \times \frac{V_a}{(V_0 + V_a)}$$

With ρ_{MeOH} : the density of methanol (g.L⁻¹) and M_{MeOH}: the molar mass of methanol (g.mol¹)

By following the decrease in the emission intensity originating from the unbound ketoderivative, it is possible to calculate the concentration of the hydrogen-bonded complex [C-S] using the formula

$$[C-S] = Co \times \frac{(I_o - I)}{I_0}$$

Where I is the measured fluorescence intensity, and I_0 the "dilution corrected" initial emission intensity of the pure non-bonded form, which is related to the initial emission intensity by:

$$I_0 = I_{in} \times \frac{Co}{C_{in}}$$

From the [C-S] concentration, it is possible to calculate [C] and [S], concentration fort the unbound keto species and unbound methanol, respectively, following

$$[C] = Co - [C - S]$$
$$[S] = So - [C - S]$$
inding constant

Assuming a 1:1 binding isotherm, the binding constant K_a can be expressed as follows:

$$K_a = \frac{[C-S]}{[C]x[S]}$$

Or alternatively

$$[C]x[S]K_a = [C - S]$$

Upon plotting the evolution of [C]x[S] vs the product [C-S], a straight line should be obtained in case of a 1:1 association, which slope corresponds to K_a.



Figure S1. 2D DOSY NMR of DDPHEA=O in CD₃OD



Figure S2. SEC analysis of **DD**^{PHEA}=**O** in LiBr-DMF (concentration: 3.58 mg.mL⁻¹). Top: with refractive index detection Bottom: with viscosimetry detection. Mn, M_w and PDI determination are based on the data obtained with refractive index detection

Additional simulations details. The parametrization of the SCC-DFTB CM3 charges used in the present work is the same used by Simon and co-workers for OH, *i.e.* $D_{OH} = 0.12873$. $D_{CO} = 0.1$ and $D_{CH} = 0.0$ were used as they well reproduce the M062X-D3 geometries and binding energies of small methanol clusters. For instance, the binding energy of the (CH₃OH)₂ dimer is -5.53 kcal.mol⁻¹ at the SCC-DFTB level of theory as compared to -5.89 and -6.37 kcal.mol⁻¹ at the M062X-D3/aug-cc-pVTZ and B3LYP-D3/QZVP levels of theory, respectively. These latter results were corrected for basis set superposition error (BSSE) and were obtained using the Gaussian 09 package.⁷ For chlorine, the D_{CC1} = 0.0 value was used.



Figure S3. Radial distribution functions for O*-H in methanol (black curve, plain line) and dichloromethane (red curve, dashed line). O* stands for the oxygen atoms of the model $DD^{Me}=O$ and H for the hydrogen atoms of the solvent.



Figure S4. Transmission (left) and two-photon fluorescence (right) microscopy images of T24 cancer cells after their incubation with $D^2A=O$ (top), $D^5A=O$ (middle) and AA=O (bottom). Two-photon excitation was performed at 950 nm.



Figure S5. Transmission (left) and fluorescence (right) confocal microscopy images of T24 cancer cells after their incubation with $DD^{PHEA}=O$ (top), $D^2D^2=O$ (middle) and $D^2D^5=O$ (bottom). One photon excitation was performed at 560 nm.

Compound D¹D¹



To a solution of 961 mg of 1 (4.20 mmol, 1 equiv.) and 2.86 g of D^1 (8.82 mmol, 2.1 equiv.) in 60 mL of absolute ethanol was added 0.85 mL of distilled pyridine (10.5 mmol, 2.5 equiv.). The solution was stirred for 15 h at 40 °C. The resulting greenish solution was diluted with 100 mL of dichloromethane (DCM) and washed with a dilute aqueous solution of HCl (50 mL), then water (50 mL) and brine (50 mL). The organic layer was dried over Na₂SO₄ and concentrated. The crude solid was dissolved in a minimal amount of DCM and precipitated by slow addition of pentane to afford the product as a dark greenish solid in 64% yield (2.034 g).

¹H NMR (CDCl₃, 500.10 MHz): δ 8.35 (d, ³J = 14 Hz, 2H, =CH), 7.40 (m, 4H, CH_{Ar}), 7.26-7.23 (m, 4H, CH_{Ar}), 6.17 (d, ³J = 14 Hz, 2H, =CH), 4.19 (m, 4H, N-CH₂), 2.88 (m, 2H, H_{eq}), 2.23 (dd, ²J = 14 Hz, ³J = 14 Hz, 2H, H_{ax}), 1.86 (m, 4H, CH₂), 1.73 (s, 12H, C(CH₃)₂), 1.60 (m, 1H, CH), 1.46 (m, 4H, CH₂), 1.34 (m, 8H, CH₂), 1.08 (s, 9H, C(CH₃)₃), 0.88 (t, ³J = 7 Hz, 6H, CH₃-CH₂).

¹³C NMR (CDCl₃, 125.75 MHz): δ 172.5 (C_{quat}), 150.4 (C_{quat}), 144.3 (CH), 142.3 (C_{quat}), 141.2 (C_{quat}), 129.0 (CH), 127.4 (C_{quat}), 125.6 (CH), 122.4 (CH), 111.2 (CH), 101.1 (CH), 49.6 (C_{quat}), 45.0 (N-CH₂), 42.5 (CH), 32.6 (C_{quat}), 31.5 (CH₂), 28.3 (CH₃), 28.2 (CH₃), 27.7 (CH₂), 27.6 (CH₃), 27.4 (CH₂), 26.8 (CH₂), 22.6 (CH₂), 14.1 (CH₃).

UV-Vis (CH₃OH): $\lambda_{max} = 780$ nm ($\varepsilon_{max} = 257000$ L.mol⁻¹.cm⁻¹).

HRMS (ESI+): $[M-Br]^+ = 679.4758$ (calcd for $C_{46}H_{64}ClN_2^+$: 679.4753).





To a solution of 200 mg of D^1D^1 (0.26 mmol, 1 equiv.) and 61 mg of *N*-hydroxysuccinimide (0.53 mmol, 2 equiv.) in 5 mL of anhydrous DMF were added 90 µL of distilled disopropylethylamine (DIEA) (0.53 mmol, 2 equiv.) and the mixture was stirred for 3 h at RT. Then the solution was added to 10 mL of DCM and washed with an aqueous saturated solution of NH₄Cl (10 mL) then with water (10 mL). The organic layer was dried over Na₂SO₄ and concentrated. After filtration trough an activated alumina plug (75 g Al₂O₃ with 6% H₂O) and elution with DCM/PE (1:1, R_f = 0.26), the product was isolated as a reddish solid in 72% yield (126 mg).

¹H NMR (CDCl₃, 500.10 MHz): δ 8.16 (d, ³J = 13 Hz, 2H, =CH), 7.26-7.17 (m, 4H, CH_{Ar}), 6.90 (d, ³J = 7 Hz, 2H, CH_{Ar}), 6.68 (d, ³J = 8 Hz, 2H, CH_{Ar}), 5.48 (d, ³J = 13 Hz, 2H, =CH), 3.67 (t, ³J = 7 Hz, 4H, N-CH₂), 2.87 (d, ²J = 13 Hz, 2H, H_{eq}), 2.13 (dd, ²J = 14 Hz, ³J = 14 Hz, 2H, H_{ax}), 1.73 (t, ³J = 7 Hz, 4H, CH₂), 1.68 (s, 6H, C(CH₃)₂), 1.67 (s, 6H, C(CH₃)₂), 1.45-1.32 (m, 13H, CH₂ and CH), 1.06 (s, 9H, C(CH₃)₃), 0.91 (t, ³J = 7 Hz, 6H, CH₃-CH₂).

¹³C NMR (CDCl₃, 125.75 MHz): δ 186.6 (C_{quat}), 162.4 (C_{quat}), 144.4 (CH), 139.8 (C_{quat}), 141.2 (C_{quat}), 132.9 (CH), 127.7 (CH), 126.6 (C_{quat}), 121.9 (CH), 120.5 (CH), 106.7 (CH), 46.6 (C_{quat}), 43.7 (CH), 42.7 (N-CH₂), 32.7 (C_{quat}), 31.6 (CH₂), 28.9 (CH₃), 28.8 (CH₃), 27.6 (CH₃), 27.0 (CH₂), 26.9 (CH₂), 26.2 (CH₂), 22.7 (CH₂), 14.1 (CH₃).

UV-Vis (CH₃OH): $\lambda_{max} = 532 \text{ nm} (\epsilon_{max} = 56000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

UV-Vis (CH₃OH + CH₃CO₂H): $\lambda_{max} = 712 \text{ nm} (\epsilon_{max} = 99000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

HRMS (ESI+): $[M+H]^+ = 661.5081$ (calcd for C₄₆H₆₅N₂O⁺: 661.5091).



Compound D²



A solution of 6 g of freshly distilled 2,3,3-trimethylindolenine (37.68 mmol, 1 equiv.) and 18.1 mL of 1-bromododecane (75.36 mmol, 2 equiv.) in 30 mL of anhydrous toluene was stirred for 3 days at 110 °C. The purple solution was concentrated and the remaining starting material was removed using dynamic trap-to-trap technique. The obtained dark reddish residue was recrystallized in EtOAc to afford the product as a dark reddish solid in 21% yield (3.253 g).

¹H NMR (DMSO-d₆, 500.10 MHz): δ 7.98 (m, 1H, CH_{Ar}), 7.84 (m, 1H, CH_{Ar}), 7.61 (m, 2H, CH_{Ar}), 4.45 (t, ³J = 8 Hz, 2H, N-CH₂), 2.84 (s, 3H, N=C-CH₃), 1.82 (quint, ³J = 8 Hz, 2H, CH₂), 1.53 (s, 6H, C(CH₃)₂), 1.40-1.22 (m, 18H, CH₂), 0.85 (t, ³J = 7 Hz, 3H, CH₂-CH₃).

¹³C NMR (DMSO-d₆, 125.75 MHz): δ 196.4 (C_{quat}), 141.9 (C_{quat}), 141.1 (C_{quat}), 129.4 (CH), 128.9 (CH), 123.5 (CH), 115.5 (CH), 54.1 (C(CH₃)₂), 47.6 (N-CH₂), 31.2 (CH₂), 29.0 (2 CH₂), 28.9 (CH₂), 28.8 (CH₂), 28.7 (CH₂), 28.6 (CH₂), 27.2 (CH₂), 25.8 (CH₂), 22.4 (CH₂), 22.0 (C(CH₃)₂), 14.0 (CH₃), 13.9 (CH₃).

HRMS (ESI+): $[M-Br]^+ = 328.2998$ (calcd for $C_{23}H_{38}N^+$: 328.2999).



Compound D²D²



To a solution of 234 mg of 1 (1.02 mmol, 1 equiv.) and 920 mg of D^2 (2.25 mmol, 2.2 equiv.) in 10 mL of absolute ethanol was added 0.21 mL of distilled pyridine (2.56 mmol, 2.5 equiv.). The reaction was stirred for 17 h at 40 °C and then allowed to cool to RT. Ethanol was evaporated and the residue was dissolved in DCM and washed with an aqueous solution of HBr 1M then with water. The organic layer was dried over Na₂SO₄ and concentrated. The crude solid was purified by flash chromatography on silica, using DCM/MeOH as eluent (95:5, $R_f = 0.63$) to afford the product as a greenish solid in 68% yield (649 mg).

¹H NMR (CDCl₃, 500.10 MHz): δ 8.34 (d, ³J = 14 Hz, 2H, =CH), 7.42-7.39 (m, 4H, CH_{Ar}), 7.27-7.24 (m, 2H, CH_{Ar}), 7.22 (d, ³J = 8 Hz, 2H, CH_{Ar}), 6.19 (d, ³J = 14 Hz, 2H, =CH), 4.22 (m, 4H, CH₂), 2.89 (dd, ²J = 14 Hz, ³J = 3 Hz, 2H, H_{eq}), 2.23 (dd, ²J = 14 Hz, ³J = 14 Hz, 2H, H_{ax}), 1.87 (m, 4H, CH₂), 1.73 (s, 6H, C(CH₃)₂), 1.72 (s, 6H, C(CH₃)₂), 1.60 (m, 1H, CH), 1.47-1.42 (m, 4H, CH₂), 1.40-1.35 (m, 4H, CH₂), 1.28 (m, 28H, CH₂), 1.10 (s, 9H, C(CH₃)₃), 0.87 (t, ³J = 7 Hz, 6H, CH₃-CH₂).

¹³C NMR (CDCl₃, 125.75 MHz): δ 172.5 (C_{quat}), 150.1 (C_{quat}), 144.1 (CH), 142.4 (C_{quat}), 141.2 (C_{quat}), 129.0 (CH), 127.5 (C_{quat}), 125.5 (CH), 122.4 (CH), 111.2 (CH), 101.3 (CH), 49.5 (C_{quat}), 45.0 (N-CH₂), 42.5 (CH), 32.6 (C_{quat}), 32.0 (CH₂), 29.7 (2 CH₂), 29.6 (2 CH₂), 29.4 (2 CH₂), 28.3 (CH₃), 28.2 (CH₃), 27.7 (CH₂), 27.6 (CH₃), 27.6 (CH₂), 27.2 (CH₂), 22.8 (CH₂), 14.3 (CH₃).

UV-Vis (CH₃OH): $\lambda_{max} = 782 \text{ nm} (\epsilon_{max} = 266000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

HRMS (ESI+): $[M-Br]^+ = 874.6606$ (calcd for $C_{58}H_{88}ClN_2^+$: 847.6631).





To a solution of 90 mg of D^2D^2 (0.10 mmol, 1 equiv.) and 17 mg of *N*-hydroxysuccinimide (0.15 mmol, 1.5 equiv.) in 5 mL of anhydrous DMF were added 30 µL of distilled DIEA (0.19 mmol, 2 equiv.) and the mixture was stirred for 5 h at RT. The solution was evaporated and the residue was dissolved in 10 mL of DCM and washed with an aqueous saturated solution of NH₄Cl (10 mL). The organic layer was dried over MgSO₄ and concentrated. The crude solid was purified by flash chromatography on silica, using EtOAc/PE as eluent (1:9, R_f = 0.47) to afford the product as reddish solid in 79% yield (63 mg).

¹H NMR (CDCl₃, 500.10 MHz): δ 8.14 (d, ³J = 13 Hz, 2H, =CH), 7.19-7.17 (m, 4H, CH_{Ar}), 6.90 (dd, ³J = 7 Hz, 2H, CH_{Ar}), 6.67 (d, ³J = 8 Hz, 2H, CH_{Ar}), 5.47 (d, ³J = 13 Hz, 2H, =CH), 3.66 (t, ³J = 7 Hz, 4H, N-CH₂), 2.85 (d, ²J = 13 Hz, 2H, H_{eq}), 2.12 (dd, ²J = 14 Hz, ³J = 14 Hz, 2H, H_{ax}), 1.72 (t, ³J = 7 Hz, 4H, CH₂), 1.67 (s, 6H, C(CH₃)₂), 1.66 (s, 6H, C(CH₃)₂), 1.55 (m, 1H, CH), 1.44-1.33 (m, 8H, CH₂), 1.26 (m, 28H, CH₂), 1.05 (s, 9H, C(CH₃)₃), 0.88 (t, ³J = 7 Hz, 6H, CH₃-CH₂).

¹³C NMR (CDCl₃, 125.75 MHz): δ 186.6 (C_{quat}), 162.4 (C_{quat}), 144.4 (C_{quat}), 139.9 (C_{quat}), 132.9 (CH), 127.8 (CH), 126.6 (C_{quat}), 121.9 (CH), 120.5 (CH), 106.8 (CH), 92.5 (CH), 46.7 (C_{quat}), 43.7 (CH), 42.8 (N-CH₂), 32.8 (C_{quat}), 32.1 (CH₂), 29.8 (CH₂), 29.8 (CH₂), 29.8 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 28.9 (CH₂), 28.9 (CH₃), 27.7 (CH₃), 27.4 (CH₂), 26.9 (CH₂), 26.3 (CH₂), 22.8 (CH₂), 14.2 (CH₃).

UV-Vis (CH₃OH): $\lambda_{max} = 531 \text{ nm} (\epsilon_{max} = 54000 \text{ L.mol}^{-1} \text{.cm}^{-1}).$

UV-Vis (CH₃OH+CH₃CO₂H): $\lambda_{max} = 712 \text{ nm} (\epsilon_{max} = 116000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

HRMS (ESI+): $[M+H]^+ = 829.6936$ (calcd for $C_{58}H_{89}N_2O^+$: 829.6969).



Compound D⁵D⁵



To a solution of 74 mg of 1 (0.32 mmol, 1 equiv.) and 200 mg of D^5 (0.71 mmol, 2.2 equiv.) in 10 mL of anhydrous ethanol were added 70 µL of distilled pyridine (0.81 mmol, 2.5 equiv.). The solution was stirred for 15 h at 80 °C. The resulting solution was concentrated under reduced pressure, and the crude residue was submitted to flash chromatography on silica gel using CH₃CN/MeOH/H₂O (85:10:5, R_f = 0.36) as eluent to afford the product as a dark greenish solid in 90% yield (220 mg).

¹H NMR (CD₃OD, 500.10 MHz): δ 8.46 (d, ³J = 14 Hz, 2H, =CH), 7.51 (d, ³J = 7 Hz, 2H, CH_{Ar}), 7.43-7.41 (m, 4H, CH_{Ar}), 7.29-7.26 (m, 2H, CH_{Ar}), 7.50 (d, ³J = 14 Hz, 2H, =CH), 4.42 (m, 4H, CH₂), 3.07 (d, ²J = 15 Hz, 2H, H_{eq}), 2.95 (t, ³J = 6 Hz, 4H, CH₂), 2.30-2.24 (m, 6H, CH₂ and H_{ax}), 1.75 (s, 12H, C(CH₃)₂), 1.54 (m, 1H, CH-C(CH₃)₃), 1.14 (s, 9H, C(CH₃)₃).

¹³C NMR (CD₃OD, 125.75 MHz): δ 174.3 (C_{quat}), 151.3 (C_{quat}), 145.9 (CH), 143.6 (C_{quat}), 142.7 (C_{quat}), 129.9 (CH), 129.3 (C_{quat}), 126.4 (CH), 123.4 (CH), 112.3 (CH), 102.6 (CH), 49.7 (C_{quat}), 49.0 (CH₂), 44.4 (CH), 46.0 (CH₂), 33.5 (C_{quat}), 29.0 (CH₂), 28.4 (CH₃), 28.1 (CH₃), 24.2 (CH₂).

UV-Vis (H₂O): $\lambda_{max} = 774$ nm ($\varepsilon_{max} = 130000$ L.mol⁻¹.cm⁻¹).

UV-Vis (CH₃OH): $\lambda_{max} = 782 \text{ nm} (\epsilon_{max} = 223000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

HRMS (ESI-): $[M-Na]^{-} = 753.2788$ (calcd for $C_{40}H_{50}CIN_2O_6S_2^{-}$: 753.2804).



Compound D⁵D⁵=O



To a solution of 100 mg of D^5D^5 (0.13 mmol, 1 equiv.) and 30 mg of *N*-hydroxysuccinimide (0.17 mmol, 2 equiv.) in 5 mL of anhydrous DMF were added 50 µL of distilled DIEA (0.29 mmol, 2.2 equiv.) and the mixture was stirred for 3 h at RT. The solvent was removed under reduced pressure and the crude residue was submitted to flash chromatography on silica gel using acetone/MeOH/H₂O (90:5:5, R_f = 0.52) as eluent. The product was isolated as deep purple solid in 85% yield (83 mg).

¹H NMR (DMSO-d6, 500.10 MHz): δ 7.90 (d, ³J = 13 Hz, 2H, =CH), 7.31 (d, ³J = 7 Hz, 2H, CH_{Ar}), 7.17 (t, ³J = 7 Hz, 2H, CH_{Ar}), 6.95 (d, ³J = 7 Hz, 2H, CH_{Ar}), 6.88 (t, ³J = 7 Hz, 2H, CH_{Ar}), 5.59 (d, ³J = 13 Hz, 2H, =CH), 3.90 (m, 4H, N-CH₂), 2.86 (m, 2H, H_{eq}), 2.51 (m, 4H, CH₂-SO₃⁻), 2.00 (dd, ²J = 14 Hz, ³J = 14 Hz, 2H, H_{ax}), 1.92 (m, 4H, CH₂), 1.55 (s, 12H, C(CH₃)₂), 1.30 (m, 1H, CH-C(CH₃)₃), 1.02 (s, 9H, C(CH₃)₃).

¹³C NMR (DMSO-d6, 125.75 MHz): δ 184.9 (C=O), 161.6 (C_{quat}), 144.0 (C_{quat}), 138.9 (C_{quat}), 132.0 (=CH), 127.9 (CH), 126.1 (C_{quat}), 121.7 (CH), 120.4 (CH), 107.4 (CH), 92.3 (=CH), 46.2 (CH₂), 45.9 (C_{quat}), 43.6 (CH), 40.9 (CH₂), 32.5 (C_{quat}), 28.3 (CH₃), 27.5 (CH₃), 26.5 (CH₂), 22.4 (CH₂).

UV-Vis (H₂O): $\lambda_{max} = 567 \text{ nm} (\epsilon_{max} = 38000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

UV-Vis (CH₃OH): $\lambda_{max} = 527$ nm ($\epsilon_{max} = 35000$ L.mol⁻¹.cm⁻¹).

HRMS (ESI-): $[M-2Na]^{2}/2 = 367.1518$ (calcd for $C_{40}H_{50}N_2O_7S_2^{2}$: 367.1535).



Compound 3



To a solution of 244 mg of 1 (1.07 mmol, 1 equiv.) in 10 mL of absolute ethanol were added dropwise 300 mg of D^5 (1.07 mmol, 1 equiv.) in 10 mL of anhydrous ethanol over 1 h. The mixture was stirred for 18 h at 80 °C. The resulting solution was concentrated under reduced pressure, and the crude residue was submitted to flash chromatography on silica gel using CH₃CN/MeOH/H₂O (85:5:5) as eluent to afford the product as a dark orange solid in a 74% yield (386 mg).

¹H NMR (DMSO-d6, 500.10 MHz): δ 10.10 (s, 1H, CHO), 7.78 (d, ³J = 14 Hz, 1H, =CH), 7.33 (d, ³J = 7 Hz, 1H, CH_{Ar}), 7.20 (t, ³J = 7 Hz, 1H, CH_{Ar}), 7.00 (d, ³J = 7 Hz, 1H, CH_{Ar}), 6.92 (t, ³J = 7 Hz, 1H, CH_{Ar}), 5.83 (d, ³J = 14 Hz, 1H, =CH), 3.97 (m, 2H, N-CH₂), 2.98 (d, ³J = 15 Hz, 1H, H_{eq}), 2.69 (d, ³J = 15 Hz, 1H, H_{eq}), 2.50 (revealed by HSQC, 2H, CH₂-SO₃⁻), 1.96 (dd, ²J = 13 Hz, ³J = 13 Hz, 1H, H_{ax}), 1.92 (m, 2H, CH₂), 1.83 (dd, ²J = 13 Hz, ³J = 13 Hz, 1H, H_{ax}), 1.28 (m, 1H, CH₂), 0.96 (s, 9H, C(CH₃)₃).

¹³C NMR (DMSO-d6, 125.75 MHz): δ 189.1 (CHO), 162.4 (C_{quat}), 147.5 (C_{quat}), 143.7 (C_{quat}), 138.8 (C_{quat}), 131.7 (=CH), 127.9 (CH), 127.4 (C_{quat}), 122.8 (C_{quat}), 121.8 (CH), 120.8 (CH), 107.9 (CH), 93.2 (=CH), 48.3 (CH₂), 46.1 (C_{quat}), 42.1 (CH), 40.9 (CH₂), 32.1 (C_{quat}), 28.0 (CH₃), 27.9 (CH₃), 27.4 (CH₂), 27.2 (CH₃), 25.7 (CH₂), 22.5 (CH₂).

UV-Vis (CH₃OH): $\lambda_{max} = 488 \text{ nm} (\epsilon_{max} = 46000 \text{ L.mol}^{-1} \text{.cm}^{-1}).$

HRMS (ESI-): $[M-Na]^{-} = 490.1839$ (calcd for $C_{26}H_{33}CINO_4S^{-}: 490.1824$).





To a solution of 200 mg of compound **3** (0.41 mmol, 1 equiv.) and 200 mg of **D**² (0.49 mmol, 1.2 equiv.) in 5 mL of anhydrous ethanol were added 50 μ L of pyridine (0.61 mmol, 1.5 equiv.). The mixture was stirred for 1.5h at 80 °C. The resulting solution was concentrated under reduced pressure, and the crude residue was submitted to flash chromatography on silica gel using DCM/MeOH (9:1, R_f = 0.47) as eluent to afford the product as a dark greenish solid in a 88% yield (288 mg).

¹H NMR (CDCl₃, 500.10 MHz): δ 8.51 (d, ³J = 14 Hz, 1H, =CH), 8.17 (d, ³J = 13 Hz, 1H, =CH), 7.46-7.40 (m, 2H, CH_{Ar}), 7.37-7.31 (m, 4H, CH_{Ar}), 7.16-7.12 (m, 2H, CH_{Ar} and =CH), 6.95 (d, ³J = 8 Hz, 1H, CH_{Ar}), 5.87 (d, ³J = 13 Hz, 1H, =CH), 4.84 (dt, ²J = 13 Hz, ³J = 7Hz, 1H, N-CH₂), 4.51 (dt, ²J = 13 Hz, ³J = 7Hz, 1H, N-CH₂), 3.88 (t, ³J = 7 Hz, 2H, N-CH₂), 3.22 (d, ²J = 16 Hz, 1H, H_{eq}), 2.99 (m, 2H, CH₂), 2.84 (d, ²J = 16 Hz, 1H, H_{eq}), 2.44 (dd, ²J = 15 Hz, ³J = 7 Hz, 2H, CH₂), 2.12 (dd, ²J = 13 Hz, ³J = 13 Hz, 1H, H_{ax}), 1.80 (t, ³J = 7 Hz, 2H, CH₂), 1.73 (s, 3H, C(CH₃)₂), 1.71 (s, 3H, C(CH₃)₂), 1.62 (s, 6H, C(CH₃)₂), 1.56 (m, 1H, CH), 1.46-1.34 (m, 4H, CH₂), 1.32-1.22 (m, 14H, CH₂), 1.14 (s, 9H, C(CH₃)₃), 0.87 (t, ³J = 7 Hz, 3H, CH₃).

¹³C NMR (CDCl₃, 125.75 MHz): δ 176.0 (C_{quat}), 168.6 (C_{quat}), 150.5 (C_{quat}), 148.7 (CH), 142.9 (C_{quat}), 142.0 (C_{quat}), 141.8 (C_{quat}), 140.7 (CH), 140.5 (C_{quat}), 130.7 (C_{quat}), 129.3 (CH), 128.6 (CH), 127.8 (C_{quat}), 126.6 (CH), 123.8 (CH), 122.4 (CH), 122.3 (CH), 112.2 (CH), 109.4 (CH), 106.2 (CH), 98.0 (CH), 50.2 (C_{quat}), 48.4 (C_{quat}), 47.0 (CH₂), 44.3 (CH₂), 44.0 (CH₂), 42.8 (CH), 32.7 (C_{quat}), 32.0 (CH₂), 29.7 (CH₂), 29.6 (CH₂), 29.4 (CH₂), 29.4 (CH₂), 28.5 (CH₂), 28.4 (CH₂), 28.3 (CH₂), 28.3 (CH₃), 28.1 (CH₃), 27.7 (CH₃), 27.3 (CH₂), 27.0 (CH₂), 24.4 (CH₂), 22.8 (CH₂), 14.3 (CH₃).

UV-Vis (CH₃OH): $\lambda_{max} = 782 \text{ nm} (\epsilon_{max} = 270000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

HRMS (ESI+): $[M+H]^+ = 801.4771$ (calcd for $C_{49}H_{70}ClN_2O_3S^+$: 801.4790).



[ppm]

Compound D²D⁵=O



To solution of 130 mg of D^2D^5 (0.16 mmol, 1 equiv.) and 37 mg of *N*-hydroxysuccinimide (0.32 mmol, 2 equiv.) in 3 mL of anhydrous DMF were added 70 µL of distilled DIEA (0.41 mmol, 2.5 equiv.) and the mixture was stirred for 3 h at RT. The solvent was removed under reduced pressure, the residue was dissolved in DCM and washed with a saturated aqueous ammonium chloride solution, dried over Na₂SO₄ and concentrated. The crude residue was submitted to flash chromatography on silica gel using CHCl₃/MeOH (85:15, R_f = 0.29) as eluent. The product was isolated as deep purple solid in a 69% yield (88 mg).

¹H NMR (DMSO-d6, 500.10 MHz): δ 7.92 (d, ³J = 14 Hz, 1H, =CH), 7.89 (d, ³J = 14 Hz, 1H, =CH), 7.32 (d, ³J = 7 Hz, 2H, CH_{Ar}), 7.18 (m, 2H, CH_{Ar}), 6.98 (d, ³J = 7 Hz, 2H, CH_{Ar}), 6.89 (m, 2H, CH_{Ar}), 5.64 (d, ³J = 13 Hz, 1H, =CH), 5.49 (d, ³J = 13 Hz, 1H, =CH), 3.92 (m, 2H, CH₂), 3.75 (m, 2H, CH₂), 2.86 (d, ²J = 15 Hz, 1H, H_{eq}), 2.80 (d, ²J = 14 Hz, 1H, H_{eq}), 2.01 (m, 2H, CH₂), 1.94 (m, 2H, CH₂), 1.62 (m, 2H, CH₂), 1.55 (s, 12H, C(CH₃)₂), 1.34 (m, 5H, CH₂ and CH), 1.22 (m, 16H, CH₂), 1.02 (s, 9H, C(CH₃)₃), 0.84 (t, ³J = 7 Hz, 3H, CH₃).

¹³C NMR (DMSO-d6, 125.75 MHz): Compound was too insoluble to record a ¹³C spectrum.

UV-Vis (CH₃OH): $\lambda_{max} = 530 \text{ nm} (\epsilon_{max} = 67000 \text{ L.mol}^{-1}.\text{cm}^{-1})$

UV-Vis (CH₃OH + CH₃CO₂H): $\lambda_{max} = 711$ nm ($\varepsilon_{max} = 170000$ L.mol⁻¹.cm⁻¹).

HRMS (ESI-): $[M-Na]^{-} = 781.4998$ (calcd for $C_{49}H_{69}N_2O_4S^{-}$: 781.4984).



Compound D⁴D⁴



To solution of 628 mg of 1 (2.75 mmol, 1 equiv.) and 2 g of D^4 (6.04 mmol, 2.2 equiv.) in 20 mL of absolute ethanol was added 0.55 mL of pyridine (6.86 mmol, 2.5 equiv.). The solution was stirred for 17 h at 80 °C. The resulting green solution was then concentrated, added by 20 mL DCM and washed with an aqueous solution of HBr 1M and water. The organic layer was dried over Na₂SO₄, filtered and concentrated. The crude residue was purified by flash chromatography on silica gel using DCM/MeOH as eluent (from 95:5 to 90:10, $R_f = 0.42$) to afford a greenish solid in a 71% yield (1.335 g).

¹H NMR (CDCl₃, 500.10 MHz): δ 8.35 (d, ³J = 14 Hz, 2H, =CH), 7.39-7.33 (m, 6H, CH_{Ar}), 7.23-7.19 (m, 2H, CH_{Ar}), 6.38 (d, ³J = 14 Hz, 2H, =CH), 4.35 (m, 4H, CH₂), 4.05 (m, 4H, CH₂), 2.91 (dd, ²J = 15 Hz, ³J = 2 Hz, 2H, H_{eq}), 2.26 (dd, ²J = 14 Hz, ³J = 14 Hz, 2H, H_{ax}), 1.73 (s, 6H, C(CH₃)₂), 1.72 (s, 6H, C(CH₃)₂), 1.53 (m, 1H, CH-C(CH₃)₃), 1.10 (s, 9H, C(CH₃)₃).

¹³C NMR (CDCl₃, 125.75 MHz): δ 173.3 (C_{quat}), 150.5 (C_{quat}), 144.7 (CH), 142.8 (C_{quat}), 141.0 (C_{quat}), 129.0 (CH), 128.2 (C_{quat}), 125.3 (CH), 122.1 (CH), 111.8 (CH), 101.9 (CH), 58.9 (CH₂), 49.5 (C_{quat}), 47.5 (CH₂), 43.0 (CH), 32.7 (C_{quat}), 28.4 (CH₃), 28.4 (CH₃), 28.1 (CH₂), 27.9 (CH₃).

UV-Vis (CH₃OH): $\lambda_{max} = 780$ nm ($\varepsilon_{max} = 161000$ L.mol⁻¹.cm⁻¹).

HRMS (ESI+): $[M-Br]^+ = 599.3392$ (calcd for $C_{38}H_{48}ClN_2O_2^+$: 599.3399).



Compound D⁶D⁶



To a solution of 500 mg of D^4D^4 (0.74 mmol, 1 equiv.) in 6 mL of anhydrous DCM at 0 °C were added 1.09 mL of distilled DIEA (6.62 mmol, 9 equiv.) and 0.73 mL of 2bromoisobutyrylbromide (5.88 mmol, 8 equiv.). The solution was stirred for 15 min at 0 °C and for 1 h at RT. The reaction was quenched with an aqueous solution of HBr 1M. The organic layer was then washed with water, dried over Na₂SO₄ and concentrated. The crude was purified by flash chromatography on silica gel with DCM/MeOH as eluent (from 95:5 to 90:10) to afford a greenish solid in a 87% yield (623 mg).

¹H NMR (CDCl₃, 500.10 MHz): δ 8.37 (d, ³J = 13 Hz, 2H, =CH), 7.40-7.30 (m, 6H, CH_{Ar}), 7.24 (m, 2H, CH_{Ar}), 6.46 (d, ³J = 13 Hz, 2H, =CH), 4.79 and 4.68 (m, 8H, CH₂), 3.00 (d, ²J = 12 Hz, 2H, H_{eq}), 2.72 (dd, ²J = 12 Hz, ³J = 12 Hz, 2H, H_{ax}), 1.77 (s, 12H, C(CH₃)₂), 1.73 (s, 12H, C(CH₃)₂), 1.56 (m, 1H, CH), 1.12 (s, 9H, C(CH₃)₃).

¹³C NMR (CDCl₃, 125.75 MHz): δ 172.9 (C_{quat}), 171.7 (C_{quat}), 150.6 (C_{quat}), 144.5 (CH), 142.3 (C_{quat}), 141.0 (C_{quat}), 129.0 (C_{quat}), 128.9 (CH), 125.6 (CH), 122.3 (CH), 111.4 (CH), 102.3 (CH), 62.4 (CH₂), 55.1 (C_{quat}), 49.6 (C_{quat}), 43.7 (CH₂), 42.8 (CH), 32.7 (C_{quat}), 30.7 (CH₃), 28.3 (CH₃), 28.1 (CH₂), 27.7 (CH₃).

UV-Vis (CH₃OH): $\lambda_{max} = 779 \text{ nm} (\epsilon_{max} = 214000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

HRMS (ESI+): $[M-Br]^+ = 895.2408$ (calcd for $C_{46}H_{58}Br_2ClN_2O_4^+$: 895.2446).







To a solution of 60 mg of D^6D^6 (0.06 mmol, 1 equiv.) and 11 mg of *N*-hydroxysuccinimide (0.09 mmol, 1.5 equiv.) in 3 mL of anhydrous DMF were added 20 µL of distilled DIEA (0.12 mmol, 2 equiv.) and the mixture was stirred for 3 h at 40 °C. Then the solvent was removed under reduced pressure, the residue was dissolved in DCM, washed with a saturated aqueous solution of NH₄Cl, then with brine, dried over Na₂SO₄ and finally concentrated. The crude residue was submitted to flash chromatography on silica gel using DCM/MeOH (98:2, $R_f = 0.90$) as eluent. The product was isolated as an orange-reddish solid in 74% yield (40 mg).

¹H NMR (CDCl₃, 500.10 MHz): $\delta 8.13$ (d, ³J = 13 Hz, 2H, =CH), 7.19 (m, 4H, CH_{Ar}), 6.93 (t, ³J = 8 Hz, 2H, CH_{Ar}), 6.74 (t, ³J = 8 Hz, 2H, CH_{Ar}), 5.62 (d, ³J = 13 Hz, 2H, =CH), 4.47 (t, ³J = 7 Hz, 2H, CH₂), 4.04 (t, ³J = 7 Hz, 2H, CH₂), 2.92 (d, ²J = 15 Hz, 2H, H_{eq}), 2.14 (dd, ²J = 14 Hz, ³J = 14 Hz, 1H, H_{ax}), 1.81 (s, 12H, C(CH₃)₂), 1.67 (s, 6H, C(CH₃)₂), 1.67 (s, 6H, C(CH₃)₂), 1.52 (m, 1H, CH), 1.06 (s, 9H, C(CH₃)₃).

¹³C NMR (CDCl₃, 125.75 MHz): δ 187.0 (C_{quat}), 171.8 (C_{quat}), 162.0 (C_{quat}), 144.0 (C_{quat}), 139.5 (C_{quat}), 132.5 (CH), 127.8 (CH), 127.6 (C_{quat}), 122.1 (CH), 121.0 (CH), 106.8 (CH), 93.1 (CH), 61.6 (CH₂), 55.4 (C_{quat}), 46.7 (C_{quat}), 43.7 (CH), 40.8 (CH₂), 32.8 (C_{quat}), 30.7 (CH₃), 29.0 (CH₃), 28.9 (CH₃), 27.7 (CH₃), 27.0 (CH₂).

UV-Vis (CH₃OH): $\lambda_{max} = 522 \text{ nm} (\epsilon_{max} = 58000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

UV-Vis (CH₃OH + CH₃CO₂H): $\lambda_{max} = 707 \text{ nm} (\epsilon_{max} = 133000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

HRMS (ESI+): $[M+H]^+ = 877.2762$ (calcd for $C_{46}H_{59}Br_2N_2O_5^+$: 877.2785).





A glass tube was filled with 1.6 mL of 2-hydroxyethyl acrylate (15.24 mmol, 335 equiv.). The solution was degassed using Argon bubbling for 10 min and 40 mg of $D^6D^6=O$ (0.05 mmol, 1 equiv.), 13 mg of CuBr (0.09 mmol, 2 equiv.) and 0.28 mg of 2,2'-bipyridine (0.18 mmol, 4 equiv.) were added. The glass tube was sealed and the mixture was stirred for exactly 1 h at 85 °C. The reaction was quenched by addition of distilled water and the crude solution was transferred to a dialysis bag (MWCO = 2000 Da) and dialyzed against a large volume of distilled water (which was replaced at 8H intervals) for 24 hours. The resulting reddish solution was lyophilized, yielding reddish fibers (390 mg).

¹H NMR (CD₃OD, 500.10 MHz): δ 8.12 (m, 2H, =CH), 7.24 (m, 4H, CH_{Ar}), 6.94 (m, 4H, CH_{Ar}), 5.73 (m, 2H, =CH), 4.13 (m, CH₂ PHEA), 3.71 (m, CH₂ PHEA), 2.40 (m, CH PHEA), 1.95-1.58 (m, CH₂ PHEA), 1.06 (s, 9H, C(CH₃)₃). Unexpected aromatic signals were observed on the ¹H NMR. DOSY experiment confirmed that these signals were part of the compound. The remaining aliphatic signals (2 C(CH₃)₂) and CH) generally observed *ca*. 1-2 ppm lie under the dominant PHEA signal.

¹³C NMR (CD₃OD, 125.75 MHz): δ 178.6 (C_{quat}), 177.1 (C_{quat}), 176.3 (C_{quat} PHEA), 167.4 (C_{quat}), 165.2 (C_{quat}), 145.1 (C_{quat}), 140.6 (C_{quat}), 136.1 (=CH), 129.3 (CH), 122.9 (CH), 122.4 (CH), 108.9 (CH), 94.4 (=CH), 67.2 (CH₂ PHEA), 60.9 (CH₂ PHEA), 42.6 (CH PHEA), 35.9 (CH₂ PHA), 29.1 (CH₃), 28.1 (CH₃), 27.9 (CH₂). 1 CH₃, 2 CH₂, 1 CH and 3 C_{quat} signals are hidden between polymer signals in the aliphatic part of the spectrum.

 $n \sim 80$, determined by ¹H NMR.

UV-Vis (CH₃OH): $\lambda_{max} = 525$ nm.

UV-Vis (H₂O): $\lambda_{max} = 541$ nm.

GPC: M_n = 32650 g.mol⁻¹; PDI = 1.33 (GPC); 1.09 (DOSY).





To a solution of 200 mg of D^3A (0.31 mmol, 1 equiv.) and 54 mg of *N*-hydroxysuccinimide (0.47 mmol, 2.5 equiv.) in 5 mL of anhydrous DMF was added 0.10 mL of distilled DIEA (0.62 mmol, 2 equiv.) and the mixture was stirred for 2 h at RT. The solvent was evaporated under reduced pressure and the residue was dissolved in 10 mL of MeOH. 234 mg of sodium iodide (1.56 mmol, 5 equiv.) were added and the resulting mixture was stirred for 30 min at RT. The solvent was evaporated and the residue was dissolved in 50 mL of DCM and washed with water. The organic layer was dried over Na₂SO₄ and concentrated. The crude residue was purified by flash chromatography on silica gel with EtOAc/MeOH (98:2, R_f = 0.49) as eluent to afford the target compound as a deep purple solid in a 70% yield (141 mg).

¹H NMR (CD₃CN, 500.10 MHz): δ 8.19 (m, 1H, =CH), 7.98 (d, ³J = 12 Hz, 1H, =CH), 7.30-7.21 (m, 7H, CH_{Ar}), 7.00 (t, ³J = 8 Hz, 1H, CH_{Ar}), 6.94 (d, ³J = 8 Hz, 1H, CH_{Ar}), 5.66 (d, ³J = 12 Hz, 1H, =CH), 5.52 (d, ³J = 12 Hz, 1H, =CH), 5.06 (d, ²J = 17 Hz, 2H, CH₂), 4.96 (d, ²J = 17 Hz, 2H, CH₂), 2.75 (d, ²J = 15 Hz, 1H, H_{eq}), 2.51 (d, ²J = 15 Hz, 1H, H_{eq}), 1.84 (dd, ²J = 14 Hz, ³J = 14 Hz, 1H, H_{ax}), 1.58 (m, 1H, H_{ax}), 1.58 (s, 3H, C(CH₃)₂), 1.55 (s, 3H, C(CH₃)₂), 1.50 (s, 6H, C(CH₃)₂), 1.28 (m, 1H, CH), 0.95 (s, 9H, C(CH₃)₃).

¹³C NMR (CDCl₃, 125.75 MHz): Compound was too insoluble to record a ¹³C spectrum.

UV-Vis (CH₃OH): $\lambda_{max} = 549 \text{ nm} (\epsilon_{max} = 66000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

UV-Vis (CH₃OH + CH₃CO₂H): $\lambda_{max} = 757 \text{ nm} (\epsilon_{max} = 160000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

HRMS (ESI-): $[M-Na]^{-} = 621.3218$ (calcd for $C_{41}H_{41}N_4O_2^{-}: 621.3235$).



Compound D²A



To a solution of 200 mg of precursor **2** (0.46 mmol, 1 equiv.) and 224 mg of **D**² (0.55 mmol, 1.2 equiv.) in 30 mL of absolute ethanol were added 60 μ L of distilled pyridine (0.68 mmol, 1.5 equiv.). The reaction was stirred for 16 h at 80 °C. The solution was evaporated and the residue was dissolved in 20 mL of DCM and washed with 20 mL of water. The organic layer was dried over Na₂SO₄ and concentrated. The crude solid was purified by flash chromatography on silica gel, using DCM as eluent (R_f = 0.20) to afford the product as a greenish solid in a 74% yield (244 mg).

¹H NMR (CDCl₃, 500.10 MHz): δ 8.12 (d, ³J = 15 Hz, 1H, =CH), 7.95 (d, ³J = 13 Hz, 1H, =CH), 7.29-7.26 (m, 2H, CH_{Ar}), 7.06 (t, ³J = 8 Hz, 1H, CH_{Ar}), 6.85 (d, ³J = 8 Hz, 1H, CH_{Ar}), 6.19 (d, ³J = 15 Hz, 1H, =CH), 5.70 (d, ³J = 13 Hz, 1H, =CH), 3.79 (m, 2H, N-CH₂), 2.86 (d, ²J = 14 Hz, 1H, H_{eq}), 2.74 (d, ²J = 14 Hz, 1H, H_{eq}), 2.13 (dd, ²J = 13 Hz, ³J = 13 Hz, 1H, H_{ax}), 2.06 (dd, ²J = 13 Hz, ³J = 13 Hz, 1H, H_{ax}), 1.76 (s, 6H, C(CH₃)₂), 1.75 (m, 2H, CH₂), 1.66 (s, 6H, C(CH₃)₂), 1.63 (m, 2H, CH₂), 1.55 (m, 1H, CH), 1.39 (m, 4H, CH₂), 1.29 (m, 4H, CH₂), 1.25 (m, 8H, CH₂), 1.04 (s, 9H, C(CH₃)₃), 0.88 (t, ³J = 7 Hz, 3H, CH₃).

¹³C NMR (CDCl₃, 125.75 MHz): δ 176.4 (C_{quat}), 173.1 (C_{quat}), 165.8 (C_{quat}), 147.4 (C_{quat}), 144.4 (CH), 143.3 (C_{quat}), 140.0 (C_{quat}), 136.8 (CH), 128.4 (CH), 127.7 (C_{quat}), 126.0 (C_{quat}), 122.7 (CH), 122.2 (CH), 113.5 (C_{quat}), 112.6 (C_{quat}), 111.9 (C_{quat}), 110.6 (CH), 108.4 (CH), 99.7 (C_{quat}), 96.5 (CH), 93.3 (C_{quat}), 53.4 (C_{quat}), 47.7 (C_{quat}), 43.4 (N-CH₂), 42.5 (CH), 32.5 (C_{quat}), 32.0 (CH₂), 29.7 (2 CH₂), 29.6 (2 CH₂), 29.5 (2 CH₂), 28.4 (CH₃), 27.7 (CH₂), 27.5 (CH₃), 27.3 (CH₂), 27.2 (CH₂), 27.2 (CH₃), 24.6 (CH₂), 22.8 (CH₂), 14.2 (CH₃).

UV-Vis (CH₂Cl₂): $\lambda_{max} = 832 \text{ nm} (\epsilon_{max} = 136000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

HRMS (ESI+): $[M+Na]^+ = 741.4237$ (calcd for $C_{46}H_{59}CIN_4NaO^+$: 741.4270).





To a solution of 150 mg of D^2A (0.21 mmol, 1 equiv.) and 48 mg of *N*-hydroxysuccinimide (0.42 mmol, 2 equiv.) in 3 mL of anhydrous DMF were added 90 µL of distilled DIEA (0.52 mmol, 2.5 equiv.) and the mixture was stirred for 3 h at 40 °C. The solvent was evaporated under reduced pressure and the residue was dissolved in DCM, washed with water and brine. The organic layer was dried over Na₂SO₄ and concentrated. The crude solid was purified by flash chromatography on silica gel with DCM/MeOH (95:5, $R_f = 0.26$) as eluent to afford product as a deep purple solid in 62% yield (94 mg).

¹H NMR (CD₃CN, 500.10 MHz): δ 8.05 (d, ³J = 13 Hz, 2H, =CH), 7.31 (d, ³J = 7 Hz, 1H, CH_{Ar}), 7.25 (dd, ³J = 8 Hz, ³J = 7 Hz, 1H, CH_{Ar}), 7.01 (dd, ³J = 8 Hz, ³J = 7 Hz, 1H, CH_{Ar}), 6.95 (d, ³J = 8 Hz, 1H, CH_{Ar}), 5.72 (d, ³J = 13 Hz, 2H, =CH), 3.84 (t, ³J = 7 Hz, 2H, N-CH₂), 2.79 (m, 2H, H_{eq}), 2.07 (m, 2H, H_{ax}), 1.71 (m, 2H, CH₂), 1.60 (s, 6H, C(CH₃)₂), 1.54 (s, 7H, CH and C(CH₃)₂), 1.38 (m, 6H, CH₂), 1.25 (m, 12H, CH₂), 1.03 (s, 9H, C(CH₃)₃), 0.87 (t, ³J = 7 Hz, 3H, CH₃).

¹³C NMR (CD₃CN, 125.75 MHz): Compound was too insoluble to record a ¹³C spectrum.

UV-Vis (CH₃OH): $\lambda_{max} = 552 \text{ nm} (\epsilon_{max} = 67000 \text{ L.mol}^{-1} \text{.cm}^{-1}).$

HRMS (ESI-): $[M-Na]^{-} = 699.4621$ (calcd for $C_{46}H_{59}N_4O_2^{-}: 699.4644$).



Compound D⁵A



To a solution of 50 mg of precursor **2** (0.11 mmol, 1 equiv.) and 40 mg of **D**⁵ (0.14 mmol, 1.2 equiv.) in 6 mL of absolute ethanol were added 40 μ L of distilled pyridine (0.51 mmol, 1.5 equiv.). The reaction was stirred for 17 h at 80 °C. The solution was evaporated and the residue was purified by flash chromatography on silica gel, using CH₃CN/MeOH/H₂O as eluent (90:5:5) to afford the product as a greenish solid in 97% yield (75 mg).

¹H NMR (DMSO-d₆, 500.10 MHz): δ 8.29 (d, ³J = 14 Hz, 1H, =CH), 8.24 (d, ³J = 14 Hz, 1H, =CH), 7.62 (d, ³J = 7 Hz, 1H, CH_{Ar}), 7.53 (d, ³J = 8 Hz, 1H, CH_{Ar}), 7.43 (t, ³J = 8 Hz, 1H, CH_{Ar}), 7.29 (t, ³J = 7 Hz, 1H, CH_{Ar}), 6.67 (d, ³J = 14 Hz, 1H, =CH), 6.08 (d, ³J = 14 Hz, 1H, =CH), 4.50 (dt, ²J = 14 Hz, ³J = 7 Hz, 1H, CH₂), 4.39 (dt, ²J = 14 Hz, ³J = 7 Hz, 1H, CH₂), 2.98 (d, ²J = 15 Hz, 1H, H_{eq}), 2.85 (d, ²J = 15 Hz, 1H, H_{eq}), 2.53 (t, ³J = 6 Hz, 2H, CH₂), 2.17 (m, 1H, H_{ax}), 2.04 (m, 1H, H_{ax}), 2.03 (m, 2H, CH₂), 1.66 (s, 6H, C(CH₃)₂), 1.59 (s, 6H, C(CH₃)₂), 1.41 (m, 1H, CH), 1.04 (s, 9H, C(CH₃)₃).

¹³C NMR (DMSO-d₆, 125.75 MHz): δ 176.7 (C_{quat}), 172.9 (C_{quat}), 166.9 (C_{quat}), 147.2 (C_{quat}), 143.5 (CH), 142.0 (C_{quat}), 141.4 (C_{quat}), 139.9 (CH), 128.6 (CH), 128.0 (C_{quat}), 127.5. (C_{quat}), 125.3 (CH), 122.5 (CH), 115.4 (C_{quat}), 114.8 (C_{quat}), 114.6 (C_{quat}), 111.8 (CH), 106.0 (CH), 103.1 (CH), 95.3 (C_{quat}), 49.1 (C_{quat}), 47.6 (CH₂), 44.3 (C_{quat}), 43.0 (CH₂), 42.5 (CH), 32.5 (C_{quat}), 27.6 (CH₂), 27.5 (CH₂), 27.3 (CH₃), 27.3 (CH₃), 26.4 (CH₃), 23.6 (CH₂).

UV-Vis (CH₃CN): $\lambda_{max} = 838 \text{ nm} (\epsilon_{max} = 126000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

HRMS (ESI-): $[M-Na]^{-} = 671.2491$ (calcd for $C_{37}H_{40}CIN_4O_4S^{-}: 671.2464$).





To a solution of 70 mg of D^5A (0.10 mmol, 1 equiv.) and 18 mg of *N*-hydroxysuccinimide (0.16 mmol, 1.5 equiv.) in 2 mL of anhydrous DMF were added 30 µL of distilled DIEA (0.21 mmol, 2 equiv.) and the mixture was stirred for 2 h at RT. Then the solvent was removed under reduced pressure and the crude residue was submitted to flash chromatography on silica gel using DCM/MeOH (85:15, $R_f = 0.21$) as eluent. The product was isolated as deep purple solid in 54% yield (37 mg).

¹H NMR (DMSO-d6, 500.10 MHz): δ 8.03 and 7.91 (d, ³J = 13 Hz, 1H, =CH), 7.86 and 7.42 (d, ³J = 13 Hz, 1H, =CH), 7.32 (m, 1H, CH_{Ar}), 7.18 (m, 1H, CH_{Ar}), 6.97 (t, ³J = 7 Hz, 1H, CH_{Ar}), 6.90 (m, 1H, CH_{Ar}), 5.62 (m, 1H, =CH), 5.58 and 5.43 (m and d, ³J = 13 Hz, 1H, =CH), 3.92 (t, ³J = 7 Hz, 1H, CH₂), 2.86-2.72 (m, 2H, H_{ax}), 2.09-1.90 (m, 4H, CH₂ and H_{ax}), 1.57, 1.55 and 1.41 (s, 12H, C(CH₃)₂), 1.31 (m, 1H, CH), 1.00 (s, 9H, C(CH₃)₃). HSQC revealed a CH₂ signal under DMSO peak.

¹³C NMR (DMSO-d6, 125.75 MHz): δ 185.1 (C_{quat}), 184.9 (C_{quat}), 175.1 (C_{quat}), 173.1 (C_{quat}), 162.1 (C_{quat}), 161.7 (C_{quat}), 156.3 (C_{quat}), 154.3 (C_{quat}), 143.9 (C_{quat}), 143.9 (C_{quat}), 138.9 (C_{quat}), 138.8 (C_{quat}), 132.8 (CH), 132.2 (CH), 130.4 (CH), 129.6 (CH), 129.0 (CH), 127.8 (CH), 127.8 (CH), 127.8 (CH), 126.0 (C_{quat}), 125.6 (C_{quat}), 121.7 (CH), 120.5 (CH), 120.4 (CH), 118.2 (C_{quat}), 117.6 (C_{quat}), 115.7 (C_{quat}), 107.5 (CH), 107.4 (CH), 100.4 (CH), 99.6 (CH), 92.3 (CH), 92.3 (C_{quat}), 91.6 (C_{quat}), 45.9 (C_{quat}), 43.5 (CH), 43.4 (CH), 40.8 (broad, CH₂), 35.7 (C_{quat}), 35.6 (C_{quat}), 32.4 (C_{quat}), 32.3 (C_{quat}), 28.3 (CH₃), 28.2 (CH₃), 27.7 (CH₃), 27.7 (CH₃), 27.4 (CH₃), 27.1 (CH₃), 26.8 (CH₂), 26.5 (CH₂), 26.5 (CH₂), 26.3 (CH₂), 22.3 (CH₂), 22.3 (CH₂). Two distinct signals were observed for each carbon atom.

UV-Vis (H₂O): $\lambda_{max} = 574$ nm ($\varepsilon_{max} = 35000$ L.mol⁻¹.cm⁻¹).

UV-Vis (CH₃OH): $\lambda_{max} = 551 \text{ nm} (\epsilon_{max} = 67000 \text{ L.mol}^{-1}.\text{cm}^{-1}).$

UV-Vis (CH₃OH + CH₃CO₂H): $\lambda_{max} = 757 \text{ nm} (\epsilon_{max} = 164000 \text{ L.mol}^{-1} \text{.cm}^{-1}).$

HRMS (ESI-): $[M+H-2Na]^{-} = 653.2803$ (calcd for $C_{37}H_{41}N_4O_5S^{-}: 653.2803$).







To a solution of 200 mg of AA (0.33 mmol, 1 equiv.) and 75 mg of *N*-hydroxysuccinimide (0.65 mmol, 2 equiv.) in 10 mL of anhydrous DMF was added 0.11 mL of distilled DIEA (0.65 mmol, 2 equiv.) and the mixture was stirred for 2 h at RT. The solvent was evaporated under reduced pressure, the residue was dissolved in 10 mL of MeOH, 489 mg of sodium iodide (3.26 mmol, 10 equiv.) were added and the mixture was stirred for 30 min at RT. The solvent was evaporated and the residue was dissolved in 50 mL of DCM and washed with water. The organic layer was dried over Na₂SO₄ and concentrated. After filtration trough a silica plug using DCM/MeOH as an eluent (9:1, $R_f = 0.24$), the product was isolated as a dark purple solid in a 80% yield (160 mg).

¹H NMR (CD₃OD/CDCl₃, 1:1, 500.10 MHz): δ 8.35 (d, ³J = 13 Hz, 2H, =CH), 5.43 (d, ³J = 13 Hz, 2H, =CH), 2.79 (d, ²J = 14 Hz, 2H, H_{eq}), 2.05 (dd, ²J = 14 Hz, ³J = 14 Hz, 2H, H_{ax}), 1.48 (s, 12H, C(CH₃)₂), 1.41 (m, 1H, CH-C(CH₃)₃), 1.01 (s, 9H, C(CH₃)₃).

¹³C NMR (CD₃OD/CDCl₃, 1:1, 125.75 MHz): δ 189.0 (C_{quat}), 178.3 (C_{quat}), 159.0 (C_{quat}), 135.3 (CH), 128.9 (C_{quat}), 120.4 (C_{quat}), 118.9 (C_{quat}), 118.6 (C_{quat}), 101.6 (CH), 93.8 (C_{quat}), 44.5 (CH), 38.7 (C_{quat}), 33.0 (C_{quat}), 28.0 (CH₃), 27.8 (CH₃), 27.3 (CH₂).

UV-Vis (CH₃OH): $\lambda_{max} = 573$ nm ($\varepsilon_{max} = 60000$ L.mol⁻¹.cm⁻¹).

UV-Vis (CH₃OH + CH₃CO₂H): $\lambda_{max} = 804$ nm ($\varepsilon_{max} = 111000$ L.mol⁻¹.cm⁻¹).

HRMS (ESI-): $[M-2Na]^{2}/2 = 285.1204$ (calcd for $C_{34}H_{30}N_6O_3^{2-}$: 285.1195); $[M+H-2Na]^{-} = 571.2461$ (calcd for $C_{34}H_{31}N_6O_3^{-}$: 571.2463).



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