Supporting Information

Glycosylated Naphthalimides and Naphthalimide Tröger´s Bases as Fluorescent Aggregation Probes for Con A

Elena Calatrava-Pérez,[a] Jason M. Delente,[a] Sankarasekaran Shanmugaraju,[a] Chris S. Hawes,[b] Clive D. Williams,[c] Thorfinnur Gunnlaugsson*[a] and Eoin M. Scanlan*[a]

[a]*School of Chemistry and Trinity Biomedical Sciences Institute (TBSI), Trinity College Dublin, The University of Dublin, Dublin 2, Ireland.*

[b]*School of Chemical and Physical Sciences, Keele University, Keele ST5 5BG, UK.*

[c]*School of Biochemistry and Immunology and Trinity Biomedical Sciences Institute (TBSI), Trinity College Dublin, The University of Dublin, Dublin 2, Ireland.*

gunnlaut@tcd.ie, eoin.scanlan@tcd.ie

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Photophysical Characterisation

Compound **1** possesses a broad characteristic ICT band due to the 4-NH_2 substitution with its maximum found at 430 nm (Figure 5.2a). The fluorescence spectra recorded in PBS at 25 °C present a band centred at *ca.* 530 nm (λ_{exc} = 433 nm). In contrast, **3** presents an absorption band at 380 nm, which possesses a shoulder *ca*. 350 nm. The emission spectra showed a band centred at 510 nm (λ_{exc} = 380 nm). Therefore, both the absorption and emission spectra of compound **3** are blue shifted around 50 nm with respect to **1**.

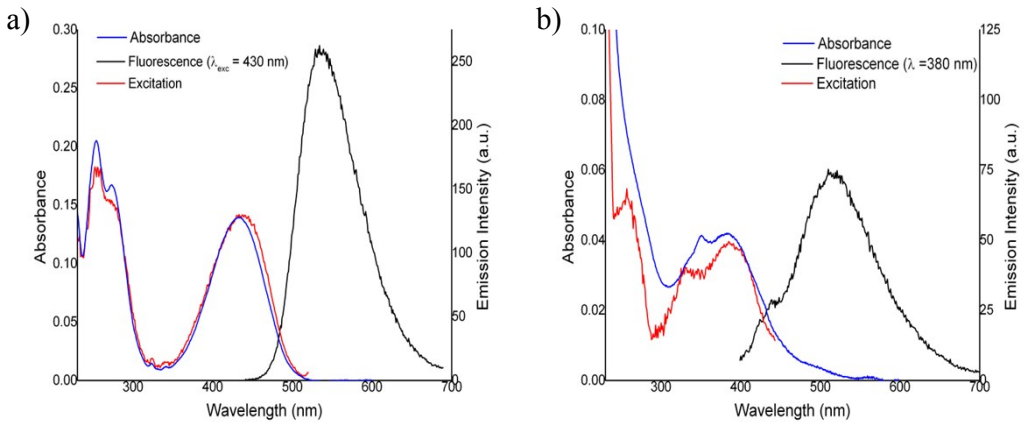


Fig S1. Absorption (blue), fluorescence (black) and excitation (red) spectra of **1** (a) and **3** (b), respectively, measured in PBS at 25 °C.

Table S.1 summarises the characteristic bands of **1** and **3**, extinction coefficient (**ε**) and fluorescent quantum yield (Φ_F) calculated in PBS. Fluorescent quantum yields were calculated using Fluorescein as standard reference.[1] Φ_F values were calculated by comparing the integrated areas underneath the emission band of the spectra using equation 1.

$$(\Phi_F)_x = (\Phi_F)_r \cdot A_r/A_x \cdot F_x/F_r \cdot (\eta_x)^2/(\eta_r)^2 \qquad (\textbf{eq. 1})$$

Table S.1.

Compound	λ_{max} (nm)		φ_F (±SEM %)
	π-π*	ICT (ε (M^{-1} cm^{-1}) ±10%)	
1	254, 273	433 (12,000)	11.5 ± 0.09
3	-	380 (8,900)	2.5 ± 0.09

Concentration studies of **1** and **3** were carried out in DPBS (Dulbecco's phosphate-buffered saline) solution. As can be seen in Figure S2, in both cases aggregation in DPBS occurred at concentrations higher than $5x10^{-5}$ M. However, the fluorescence quenching was more pronounced in the case of **1** (Fig S2b).

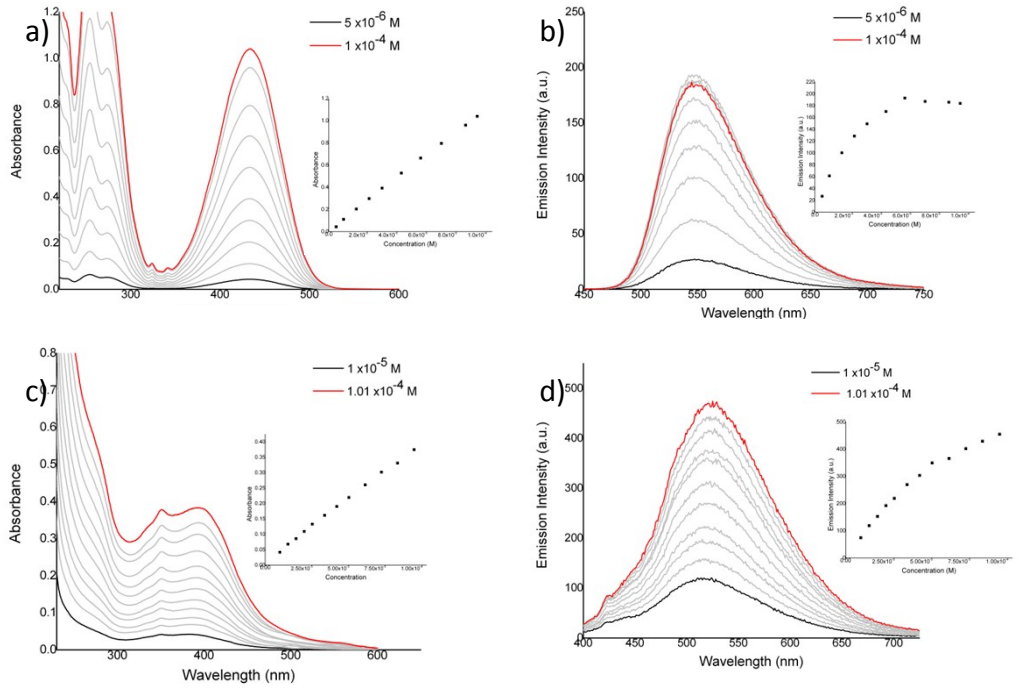


Fig S2 Absorption (a and c) and emission (b and d) spectra, respectively, of **1** (a and b) and **3** (c and d), at different concentrations. The spectra were recorded in PBS at 25 °C. Inserts in b) and d) show the quenching in fluorescent emission at concentrations higher than $5x10^{-5}$ M.

Compound **2** presents similar absorption, emission and excitation bands to compound **3** (Figure S3). An absorption band is observed at 385 nm, which also presents a shoulder at 350 nm. The emission spectrum (black line) presents a band centred at 525 nm (λ_{exc} = 385 nm). However, the excitation spectra (red line) is red-

shifter *ca*. 30 nm compared to the absorption spectra, which was not observed for compound **3**.

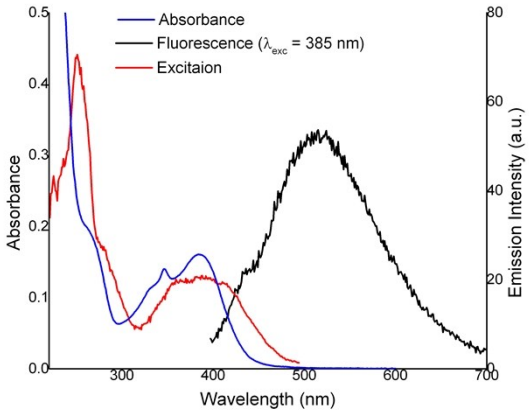


Fig S3. Absorption (blue), fluorescence (black) and excitation (red) spectra of **2** measured in PBS at 25 °C.

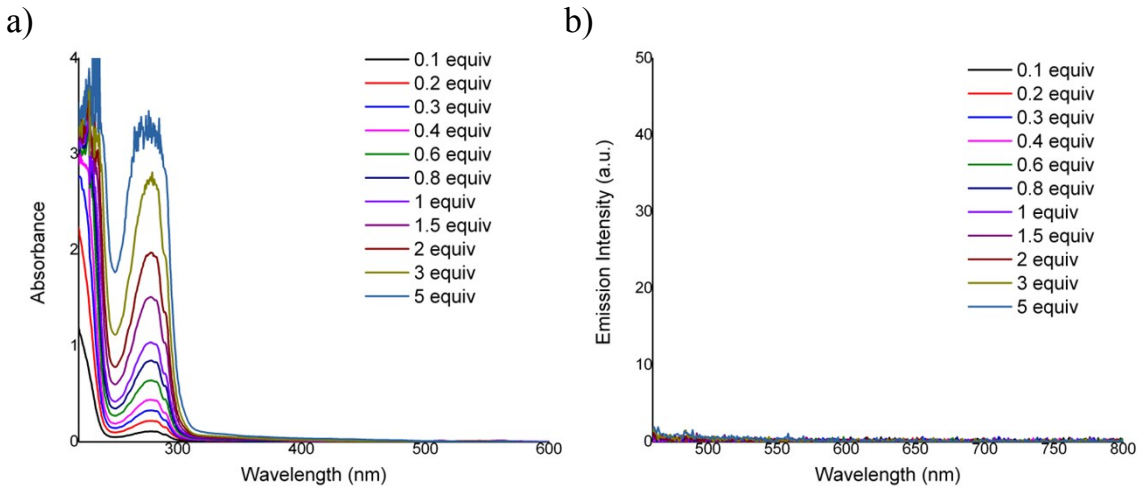


Fig S4 Changes in the absorption (a) and fluorescence emission spectra (λ_{exc} = 433 nm) of Con A at different concentrations, recorded in DPBS pH 7.2 (0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$) at 25 °C

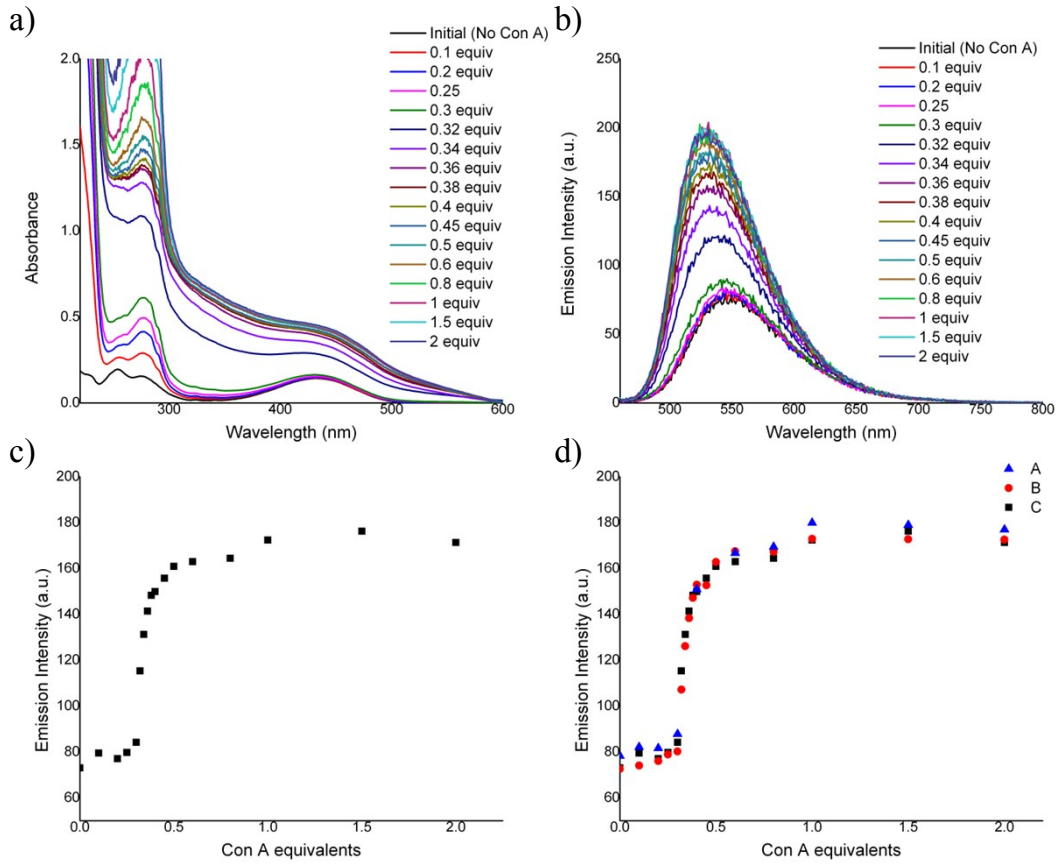


Fig S5. Changes in the absorption (a) and fluorescence emission spectra (b) of **Man-Nap** (1×10^{-5} M) upon the addition of Con A recorded in DPBS pH 7.2 (0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$) at 25 °C. c) changes in the fluorescence emission intensity (λ_{max} = 545 nm) of **Man-Nap** *vs*. Con A equivalents. Figures representative of three independent experiments. d) Changes in the fluorescence emission intensity (λ_{max} = 545 nm) of **Man-Nap** *vs*. Con A equivalents from three independent experiments.

Galactose terminated glyconaphthalimide probes were incubated with 0.1 equivalents of Con A as negative controls (Fig S4). This experiment demonstrated that only the α-mannose functionalised probes interact with Con A, provoking a change in the fluorescence emission. The synthesis of these compounds have been previously described in the literature.[2]

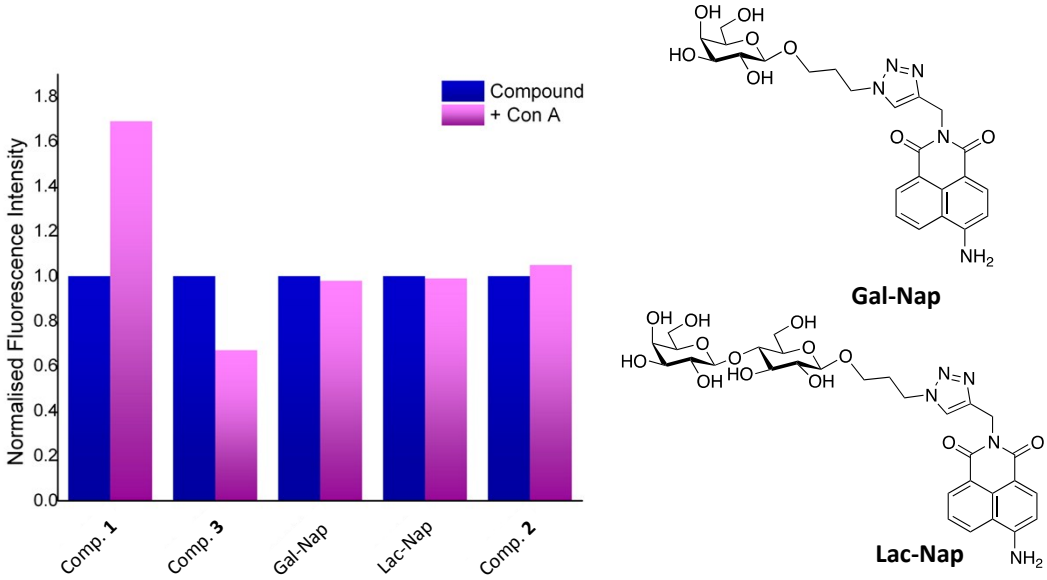


Fig S6 Normalised fluorescence intensity of a range of glyconaphthalimide probes and compound **2**, before (blue) and after (magenta) the addition of 0.1 equivalents of Con A. The experiments were carried out in DPBS in the presence of 0.1 mM $MnCl_2$ and 0.1 mM $CaCl_2$ at 25 °C.

In vitro studies of compounds 1-3

Table S2 shows the toxicity of compounds **2** and **3** in HeLa cells after 24 h and 72 h incubation. The toxicity of compound **1** has previously been reported.[2]

Table S2.

Compound	IC_{50} (μM)	
	24 h	**72 h**
3	>100	>100
2	>100	>100

Uptake studies of compound **2** (50 μM) in HeLa cells at different incubation times showed a greater uptake of the aggregates after long incubation times (Fig S7).

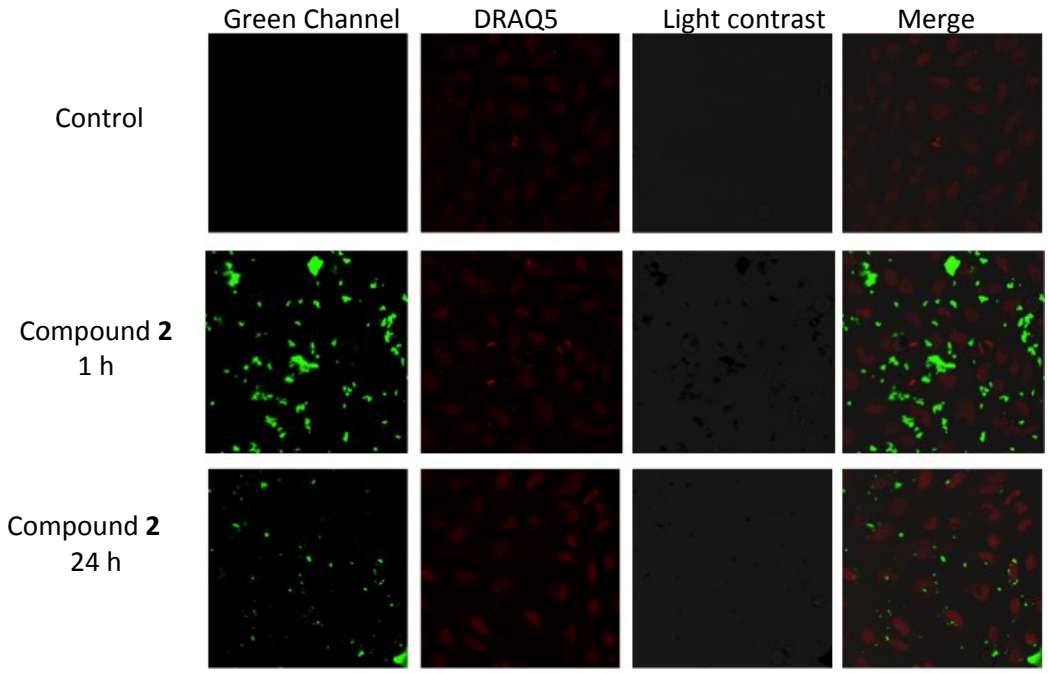
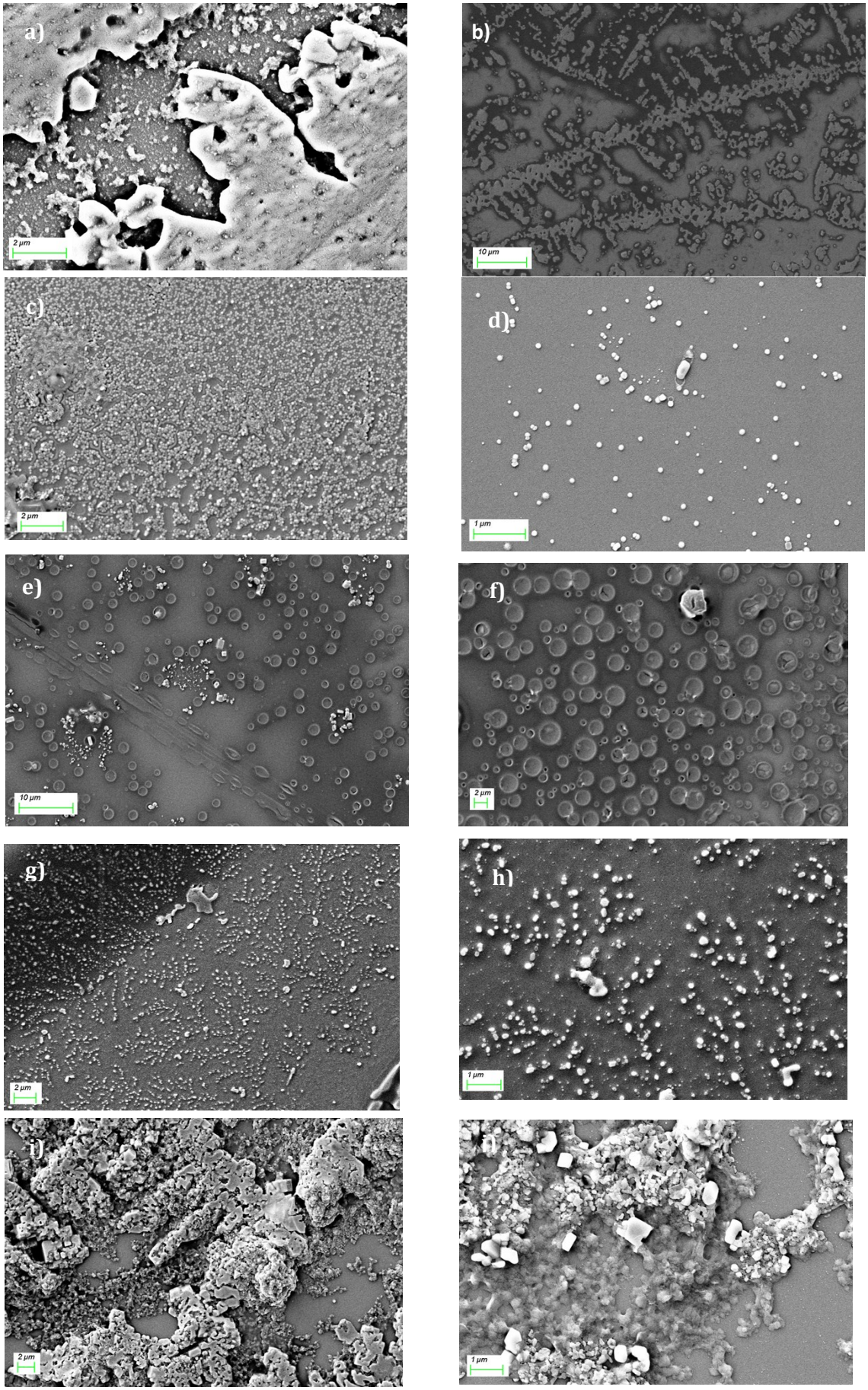


Fig S7 Confocal images of HeLa cells treated with compound **2** at different times. Compounds were excited by a 405 nm argon laser, emission 450-550 nm, DRAQ5 was excited by a 633 nm red helium-neon laser, emission >650 nm.

Morphology studies of compounds 1 and 3


a)
2 µm
b)
10 µm
c)
2 µm
d)
1 µm
e)
10 µm
f)
2 µm
g)
2 µm
h)
1 µm
i)
2 µm
1 µm

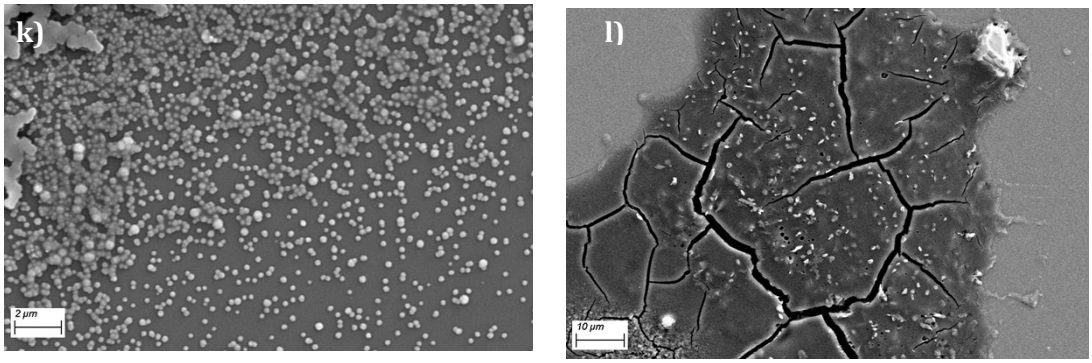


Fig S8 DPBS (a) and Con A alone ($1x10^{-5}$ M, b) were imaged under same conditions as controls. Further SEM images of **3** ($1x10^{-4}$ M in DPBS) before (c, d) and after addition of Con A (0.1 equiv) (e,f) and **1** ($1x10^{-4}$ M in DPBS) before (g, h) and after addition of Con A (0.1 equiv) (i, j), and of **3** ($1x10^{-4}$ M in water) (k) and **1** ($1x10^{-4}$ M in water) (l).

Sample	Size (d, nm)	Standard deviation (d, nm)	PDI
3 ($1x10^{-4}$ M in DPBS)	423.9	31.11	0.469
3 + ConA ($1x10^{-4}$ M in DPBS)	5390	316.2	0.182

Fig S9 DLS results of the measurement on **3** and **3 + con A** ($1*10^{-4}$ M in DPBS) demonstrating the formation of aggregates in solution

NMR data for compound 3.

Compound **3**

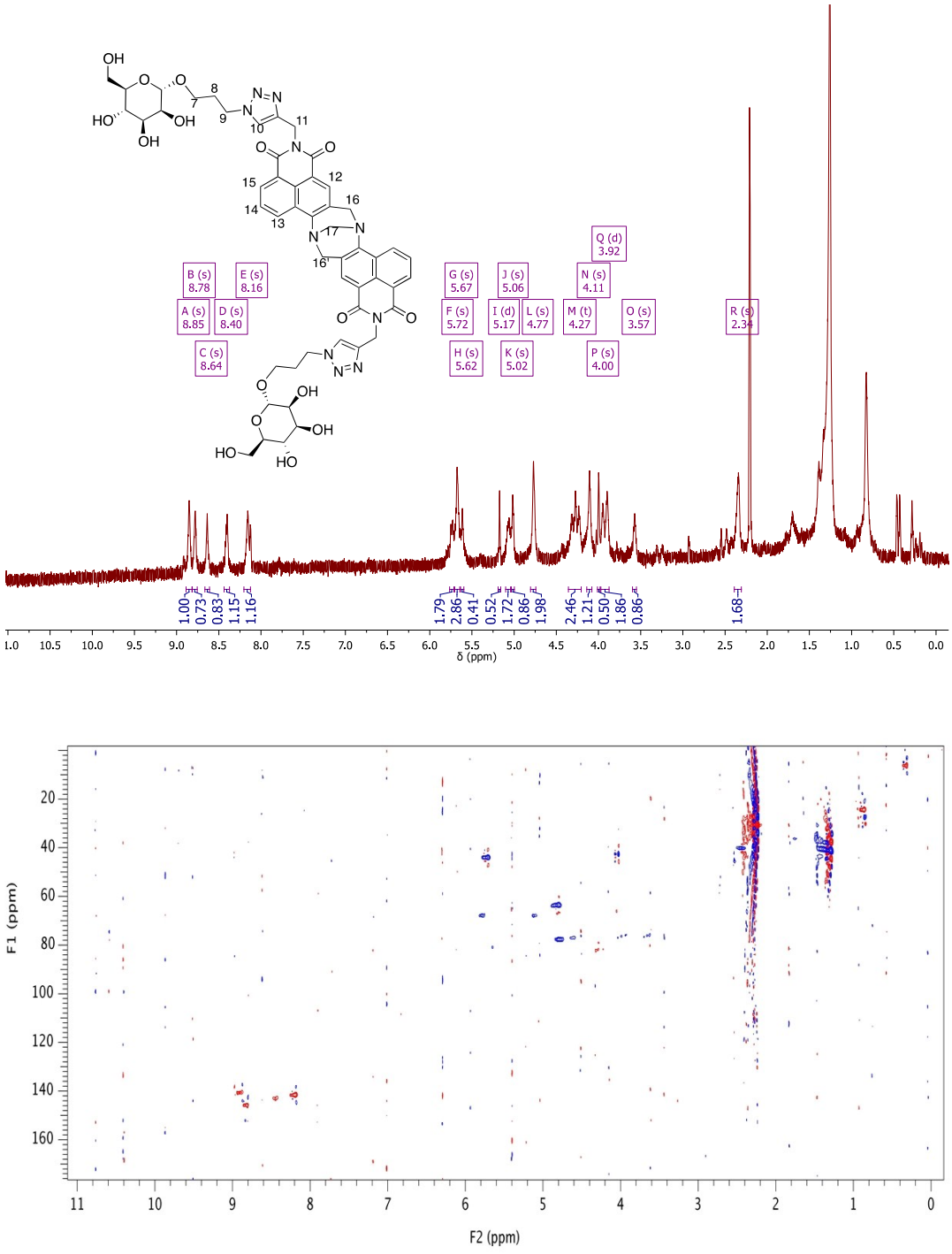


Fig S10 ^{1}H-NMR and HSQC of compound **3** in trifluoroacetic acid-d.

References:

1. Brouwer, A. M., *Pure and Appl. Chem.* 2011**, *83***, 2213-2228.
2. Calatrava-Perez, E., Bright, S. A., Achermann, S., Moylan, C., Senge, M. O., Veale, E. B.; Williams, D. C.; Gunnlaugsson, T.; Scanlan, E. M., *Chem. Commun.* 2016, ***52***, 13086-13089.