

Supporting Information for:

A toolbox for controlling the properties and functionalisation of hydrazone-based supramolecular hydrogels

Jos M. Poolman,^{#a} Chandan Maity,^{#a} Job Boekhoven,^{ab} Lars van der Mee,^a Vincent A. A. le Sage,^a **G. J. Mirjam Groenewold**,^c Sander I. van Kasteren,^c Frank Versluis,^a Jan H. van Esch^{*a} and Rienk Eelkema^{*a}

a) Advanced Soft Matter, Department of Chemical Engineering, Delft University of Technology, Julianalaan 136, 2628 BL Delft, the Netherlands. b) Institute for Advanced Study and Department of Chemistry, Technische Universität München, Lichtenbergstrasse 2A, 85748 Garching, Germany. c) Leiden Institute of Chemistry and The Institute for Chemical Immunology, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands.

* Correspondence to: r.eelkema@tudelft.nl, j.h.vanesch@tudelft.nl

[#] equal contributions

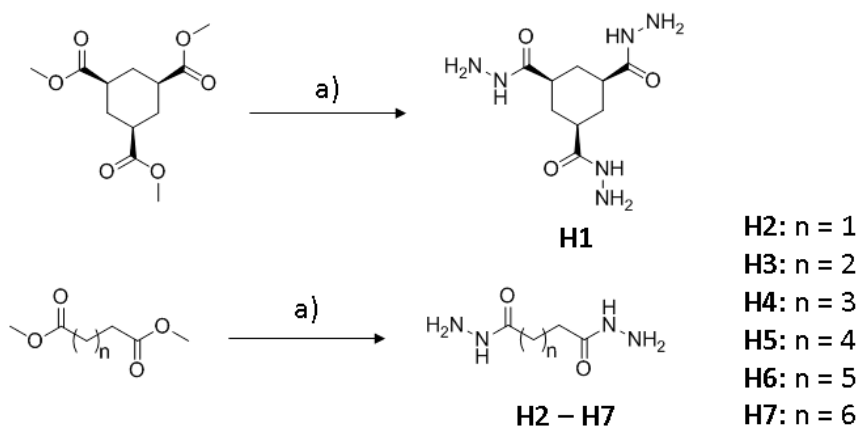
General remarks:

All reagents were purchased from commercial sources and were used as provided unless stated otherwise. 4-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)benzaldehyde (**A5**),¹ cis,cis-cyclohexane-1,3,5-tricarbohydrazide(**H1**),² 3,4-bis(2-(2-methoxyethoxy)ethoxy)benzaldehyde (**A11**), fluorescein labelled aldehyde (**A17**),³ 2,3,4,6-tetraacetylpropargylmannoside,⁴ 3-(4-methylpyridiniumyl)propane-1-sulfonate,⁵ 2,3,3-trimethyl-1-(3-sulfonatepropyl)-3H-indolium⁶ and 6-bromo-4-methyl-7-hydroxycoumarin⁷ were synthesised according to literature procedures. NMR spectra were recorded on a Bruker Avance-400 spectrometer (399.90 MHz for ¹H and 100.56 MHz for ¹³C) or a Varian Inova-300 spectrometer (operating at 300.04 MHz for ¹H) at 298 K using residual protonated solvent signals as internal standard (¹H: δ(CHCl₃) = 7.26 ppm, δ((CH₃)₂SO) = 2.50 ppm, δ(CH₃OH) = 3.31 ppm, δ(D₂O) = 4.79 ppm, and ¹³C: δ(CHCl₃) = 77.16 ppm, δ(CH₂Cl₂) = 53.80 ppm, δ((CH₃)₂SO) = 39.52 ppm, δ(CH₃OH) = 49.00 ppm, D₂O was referenced to internal dioxane, 67.19 ppm. TLC was performed on Merck Silica Gel 60 F254 TLC plates with a fluorescent indicator with a 254 nm excitation wavelength and compounds were visualized under UV light of 254 nm wavelength. HPLC-MS analysis was performed on a Shimadzu Liquid Chromatograph Mass Spectrometer, LCMS-2010, LC-8A pump with a diode array detector SPD-M20. The column used was the Xbridge Shield RP 18.5μm (4.6x150mm). UV-Vis spectroscopic measurements were performed on an Analytik Jena Specord 250 spectrophotometer. Fluorescence spectroscopy was performed on a Jasco 815 spectrophotometer. All experiments were performed using MilliQ water.

Compounds used in this study:

Hydrazide derivatives:

Succinohydrazide (**H2**), adipohydrazide (**H4**), nonanedihydrazide (**H7**) were purchased from commercial sources. Glutarohydrazide (**H3**), heptanedihydrazide (**H5**), octanedihydrazide (**H6**) were synthesised in one step from their corresponding methyl esters derivatives.

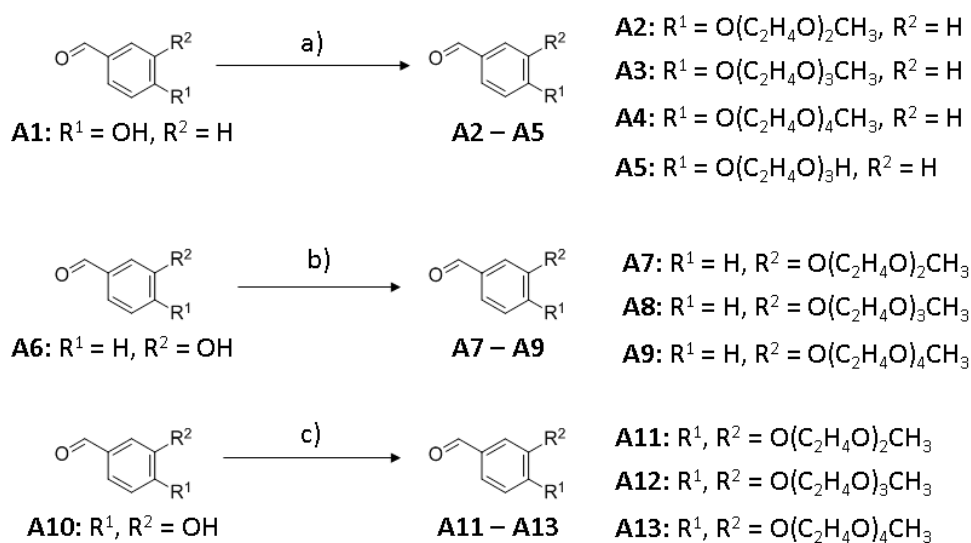


Scheme S1: Synthesis of hydrazide derivatives. Reagent and conditions: a) $N_2H_4 \cdot H_2O$, MeOH, rt, 16 h (for **H1**, **H3**, **H5**, **H6**).

General procedure for hydrazide synthesis:

Hydrazine monohydrate (194.0 mmol) was added in portions to a solution of the methyl ester derivative (19.0 mmol) in methanol. The mixture was stirred overnight at room temperature, yielding a white slurry. The solvent and excess hydrazine were evaporated *in vacuo*, yielding the desired compound (quantitative).

Benzaldehyde derivatives:

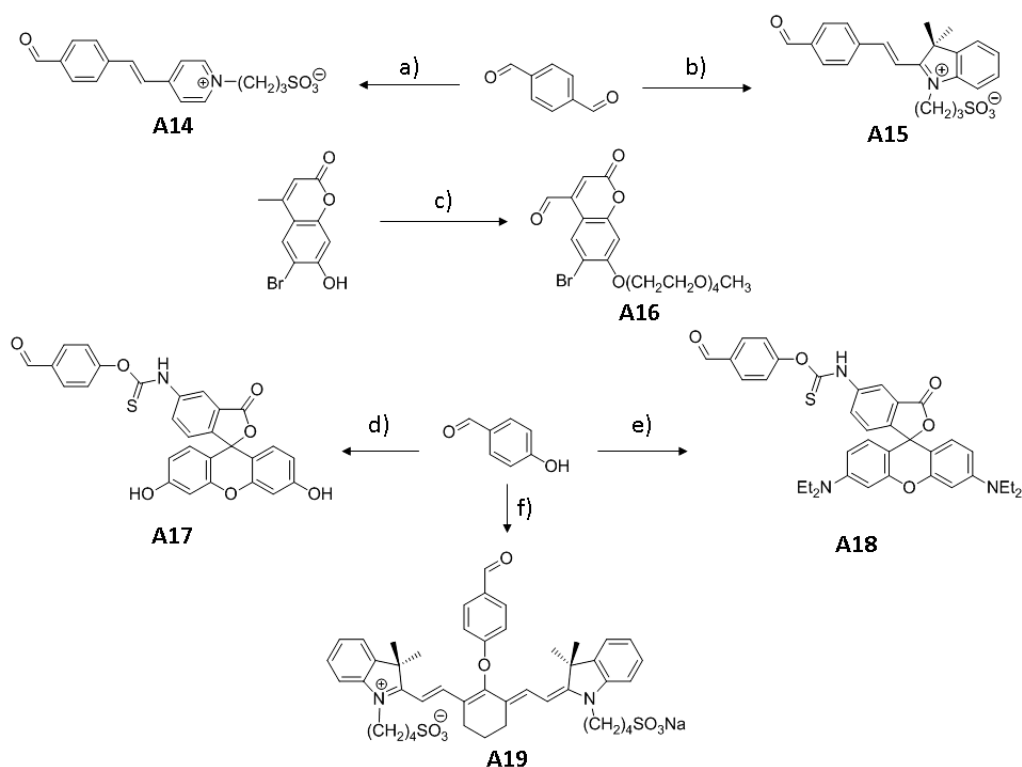


Scheme S2: Synthesis of benzaldehyde derivatives. Reagents and conditions: a) R^1Ts , K_2CO_3 , DMF, 90 °C, 16 h (for **A2 – A4**); or $Cl(C_2H_4O)_3H$, K_2CO_3 , DMF, 90 °C, 16 h (for **A5**); b) R^2Ts , K_2CO_3 , DMF, 90 °C, 16 h (for **A7 – A9**), and c) RTs ($R = R^1, R^2$), K_2CO_3 , DMF, 90 °C, 16 h (for **A11 – A13**).

General procedure for benzaldehyde derivative synthesis:

A slurry of a benzaldehyde derivative (1 eq.), the required tosyl derivative (or chloride in case of **A5**; 1.1 for monohydroxy benzaldehyde and 2.2 eq. for dihydroxy benzaldehyde), potassium carbonate (2 or 4 eq.) and DMF was stirred at 90 °C for overnight. After cooling to room temperature, ethyl acetate and water were added. The water layer was extracted with ethyl acetate, after which the combined organic layers were washed with saturated bicarbonate solution (3x), water (3x) and brine (1x). The organic layer was dried over $MgSO_4$, and removed by evaporation under reduced pressure, yielding the desired compound.

Fluorophore Labelled Aldehydes:

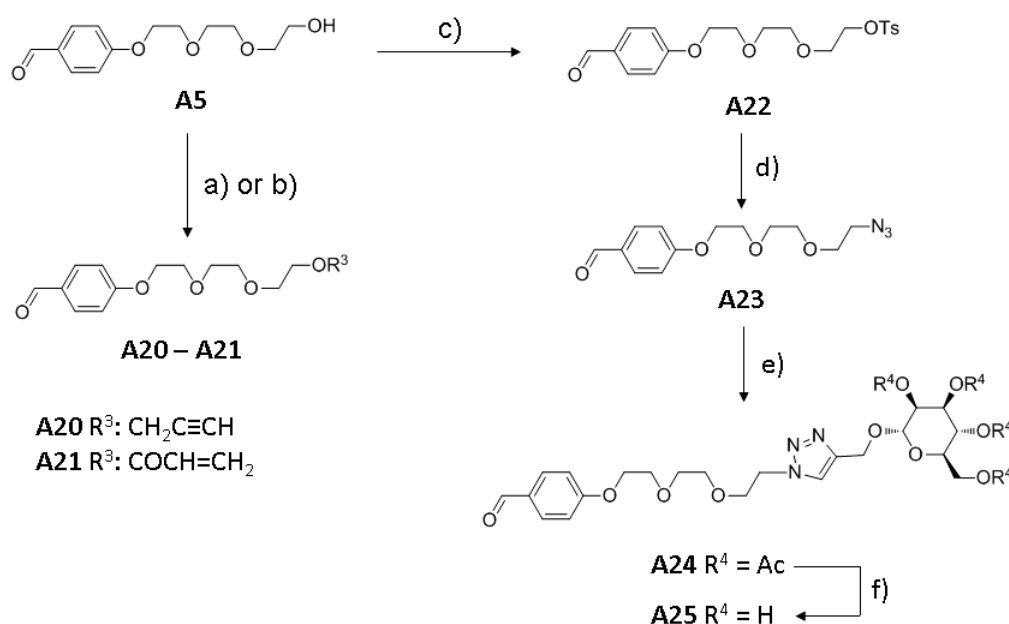


Scheme S3: Synthetic route for fluorophore labelled aldehydes. Reagents and Conditions: a) 3-(4-methylpyridiniumyl)propane-1-sulfonate, piperidine, EtOH, Δ ; b) 2,3,3-trimethyl-1-(3-sulfonatepropyl)-3H-indolium, AcOH, Δ ; c) i) 2-(2-(2-(4-formylphenoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate, K₂CO₃, DMF, Δ ; ii) SeO₂, Xylene, Δ ; d) Fluorescein isothiocyanate, K₂CO₃, DMF, Δ ; e) Rhodamine B isothiocyanate, NaH, DMF, Δ ; f) IR-783, NaH, DMF, Δ .

Synthetic procedure for Fluorophore Labelled Aldehydes

Styryl labelled aldehydes (**A14**, **A15**) were synthesised from terephthalaldehyde via condensation with the corresponding pyridinium and indolium sulfonate salts which were obtained from 1,3-propane sulfonate via condensation with 4-methylpyridine and 2,3,3-trimethyl-3H-indole respectively. Fluorescein (**A17**), rhodamine (**A18**) and cyanine dye (**A19**) labelled aldehydes were synthesised by deprotonation of 4-hydroxybenzaldehyde and subsequent reaction with fluorescein isothiocyanate, rhodamine B isothiocyanate and IR-783 respectively. Coumarine labelled aldehyde (**A16**) was obtained via SeO₂ oxidation reaction from 6-bromo-4-methyl-7-O-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)coumarin, which was synthesised via alkylation reaction of tetraethylene glycol monomethyl ether tosylate and 6-bromo-4-methyl-7-hydroxycoumarin.

Functional aldehyde derivatives:



Scheme S4: Synthesis route for functional aldehyde derivatives. Reagents and Conditions: a) $\text{Br-CH}_2\text{C}\equiv\text{CH}$, NaH , THF , rt ; b) $\text{ClCOCH}=\text{CH}_2$, Et_3N , CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{rt}$; c) Ts-Cl , Et_3N , CH_2Cl_2 , rt ; d) NaN_3 , DMF , Δ ; e) 2,3,4,6-tetraacetylpropargyl- α -mannoside, CuI , Et_3N , CH_2Cl_2 , rt ; f) NaOMe , MeOH , rt .

Synthetic procedure for functional aldehyde derivatives:

Functional aldehyde derivatives were synthesised from 4-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)benzaldehyde (**A5**). Alkyne and alkene labelled aldehyde derivatives (**A20** and **A21**) were synthesised via nucleophilic substitution of **A5** to propargyl bromide and acrylyl chloride respectively, in the presence of base (NaH and Et_3N respectively). The hydroxy group of **A5** was converted to a tosylate group (**A22**) via treatment with tosyl chloride. The azide derivative (**A23**) was obtained from **A22** via nucleophilic substitution with azide ions. Copper catalysed click reaction of **A23** and 2,3,4,6-tetraacetylpropargyl-(α)-mannoside provided acetate derivative **A24**, which, via subsequent deprotection by NaOMe , provided the mannose derivative **A25**.

Click-group functionalised fluorophores:

Rhodamine labelled azide and acetylene derivative (azide-fluor 545 and fluor 488-alkyne respectively), fluorescein labelled concanavalin A (Concanavalin A, FITC conjugate, Type IV) were purchased from commercial sources. Thiol labelled fluorescein derivative (**FITC-SH**) was synthesised following a literature procedure.⁸

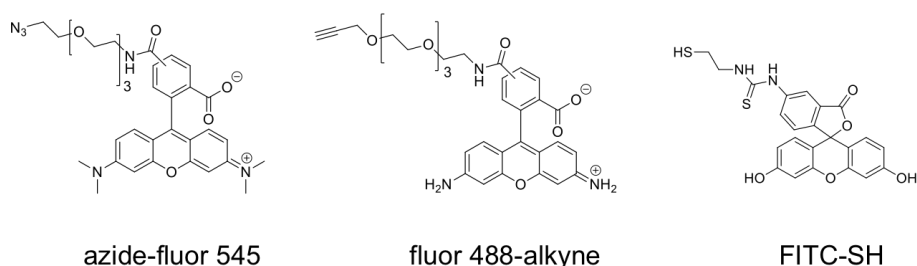


Figure S1: Chemical structure of click group functionalised fluorophores.

Cy5-Azide-Ovalbumin:

Production of azide-derived Ovalbumin (azide-**Ova**).

Ovalbumin carrying a C-terminal 6His-tag was expressed from the previously reported plasmid pMCSG7-OVA⁹ (a kind gift from N. Del Cid) in *E. coli* B834(DE3) using methods previously described¹⁰⁻¹². Briefly, *E. coli* B834(DE3) was transformed with the above plasmid and grown to an OD₆₀₀ of 0.9-1.0 in LB-medium (1L over 2 x 2L flasks). Cells were harvested by centrifugation at 3000 *g* for 10 minutes at 4°C and then resuspended in 2 x 100 mL PBS. After recentrifugation (same conditions), the cells were taken up in SelenoMet-medium (Molecular Dimensions), augmented with either 4 mM methionine or 4 mM azidohomoalanine. Cells were incubated at 30 degrees for 1h prior to induction of expression by the addition of isopropylthiogalactoside (IPTG, 1 mM final concentration). Expression was continued at 30 degrees for 18 hours. Cells were harvested by centrifugation and resuspended in 25 mL of equilibration buffer (50 mM NaH₂PO₄, 500 mM NaCl, pH 8.1) augmented with 250U benzonase + one EDTA-free protease inhibitor tablet (Roche) and lysed by sonic disruption at 4 degrees. The soluble fraction was isolated by centrifugation (40,000 *g*, 45 minutes) and the supernatant was passed over a Ni-NTA-agarose resin (2mL resin slurry per litre of culture) that had been equilibrated with equilibration buffer. The resin-bound protein was washed with 20 column volumes of wash buffer (equilibration buffer containing 10 mM imidazole) prior to elution with 2 x 2 column volumes of elution buffer (equilibration buffer augmented with 500 mM imidazole). SDS-PAGE analysis showed that elution fraction 2 contained the desired proteins in >75% purity. Both OVA expressed with methionine and OVA expressed with azidohomoalanine were used without further purification.

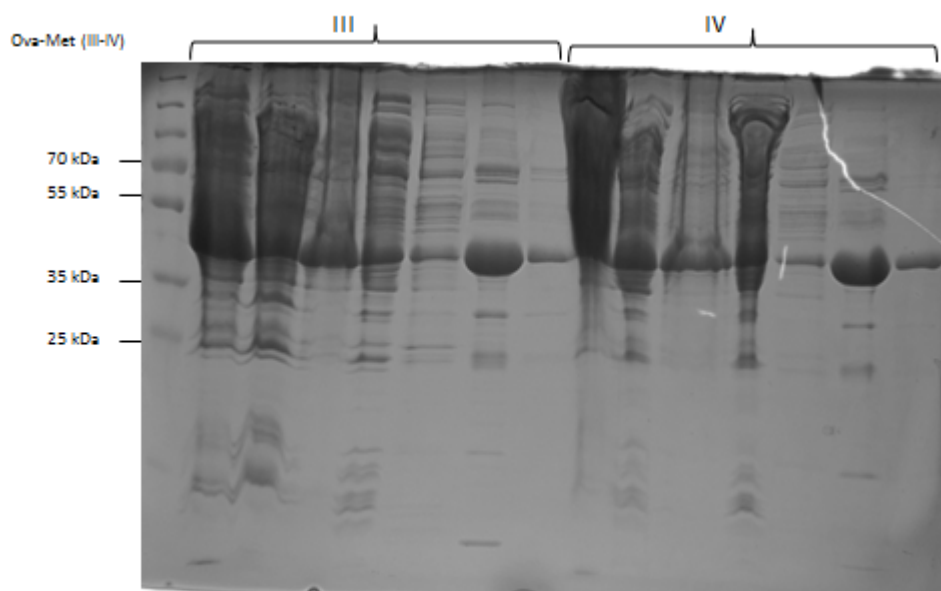


Figure S2: SDS-PAGE gel.

Fluorophore modification of azide-**Ova** and wild type-**Ova**

1 mg of protein (as determined by Bradford Assay¹³) was volume adjusted to 2.3 mL in elution buffer. 0.7 mg of Cy5-HOSu-ester (Lumiprobe, cat. No: 23020) was added to each of

the protein samples (as a freshly prepared solution in DMSO; 200 μ L). The tubes containing the mixtures were inverted 4 times and left unagitated at room temperature for 1 hour. After this time, the modified proteins were purified over sephadex G-25 resin (PD-10 column), that was pre-equilibrated with PBS according to the manufacturer's protocol. Purified proteins were used *as is* in further reactions.

General procedure of gel preparation:^{2,3}

The procedure is explained for the **H1** and **A11** compounds but holds for any combination of hydrazides and aldehydes. Also, the functionalised gel samples were prepared using this procedure, including the desired functional aldehyde by replacing that amount of aldehyde **A11**.

The trishydrazide (**H1**) (40 mM) and aldehyde (**A11**) (240 mM) derivatives were dissolved in aqueous 100 mM sodium phosphate buffer at pH 5. After mixing appropriate amounts of the two stock solutions, the resulting mixture was allowed to react overnight. All experiments were performed at room temperature. To ensure the complete conversion of all hydrazide groups, gels were always prepared in a 1:2 functional group molar ratio of the hydrazide and aldehyde, which means that there is an initial 1:6 molar ratio between **H1** and **A11**. For example, starting with 16 mM of **H1** and 96 mM of **A11** would yield close to 16 mM of hydrogelator in the final mixture accompanied by 48 mM free aldehyde in solution, which was not removed. .

Critical gelation concentration (CGC) test procedure:

A sample vial (2 ml) was filled with the appropriate volumes of aldehyde and hydrazide stock solution and made up to 1 ml total volume by addition of buffer. The vial was closed, shaken vigorously and allowed to stand overnight. The vial was inverted to see whether a gel network had formed. The CGC is the minimal amount of gelator needed to trap the solvent and form a stable gel. The CGC is defined as the average value of the lowest concentration where a gel was formed and the highest concentration where the solvent could no longer be supported. The CGC is expressed as the initial concentration of hydrazide, in mM.

General procedure for fibre network functionalization

Stock solutions of various compounds [**H1** (40.0 mM), **A11** (240.0 mM), fluorophore labelled aldehyde: **A15**, **A18** (1.0 mM), functional aldehydes: **A20**, **A21**, **A23**, **A25** (1.0 mM) and functional compounds: azide-fluor 545 (1.6 mM), thiol labelled fluorescein derivative (**FITC-SH**, 1.0 mM), Fluor 488-alkyne (1.6 mM), fluorescein labelled Concanavalin A (0.1 mM)] were prepared by dissolving compounds in phosphate buffer (pH 5.0). Functionalised gel samples were prepared as described in the general procedure above but now including a functional aldehyde (0.3-1.0 mol%, with respect to **A11**) and a corresponding functional compound (0.02–0.03 mol%, with respect to **A11**), both listed directly above. After vigorous shaking, the mixture was transferred to an imaging chamber (for confocal laser scanning microscopy) and was covered with a glass slide. Next, the sample was allowed to stand overnight in order to ensure complete conversion and gelation before imaging. For control experiment samples were prepared in similar way as functionalised gel sample, replacing the functional aldehyde derivative with equal volume of buffer solution.

Confocal laser scanning microscopy:

Confocal laser scanning micrographs were obtained with a Zeiss LSM 700 confocal laser scanning microscope equipped with a Zeiss Axio Observer inverted microscope and a 40x PlanFluor oil immersion objective lens (NA 1.3) using an incident laser. The confocal pinhole was set to 1.0 airy unit and the data files were processed using ZEN 2011 software. Image dimensions were 95.35 μm x 95.35 μm with a resolution of 1024 x 1024 pixels. Exposition time per pixel was 1.58 μs . The gel networks were imaged using confocal laser scanning microscopy via incorporating an aldehyde functionalised fluorophore derivative (**A14-A19**) into the fibre formation process. The gel samples were prepared as before, but now including 30 μM (0.02-0.1 mol%, replacing that amount of aldehyde **A11**) of the aldehyde functionalised fluorescent probe. After vigorous shaking, the sample mixture was transferred to the imaging chamber (diameter x thickness = 20 mm x 0.6 mm), which was subsequently closed off with a glass cover slide. Next, the sample was allowed to stand overnight in order to ensure complete conversion and gelation before imaging.

Microscopy on astrocytes in gels

For the microscopy experiments on astrocytes in combination with gels, a gel was first prepared in a glass vial (pH 5.0 buffer, 10 mM **H1**, 60 mM **A11**, 30 μM **A18**). This gel was allowed to equilibrate for at least 12 hours. The mouse brain-derived astrocytes were trypsinised, and washed with incubation media (10% foetal bovine serum (FBS) in Dulbecco's modified Eagle medium (DMEM)), before plating 10^5 cells on a 24 well plate and 10^5 cells on a PDL coated coverslip. After incubation for 5 hours in incubation media at 37°C and 5% CO_2 , the cells plated on the tissue culture plastic plate were washed and stained with calcein-AM following the vendor's protocol. After incubation for 30 minutes, the calcein-AM containing media was removed and 100 μL of PBS was added. Then, 100 μL of the previously prepared gel containing **A18** was added. After addition of the gel, the cells were immediately imaged using a Nikon Eclipse inverted microscope through a 10X objective. Similarly, the astrocytes plated on the PDL coverslips were incubated in incubation media for 5 hours at 37°C and 5% CO_2 . After incubation, the cells were fixed using glutaraldehyde, their membranes were permeabilised using Triton-X and they were stained with DAPI and FITC phalloidin, all following a literature procedure.¹⁴ After staining, the cells were washed and 5 μL of the previously prepared gel containing **A18** was added. The excess gel was wicked away and the coverslips were mounted prior to imaging with a confocal microscope (Zeiss LSM 510 equipped with a 40X oil immersion objective).

Fibre network functionalization with A20:

A11 was added to the mixture of CuI, azide-fluor 545, **H1**, **A15** and **A20** in pH 5 buffer (final concentrations of 10 mM **H1**, 59 mM **A11**, 30 μM **A15**, 0.2 mM **A20**, 20 μM azide-fluor 545 and 20 μM CuI). After a vigorous shake, the mixture was transferred to a reaction chamber and was allowed to stand overnight to form a gel. Control experiments were performed in a similar way as above replacing **A20** with an equal volume of buffer solution.

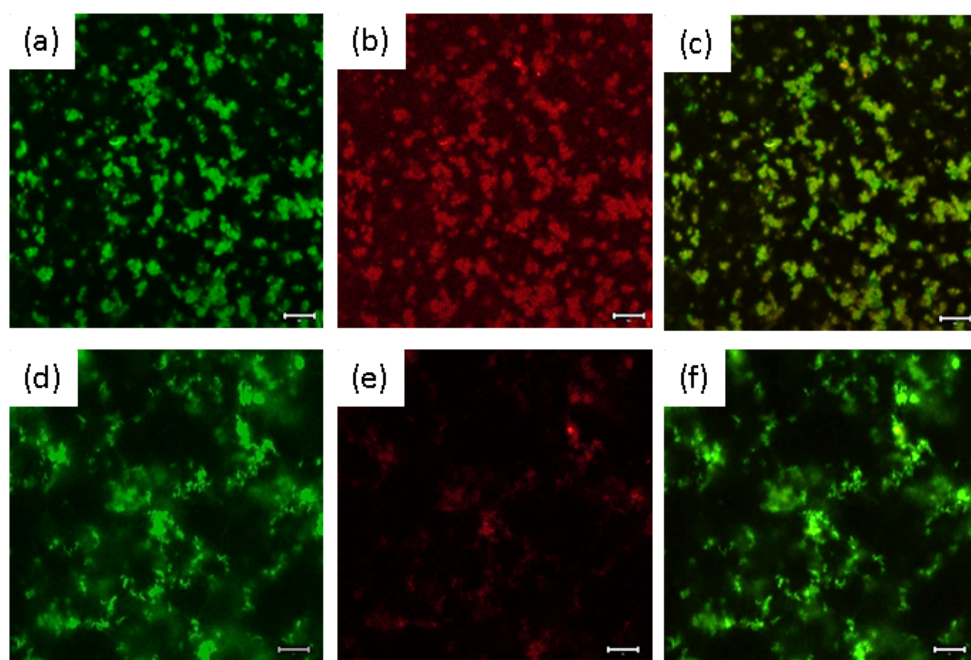


Figure S3: Confocal laser scanning micrographs of gel fibres (scale bar - 20 μm) in presence (top) or absence (bottom) of **A20**: (a, d) exciting styryl-indolium ($\lambda_{\text{exc.}} = 405 \text{ nm}$), emitted light collection: 410-471 nm range; master gain 703 V, digital gain 1.0 (for a) and master gain 817 V, digital gain 1.0, (for d), (b, e) exciting fluor 545 ($\lambda_{\text{exc.}} = 546 \text{ nm}$), emitted light collection: 646-797 nm range; master gain 1028 V, digital gain 1.0 (for b), and master gain 1141 V, digital gain 1.0 (for e), and (c, f) merged: $\lambda_{\text{exc.}} = 405 \text{ nm}$ and 546 nm.

Fibre network functionalization with A21:

H1 was added to a mixture of **A15**, **A21** and **FITC-SH**. After gentle mixing, **A11** was added to it and the mixture was *vortexed* (final concentration of 10 mM **H1**, 59 mM **A11**, 30 μM **A15**, 0.3 mM **A21**, and 10 μM **FITC-SH**). Then, it was transferred to a reaction chamber to stand overnight for complete gelation before imaging. A control experiment was performed similar as above replacing equal volume of **A21** with buffer solution.

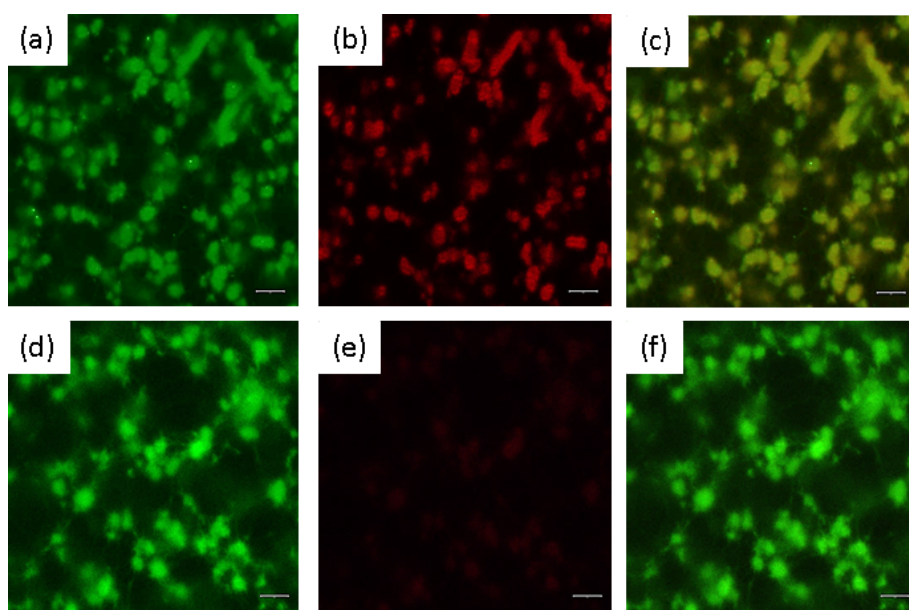


Figure S4: Confocal laser scanning micrographs of gel fibres (scale bar - 20 μM) in presence (top) or absence (bottom) of **A21**: (a, d) exciting styryl-indolium ($\lambda_{\text{exc.}} = 405 \text{ nm}$), emitted light collection: 410-460 nm range; master gain 830 V, digital gain 1.0 (for a) and master gain 957 V, digital gain 1.0 (for d), (b, e) exciting fluorescein ($\lambda_{\text{exc.}} = 488 \text{ nm}$), emitted light collection: 610-797 nm range; master gain 1000 V, digital gain 1.2 (for b) and master gain 1200 V, digital gain 1.0 (for e), and (c, f) merged: $\lambda_{\text{exc.}} = 405 \text{ nm}$ and 488 nm.

Fibre network functionalization with A23:

Similar experiment was performed as fibre network functionalization with **A20**, except **A20** and azide-fluor 545 were replaced by **A23** and Fluor 488-alkyne respectively. The gel contained the final concentration of 10 mM **H1**, 59 mM **A11**, 30 μM **A15**, 0.2 mM **A23**, 20 μM Fluor 488-alkyne and 20 μM CuI. A control experiment was performed similar way as above replacing **A23** with equal volume of buffer solution.

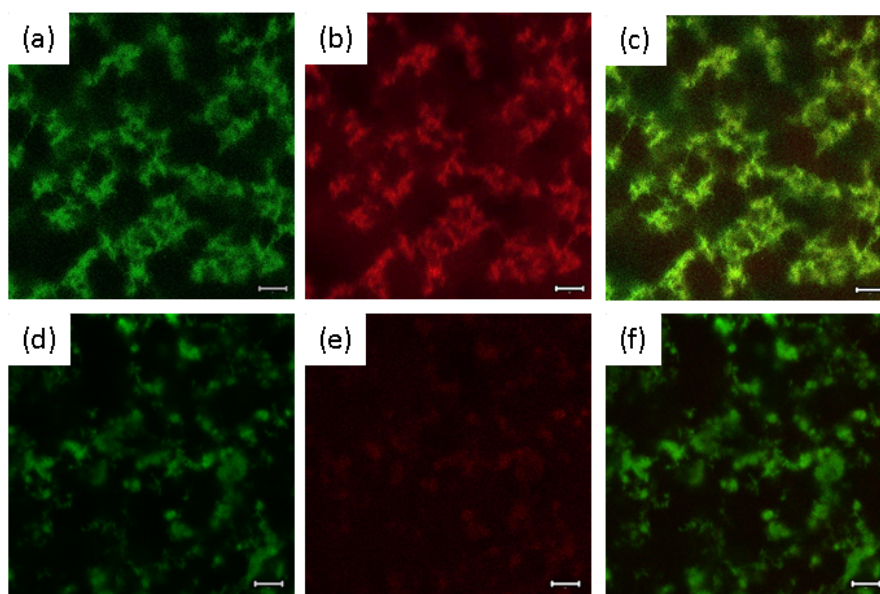


Figure S5: Confocal laser scanning micrograph of gel fibre (scale bar - 20 μM) in presence (top) or absence (bottom, blank experiment) of **A23**: (a, d) exciting styryl-indolium ($\lambda_{\text{exc.}} = 405 \text{ nm}$), emitted light collection: 410-458 nm range; master gain 904 V, digital gain 1.0 (for a) and master gain 747 V, digital gain 1.0 (for d), (b, e) exciting Fluor 488 ($\lambda_{\text{exc.}} = 488 \text{ nm}$), emitted light collection: 605-797 nm range; master gain 798 V, digital gain 1.2 (for b) and master gain 923 V, digital gain 1.2 (for e), and (c, f) merged: $\lambda_{\text{exc.}} = 405 \text{ nm}$ and 488 nm.

Fibre network functionalization with A25:

H1 was added to a mixture of **A18**, **A25** and fluorescein labelled concanavalin A. After gentle mixing, **A11** was added to the mixture and the solution was mixed vigorously. Then, it was transferred to a reaction chamber to stand overnight for complete gelation before imaging. The gel contained a final concentration of 15 mM **H1**, 89 mM **A11**, 20 μM **A18**, 0.9 mM **A25**, and 20 μM fluorescein labelled concanavalin A. A control experiment was performed similar as above replacing equal volume of **A25** with buffer solution.

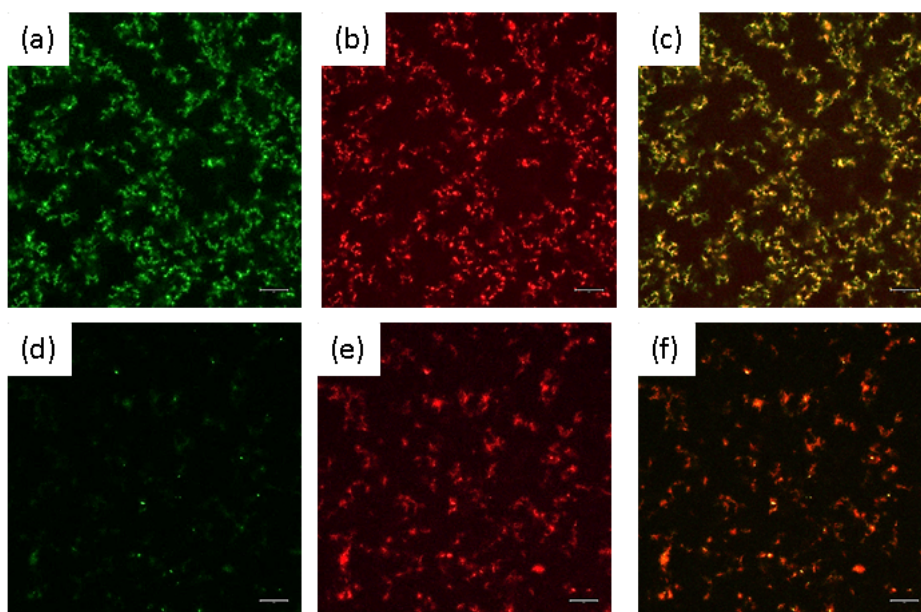


Figure S6: Confocal laser scanning micrograph of gel fibre (scale bar - 20 μM) in presence (top) or absence (bottom, blank experiment) of **A25**: (a, d) exciting fluorescein ($\lambda_{\text{exc.}} = 488 \text{ nm}$), emitted light collection: 486-534 nm range; master gain 1051 V, digital gain 1.2 (for a) and master gain 961 V, digital gain 1.2 (for d), (b, e) exciting rhodamine ($\lambda_{\text{exc.}} = 545 \text{ nm}$), emitted light collection: 581-797 nm range; master gain 946 V, digital gain 1.2 (for b) and master gain 871 V, digital gain 1.2 (for e), (c, f) merged: $\lambda_{\text{exc.}} = 488 \text{ nm}$ and 545 nm.

Fibre network functionalization with A20 and click reaction with azide functionalised Ovalbumin protein:

H1 was added to a mixture of **A15**, **A20**, **CuI** and azide-functionalised Cy5-labelled *Ovalbumin* (*azide-Ova*). After gentle mixing, **A11** was added to it and the solution was mixed vigorously. Then, it was transferred to a reaction chamber to stand overnight for complete gelation before imaging. The gel contained a final concentration of 10 mM **H1**, 59 mM **A11**, 30 μM **A15**, 0.2 mM **A20**, 20 μM **CuI** and 1 μM *azide-Ova*. Control experiments were performed similar as above replacing either an equal volume of **A20** with buffer solution (thus leaving out the alkyne) or an equal volume of *azide-Ova* solution with wild type-**Ova** solution.

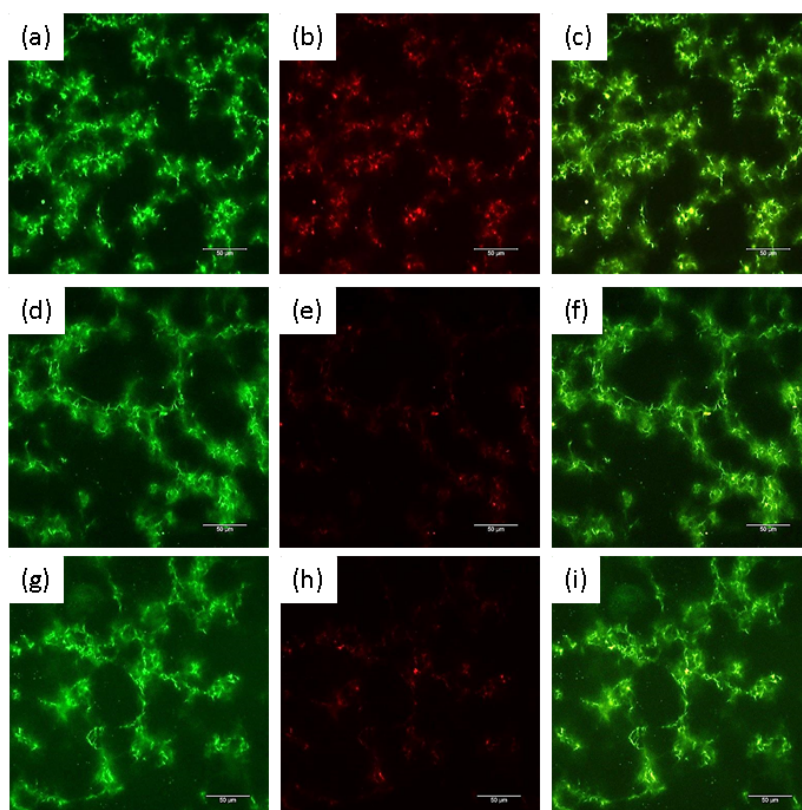


Figure S7: Confocal laser scanning micrograph of gel fibre (scale bar - 50 μM) with **A20** and azide-**Ova** (top row), only with azide-**Ova** (middle row), and with **A20** and wild type-**Ova** (bottom row): (a, d, g) exciting styryl-indolium ($\lambda_{\text{exc.}} = 405 \text{ nm}$), emitted light collection: 401-463 nm range; master gain 933 V, digital gain 1.2 (for a), master gain 970 V, digital gain 1.2 (for d), and master gain 950 V, digital gain 1.2 (for g), (b, e, h) exciting cyanine ($\lambda_{\text{exc.}} = 633 \text{ nm}$), emitted light collection: 644-797 nm range; master gain 1108 V, digital gain 1.2 (for b) and master gain 1138 V, digital gain 1.2 (for e), and master gain 1098 V, digital gain 1.2 (for h), and (c, f, i) merged: $\lambda_{\text{exc.}} = 405 \text{ nm}$ and 633 nm.

Rheology: Oscillatory experiments were performed using a AR G2 rheometer from TA Instruments in a strain-controlled mode; the rheometer was equipped with a steel plate-and- plate geometry of diameter 40 mm and a water trap. The temperature of the plates was controlled at $25 \pm 0.2 \text{ }^{\circ}\text{C}$. Measurements were performed at a frequency of 1 Hz while applying 0.05% strain. During measurement, the storage and loss moduli G' and G'' were followed as a function of the time. Total volume = 1mL and gelator concentration = 20 mM (20 mM of hydrazide derivative, 120 mM of aldehyde derivative (for **H1**) or 80 mM of aldehyde derivative (for **H2-H6**). The reported G' value is where G' reaches a plateau value.

Transmission Electron Microscopy

A few microliters of suspension were deposited on a Quantifoil R 1.2/1.3 Holey Carbon coated grid. After blotting away the excess of liquid the grids were stained with phosphotungstic acid (2 wt% in water) and dried. Samples were observed in a JEOL JEM-1400Plus electron microscope, operating at 120 kV. Micrographs were recorded under low-dose conditions on a slow-scan CCD camera (Gatan, 830)

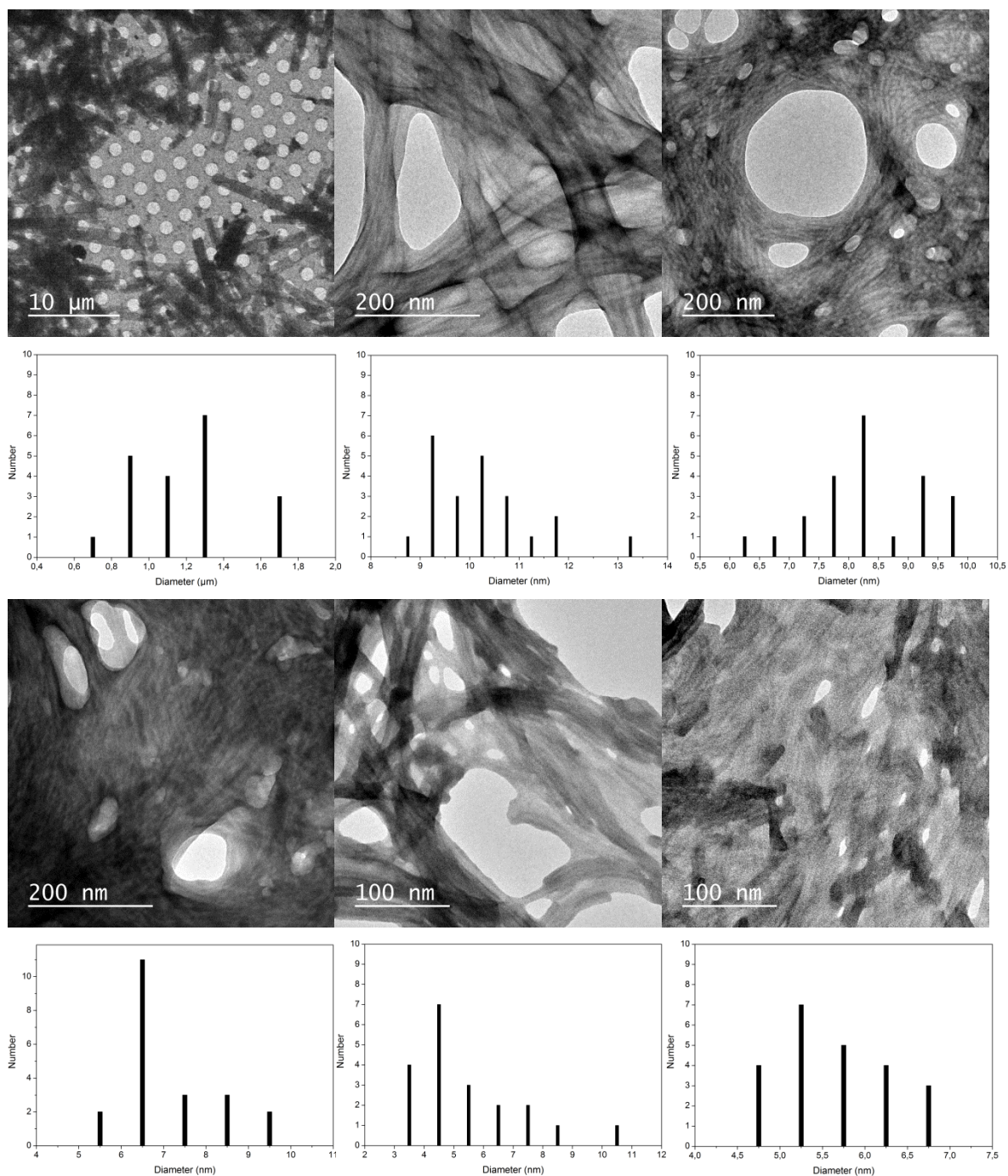
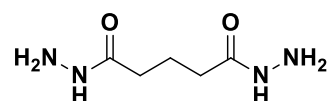


Figure S8: TEM images of fibres of hydrogelators prepared from (top, left to right) **H6** (15.5 mM) and **A2** (62 mM); **H1** (9 mM) and **A2** (54 mM); **H1** (6.5 mM) and **A7** (39 mM); (bottom, left to right) **H1** (5.5 mM) and **A11** (33 mM); **H1** (5.5 mM) and **A11** (33 mM) with **A18** (330 μ M); and Concanavalin A (7 μ M), **H1** (5.5 mM) and **A11** (33 mM) with **A25** (330 μ M). Below are the statistical distributions of single fibre diameters (obvious fibre bundles were omitted). A control with only **A11** showed no discernable structures.

Characterisation data:

Glutarohydrazide (**H3**):

Although the compound is described in the literature,¹⁵ some characterisation data were not reported. **Yield:** quantitative.



$^1\text{H-NMR}$ (400 MHz, DMSO-D_6) δ = 8.92 (s, 2H, CONH), 4.13 (s, 4H, NHNH_2), 1.98 (bs, 4H, CH_2), 1.70 (d, 2H, CH_2). **$^{13}\text{C-NMR}$** (100 MHz, DMSO-D_6): δ = 171.6 (CO), 33.3 (COCH_2^-), 21.9 ($-\text{CH}_2^-$). **MS** (ESI^+) m/z : 161.0 $[\text{M}+\text{H}]^+$, 183.1 $[\text{M}+\text{Na}]^+$.

Heptanedihydrazide (**H5**):

Yield: quantitative. **$^1\text{H-NMR}$** (400 MHz, DMSO-D_6) δ = 8.89 (s, 2H, CONH), 4.13 (s, 4H, NHNH_2), 1.97 (bs, 4H, CH_2), 1.46 (bs, 4H, CH_2), 1.19 (d, 2H, CH_2). **$^{13}\text{C-NMR}$** (100 MHz, DMSO-D_6): δ = 172.0 (CO), 33.7 (COCH_2^-), 28.7 (CH_2), 25.4 ($-\text{CH}_2^-$). **MS** (ESI^+) m/z : 189.1 $[\text{M}+\text{H}]^+$, 211.1 $[\text{M}+\text{Na}]^+$.

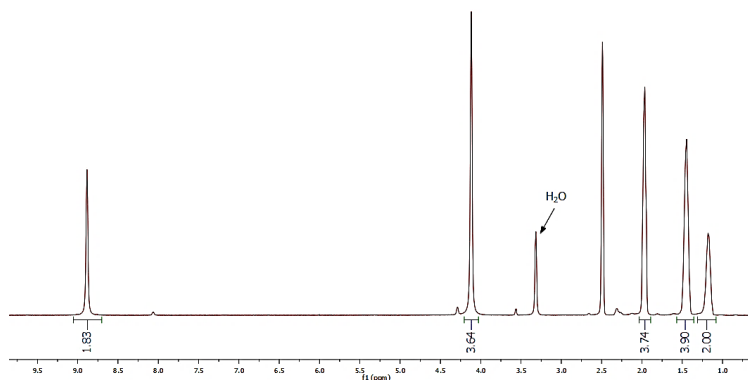
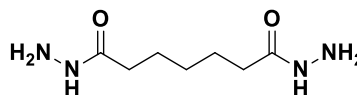


Figure S9: ^1H NMR spectrum of heptanedihydrazide (**H5**) in DMSO-D_6 at 25 °C.

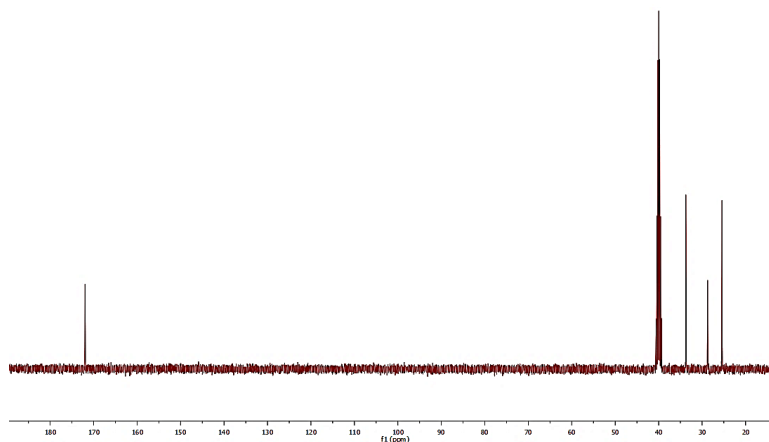
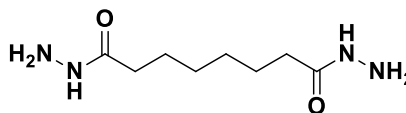


Figure S10: ^{13}C NMR spectrum of heptanedihydrazide (**H5**) in DMSO-D_6 at 25 °C.

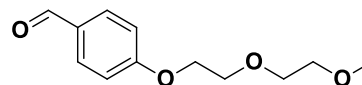
Octanedihydrazide (**H6**):

Yield: quantitative. The spectroscopic data matched with the corresponding reported data in the literature.¹⁶



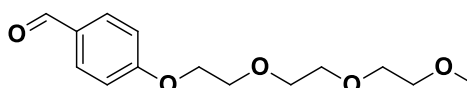
4-(2-(2-Methoxyethoxy)ethoxy)benzaldehyde (**A2**):

Yield: 78 %. The spectroscopic data matched with the corresponding reported data in the literature.¹⁷



4-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)benzaldehyde (**A3**):

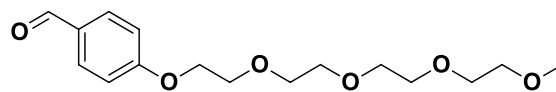
Yield: 88 %. The spectroscopic data matched with the



corresponding reported data in the literature.¹⁸

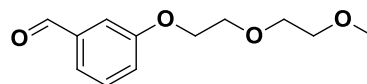
4-(2-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)ethoxy)benzaldehyde (A4):

Yield: 91 %. The spectroscopic data matched with the corresponding reported data in the literature.¹⁹



3-(2-(2-Methoxyethoxy)ethoxy)benzaldehyde (A7): Although

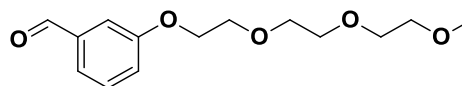
the compound is described in the literature, some characterisation data were not reported.²⁰ **Yield:** 89 %. ¹H-



NMR (400 MHz, CDCl₃): δ = 9.96 (s, 1H, CHO), 7.45 – 7.39 (m, 3H, ArH), 7.02 (dt, 1H, J = 2.1 Hz, J = 7.3 Hz, ArH), 4.20 (t, 2H, CH₂), 3.88 (t, 2H, CH₂), 3.72 (t, 2H, CH₂), 3.57 (t, 2H, CH₂), 3.38 (s, 3H, CH₃). ¹³C-NMR (100 MHz, CDCl₃): δ = 192.3 (CHO), 159.6 (C_{Ar}), 138.0 (C_{Ar}), 130.2 (C_{Ar}), 123.8 (C_{Ar}), 122.2 (C_{Ar}), 113.3 (C_{Ar}), 72.2 (CH₂), 71.0 (CH₂), 69.9 (CH₂), 67.9 (CH₂), 59.3 (CH₃). **MS** (ESI⁺) m/z : 225.3 [M+H]⁺.

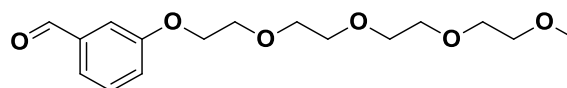
3-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)benzaldehyde (A8):

Yield: 91 %. The spectroscopic data matched with the corresponding reported data in the literature.¹⁸



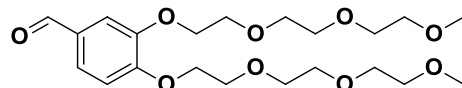
3-(2-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)ethoxy)benzaldehyde (A9):

Yield: 87 %. The spectroscopic data matched with the corresponding reported data in the literature.²¹



3,4-Bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzaldehyde (A12):

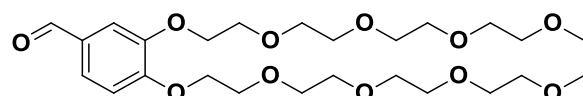
Yield: 99 %. The spectroscopic data matched with the corresponding reported data in the literature.²²



3,4-Bis(2-(2-(2-(2-

methoxyethoxy)ethoxy)ethoxy)benzaldehyde (A13):

Yield: 79 %. ¹H-NMR (400 MHz, CDCl₃): δ = 9.76 (s, 1H, CHO), 7.43 (bs, 2H, ArH), 6.94 (d, J = 6.3 Hz, 1H, ArH), 4.17-4.14 (m, 4H, CH₂),



3.83 (s, 4H, CH₂), 3.67 (s, 4H, CH₂), 3.59 (bs, 14H, CH₂), 3.47 (s, 4H, CH₂), 3.29 (s, 6H, CH₃). ¹³C NMR (100 MHz, CDCl₃): δ = 190.7 (CHO), 154.3 (C_{Ar}), 149.1 (C_{Ar}), 130.2 (C_{Ar}), 126.5 (C_{Ar}), 112.5 (C_{Ar}), 111.9 (C_{Ar}), 71.8 (2xCH₂), 70.9 (CH₂), 70.8 (CH₂), 70.6 (2xCH₂), 70.5 (4xCH₂), 70.4 (2xCH₂), 69.5 (CH₂), 69.3 (CH₂), 68.7 (CH₂), 68.3 (CH₂), 58.9 (2xCH₃). **MS** (ESI⁺) m/z : 520.0 [M+H]⁺.

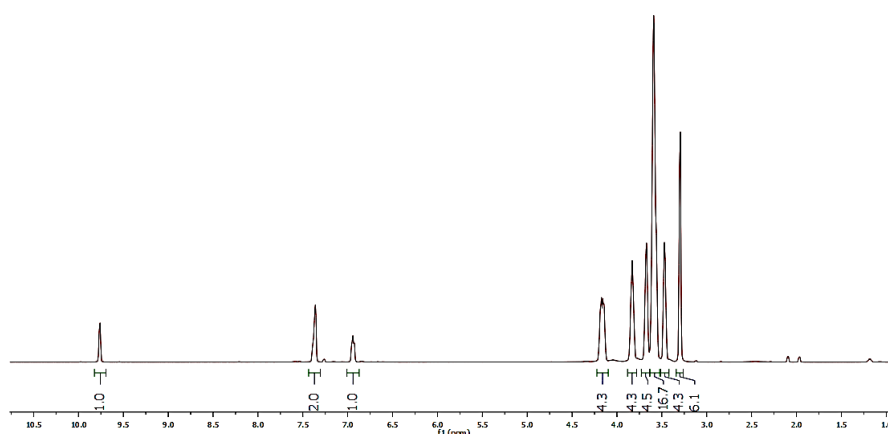


Figure S11: ^1H NMR spectrum of 3,4-bis(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)benzaldehyde (**A13**) in CDCl_3 at 25 °C.

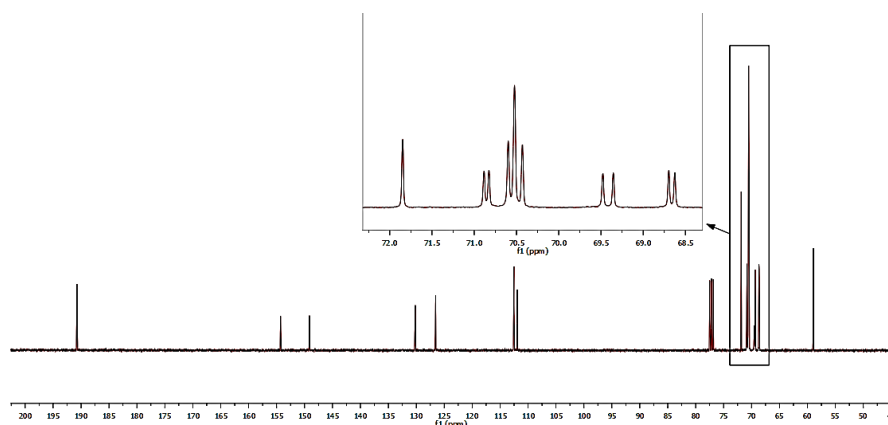
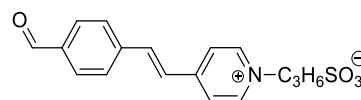


Figure S12: ^{13}C NMR spectrum of 3,4-bis(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)benzaldehyde (**A13**) in CDCl_3 at 25 °C.

3-(4-(4-Formylstyryl)pyridiniumyl)propane-1-sulfonate (**A14**):

3-(4-Methylpyridiniumyl)propane-1-sulfonate (0.97 g, 4.5 mmol) and terephthalaldehyde (1.81 g, 13.5 mmol) were dissolved in EtOH (5.0 mL). Piperidine (0.45 mL, 4.5 mmol) was added to the mixture and it was refluxed for overnight. After cooling at ambient temperature, a greenish solid was obtained by filtration, which was washed with ethanol, acetone and dried under reduced pressure to obtain the desired compound as pale greenish solid (0.70 g, 47 %). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 10.06 (s, 1H, CHO), 8.99 (d, J = 6.7 Hz, 2H, ArH), 8.27 (d, J = 6.8 Hz, 2H, ArH), 8.03 (m, 3H, ArH), 7.84 (s, 2H, ArH), 7.66 (d, J = 16.3 Hz, 1H, ArH), 4.67 (q, 2H, CH_2), 2.45 (t, 2H, CH_2), 2.24 (m, 2H, CH_2). **MS** (ESI Pos.): m/z = 284.1 $[(\text{MH}-\text{O}_3)^+]$, 209 $[(\text{MH}-\text{C}_3\text{H}_7\text{SO}_3)^+]$, 180, 152.



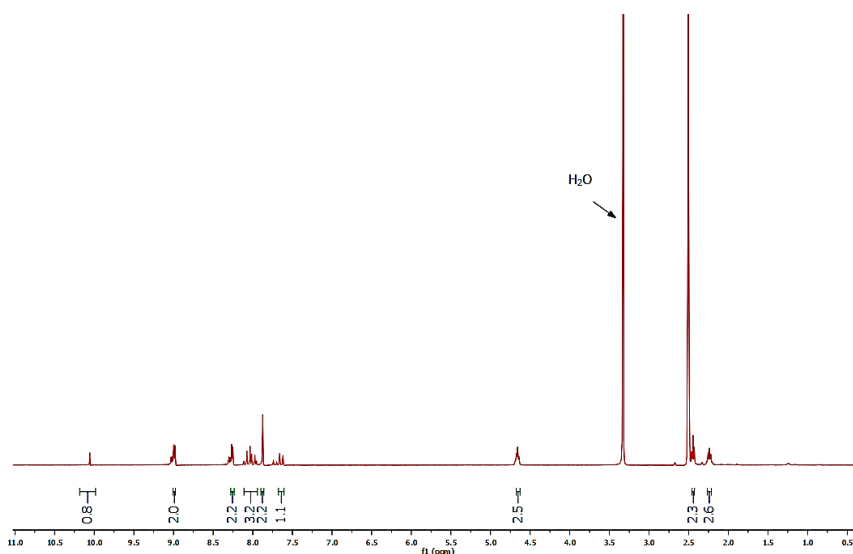


Figure S13: ^1H NMR spectrum of 3-(4-(4-formylstyryl)pyridiniumyl)propane-1-sulfonate (**A14**) in $\text{DMSO-}D_6$ at 25°C .

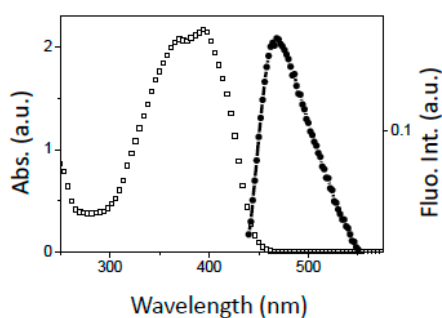
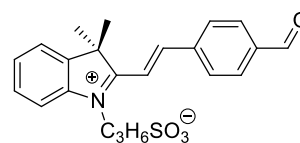


Figure S14: UV-Vis and fluorescence spectra of styryl derivative (**A14**) in water ($C = 10^{-4} \text{ M}$, $\lambda_{\text{exc.}} = 430 \text{ nm}$).

3-(2-(4-Formylstyryl)-3,3-dimethyl-3H-indoliumyl)propane-1-sulfonate (A15**):**

2,3,3-Trimethyl-1-(3-sulfonatepropyl)-3H-indolium (0.28 g, 1.0 mmol) and terephthalaldehyde (0.67 g, 5.0 mmol) were dissolved in acetic acid (5.0 mL) and the mixture was refluxed for overnight. After completion of the reaction, it was concentrated under reduced pressure and the obtained residue was washed with CHCl_3 , followed by acetone to obtain the desired compound as an orange solid (0.35 g, 88 %). ^1H NMR (400 MHz, $\text{DMSO-}D_6$): $\delta = 10.11$ (s, 1H, CHO), 8.53 (m, 3H, ArH), 8.10 (m, 4H, ArH), 7.92 (d, $J = 3.1 \text{ Hz}$, 1H, ArH), 7.69 (d, $J = 3.1 \text{ Hz}$, 2H, ArH), 4.96 (d, 2H, CH_2), 2.70 (t, 2H, CH_2), 2.23 (bs, 2H, CH_2), 1.83 (s, 6H, $\text{C}(\text{CH}_3)_2$). **MS** (ESI Pos.): $m/z = 284[\text{M} + \text{Na} - \text{C}_3\text{H}_4\text{SO}_4]^+$, 142.



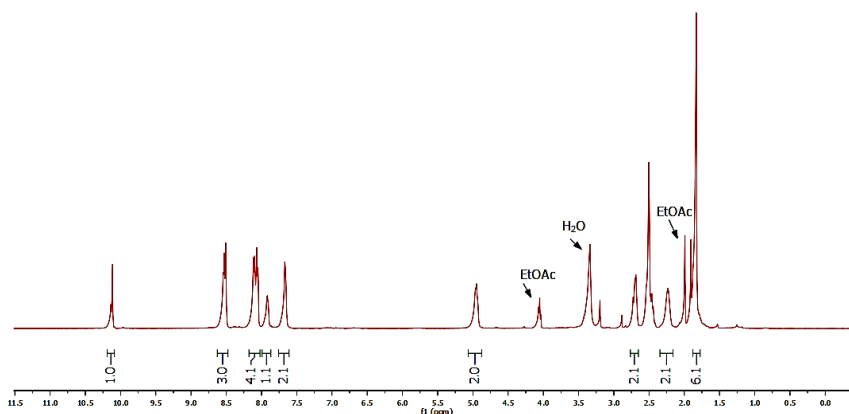


Figure S15: ^1H NMR spectrum of indolium derivative (**A15**) in $\text{DMSO-}D_6$ at 25°C .

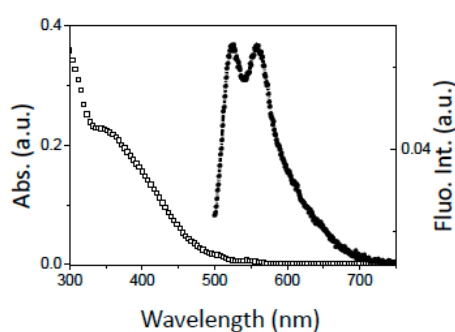
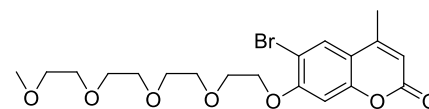


Figure S16: UV-Vis and fluorescence spectra of indolium derivative (**A15**) in water ($C = 10^{-4} \text{ M}$, $\lambda_{\text{exc.}} = 420 \text{ nm}$).

6-Bromo-4-methyl-7-O-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)coumarin:

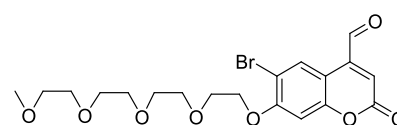
K_2CO_3 (2.76 g, 20.0 mmol) was added to a solution of 6-bromo-4-methyl-7-hydroxycoumarin (2.55 g, 10.0 mmol) in DMF (20.0 mL). The mixture was heated at 80°C for 30 minutes. After that, tetraethylene glycol monomethyl ether tosylate (5.44 g, 15.0 mmol) was added to the mixture and heating was continued for overnight. After completion of the reaction, it was concentrated under reduced pressure and diluted with EtOAc. The organic layer was washed with water, brine and dried over MgSO_4 . After removal of the solvent, the crude substance was purified by column chromatography (silica gel, $\text{CHCl}_3/\text{EtOAc}$) to obtain the desired compound as a brownish liquid (2.70 g, 61 %).

^1H NMR (400 MHz, CD_3OD): $\delta = 7.81$ (s, 1H, ArH), 6.92 (s, 1H, ArH), 6.16 (s, 1H, ArH), 4.28 (t, 2H, $\text{OCH}_2\text{CH}_2\text{O}_4\text{C}_7\text{H}_{15}$), 3.96 (t, 2H, $\text{OCH}_2\text{CH}_2\text{O}_4\text{C}_7\text{H}_{15}$), 3.81 (t, 2H, $\text{OC}_2\text{H}_4\text{OCH}_2\text{CH}_2\text{O}_3\text{C}_5\text{H}_{11}$), 3.70 – 3.63 (m, 10H, $\text{OC}_2\text{H}_4\text{OCH}_2\text{CH}_2\text{O}_2\text{C}_4\text{H}_8\text{OCH}_3$), 3.56 (s, 3H, $\text{O}_4\text{C}_8\text{H}_{16}\text{OCH}_3$), 2.40 (s, 3H, Ar- CH_3). **MS** (ESI Pos.): $m/z = 445.7$ $[(\text{M}+\text{H})]^+$.



6-Bromo-4-formyl-7-O-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)coumarin (A16**):**

A mixture of 6-bromo-4-methyl-7-O-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)coumarin (4.45g, 10.0 mmol) and SeO_2 (1.44 g, 13.0 mmol) in xylene (15.0 mL) was stirred at reflux temperature for 2 days. After completion of the reaction, it was filtered through celite and concentrated under reduced pressure. The obtained crude substance was purified by column chromatography (silica gel,



Fluorescein Labelled Aldehyde (**A17**):

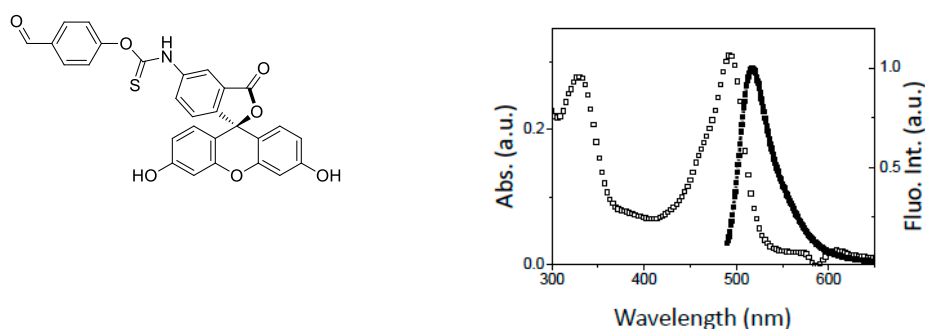


Figure S20: UV-Vis spectrum of fluorescein derivative (**A17**) in water ($C = 10^{-6}$ M, $\lambda_{exc.} = 490$ nm).

Rhodamine Labelled Aldehyde (**A18**):

NaH (0.01 g, 0.40 mmol) was added to a solution of 4-hydroxybenzaldehyde (0.02 g, 0.20 mmol) in DMF (2.0 mL) at 0 °C. A solution of rhodamine B isothiocyanate (0.05 g, 0.10 mmol, Sigma Aldrich) in DMF (1.0 mL) was added afterwards to the mixture and it was stirred for overnight at 80 °C. After that, the solvent was evaporated under reduced pressure and the obtained residue was taken in minimum amount of water and aqueous layer was washed with CH_2Cl_2 to remove the excess of benzaldehyde derivative. The aqueous layer was then concentrated under reduced pressure and dried to obtain desired compound as red solid (0.04 g, 64 %). **MS** (ESI Neg.): $m/z = 620.1$ ($[\text{M}-\text{H}]^+$).

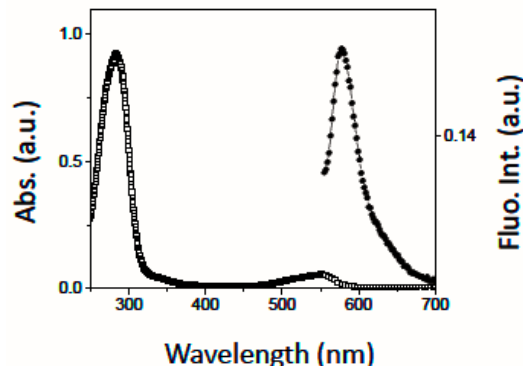
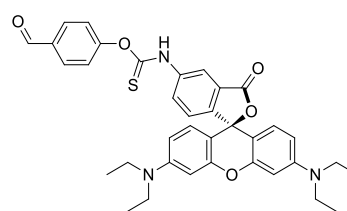
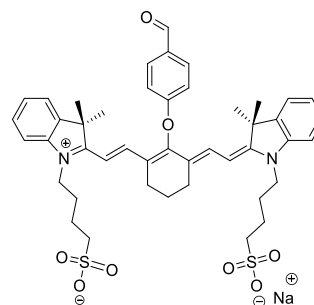


Figure S21: UV-Vis and fluorescence spectra of rhodamine derivative (**A18**) in water ($C = 10^{-5}$ M, $\lambda_{exc.} = 550$ nm).

Cyanine Dye Labelled Aldehyde (**A19**):

4-Hydroxybenzaldehyde (15.0 mg, 0.12 mmol) and NaH (3.0 mg, 0.12 mmol) were dissolved in anhydrous DMF (5.0 mL) and the mixture was stirred at room temperature for 10 min. Then a solution of IR-783 (2-[2-[2-Chloro-3-[2-[1,3-dihydro-3,3-dimethyl-1-(4-sulfobutyl)-2H-indol-2-ylidene]-ethylidene]-1-cyclohexen-1-yl]-ethenyl]-3,3-dimethyl-1-(4-sulfobutyl)-3H-indolium hydroxide; Sigma Aldrich) (37.0 mg, 0.05 mmol, Sigma Aldrich) in DMF (5.0 mL) was added to the mixture and it was further stirred for overnight. After that, the solvent was removed under reduced pressure and the obtained residue was washed with CHCl_3 to obtain the desired compound as a dark green solid (3.0



mg, 72 %). **¹H NMR** (400 MHz, CD₃OD): δ = 9.48 (bs, 3H, -CHO & ArH), 7.95 (bs, 3H, ArH), 7.59 (bs, 5H, ArH), 7.26 (bs, 1H, ArH), 6.62 (bs, 5H, ArH), 3.28 (bs, 2H, -CH₂-), 2.96 (bs, 4H, -CH₂-), 2.83 (bs, 12H, -CH₂-), 1.89 (bs, 4H, -CH₂-), 1.28 (bs, 6H, C(CH₃)₂), 0.86 (bs, 6H, C(CH₃)₂). **MS** (ESI Neg.): *m/z* = 811.3 ([M-Na]⁺).

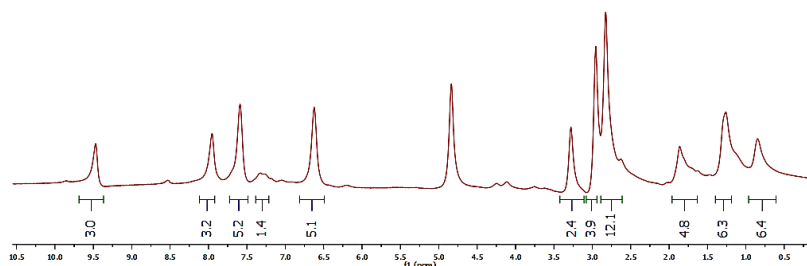


Figure S22: ¹H NMR spectrum of cyanine derivative (**A19**) in CD₃OD at 25 °C.

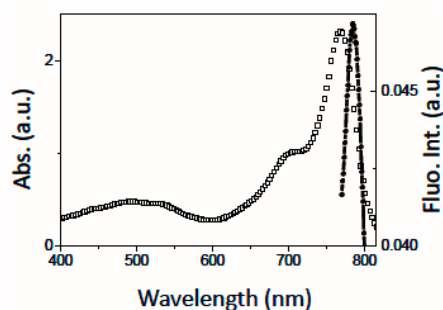
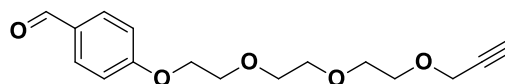


Figure S23: UV-Vis and fluorescence spectra of cyanine derivative (**A19**) in water (*C* = 10⁻⁵ M, λ_{exc.} = 765 nm).

4-(2-(2-(2-(Prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)benzaldehyde (**A20**):

4-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)benzaldehyde (5.00 g, 19.7 mmol) was dissolved in dry THF (200.0 mL) and the solution was cooled to 0 °C using an ice bath. NaH (0.94 g, 60 wt% in mineral oil, 23.60 mmol) was added to the solution and it was stirred for 1h. Propargyl bromide (2.22 mL, 80 wt% in toluene, 21.63 mmol) was added to the mixture and it was allowed to obtain ambient temperature. The reaction mixture was stirred overnight. After completion of the reaction, it was concentrated under reduced pressure and diluted with water (200 mL). The aqueous layer was extracted with CH₂Cl₂ (3 x 200 mL). The organic layers were combined and washed with brine (600 mL). The organic layer was dried over MgSO₄, then filtered off and concentrated under reduced pressure. The obtained residue was purified by column chromatography (Silica gel, EtOAc/PE 1/1) yielding the desired product as a light-yellow oil (2.52 g, 44 %). **¹H NMR** (400 MHz, CDCl₃): δ = 9.82 (s, 1H), 7.78 (d, *J* = 8.6 Hz, 2H, Ar-*H*), 6.98 (d, *J* = 8.6 Hz, 2H, Ar-*H*), 4.19-4.13 (m, 4H, CH₂), 3.87-3.82 (m, 2H, CH₂), 3.77-3.71 (m, 2H, CH₂), 3.66-3.61 (m, 6H, CH₂), 2.41 (t, *J* = 2.2 Hz, 1H, CH). **¹³C NMR** (100 MHz, CDCl₃): δ = 190.8 (CHO), 163.9 (C_{Ar}), 132.0 (2x C_{Ar}), 130.1 (C_{Ar}), 114.9 (2x C_{Ar}), 79.7 (C≡CH), 74.7 (C≡CH), 70.9 (CH₂), 70.7 (CH₂), 70.5 (CH₂), 69.5 (CH₂), 69.1 (CH₂), 67.8 (CH₂), 58.4 (CH₂). **MS** (ESI Pos.): *m/z* = 293.5 ([M+H]⁺).



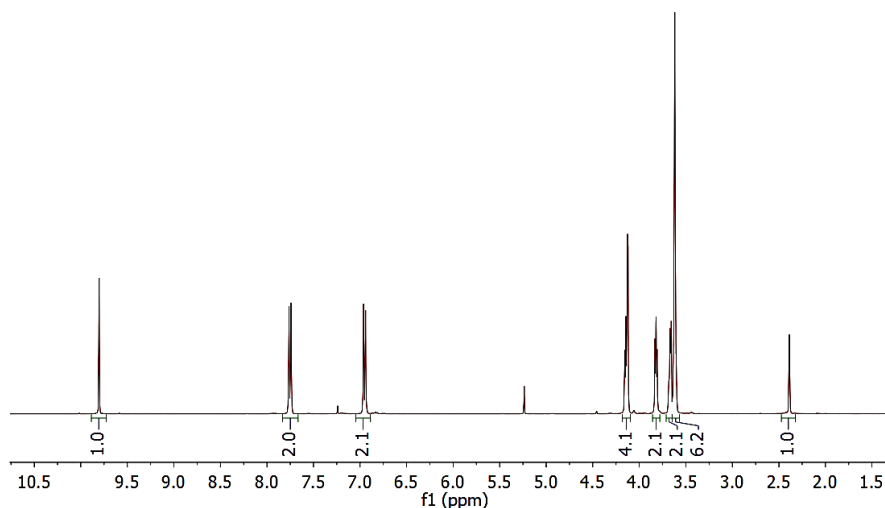


Figure S24: ^1H NMR spectrum of 4-(2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)benzaldehyde (**A20**) in CDCl_3 at 25 $^\circ\text{C}$.

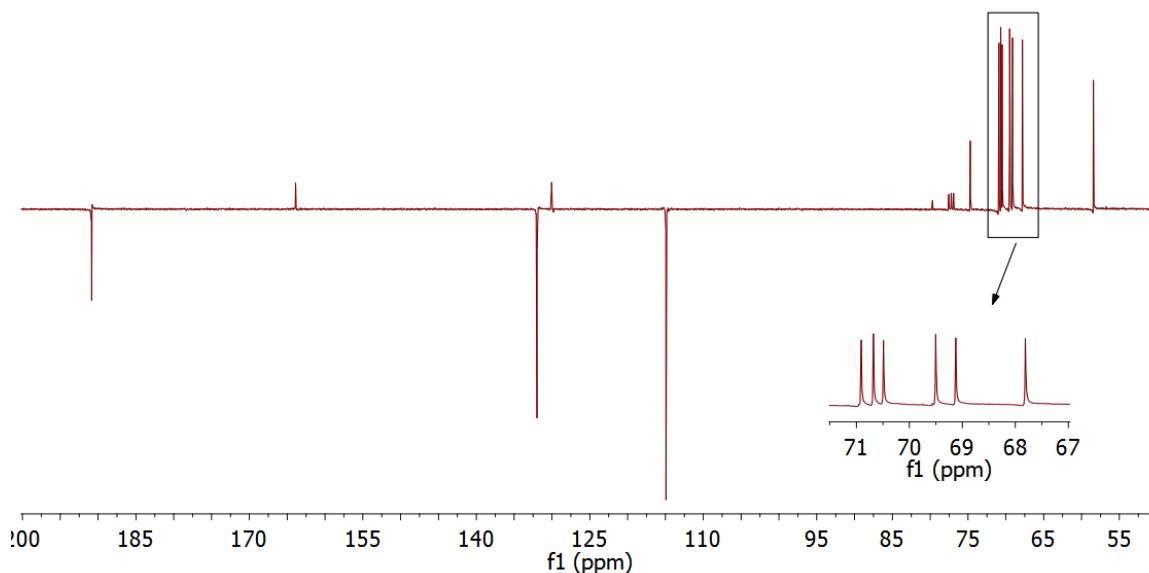
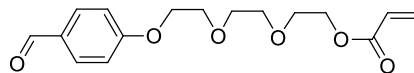


Figure S25: ^{13}C NMR spectrum of 4-(2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)benzaldehyde (**A20**) in CDCl_3 at 25 $^\circ\text{C}$.

2-(2-(2-(4-Formylphenoxy)ethoxy)ethoxy)ethyl acrylate (A21**):**

Acryloyl chloride (1.45 mL, 18.0 mmol) was added dropwise to a solution of 4-(2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)benzaldehyde (2.29 g, 9.0 mmol), triethyl amine (2.90 mL, 20.7 mmol) in CH_2Cl_2 (90.0 mL) at 0 $^\circ\text{C}$. After complete addition, it was allowed to get ambient temperature and stirred at that temperature for overnight. After completion of the reaction, it was quenched with water and diluted with CH_2Cl_2 . The organic layer was washed with water, brine and dried over MgSO_4 . After removal of the solvent under reduced pressure, the crude compound was purified by flash column chromatography (silica gel, CH_2Cl_2) to obtain the desired compound as a viscous liquid (2.77 g, 94 %). ^1H NMR (400 MHz, CDCl_3): δ = 9.88 (s, 1H, CHO), 7.83 (d, J = 8.8 Hz, 2H, Ar-H), 7.03 (d, J = 8.7 Hz, 2H, Ar-H), 6.44 (dd, J = 17.3 Hz, J = 1.4 Hz, 1H, CH=CHH), 6.15 (dd, J = 10.5 Hz, J = 17.4 Hz, CH=CH₂), 5.83 (dd,



$J = 1.4 \text{ Hz}$, $J = 10.4 \text{ Hz}$, 1H, CH=CHH), 4.31 (t, 2H, CH₂), 4.21 (t, 2H, CH₂), 3.89 (t, 2H, CH₂), 3.74–3.72 (m, 4H, CH₂), 3.69 (t, 2H, CH₂). ¹³C NMR (100 MHz, CDCl₃): $\delta = 190.8$ (CHO), 166.1 (CO), 163.8 (C_{Ar}), 131.9 (2xC_{Ar}), 131.0 (CH=CH₂), 130.0 (C_{Ar}), 128.2 (CH=CH₂), 114.8 (2xC_{Ar}), 70.9 (CH₂), 70.6 (CH₂), 69.5 (CH₂), 69.2 (CH₂), 67.7 (CH₂), 63.6 (CH₂). MS (ESI Pos.) m/z : 309.2 [(M+H)⁺], 331.2 [(M+Na)⁺].

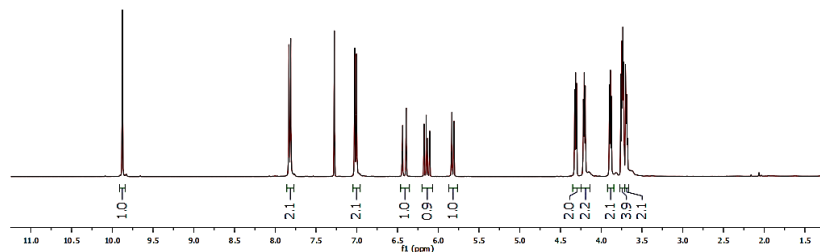


Figure S26: ¹H NMR spectrum of 2-(2-(2-(4-formylphenoxy)ethoxy)ethoxy)ethyl acrylate (**A21**) in CDCl₃ at 25 °C.

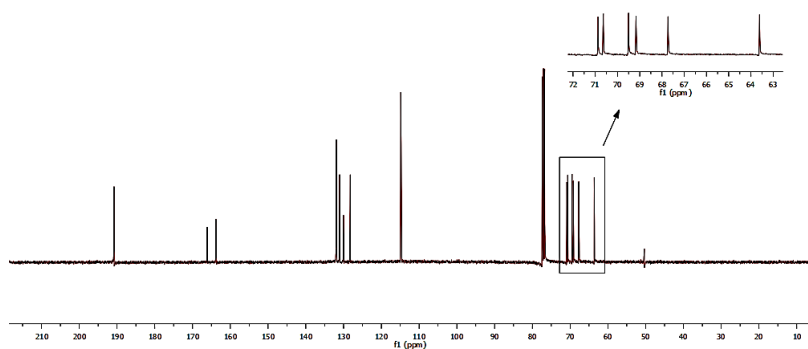
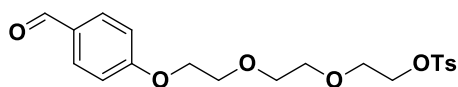


Figure S27: ¹³C NMR spectrum of 2-(2-(2-(4-formylphenoxy)ethoxy)ethoxy)ethyl acrylate (**A21**) in CDCl₃ at 25 °C.

2-(2-(2-(4-Formylphenoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**A22**):

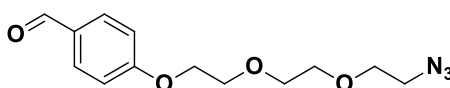
4-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)benzaldehyde (**A5**) (1.00 g, 3.9 mmol) was dissolved in CH₂Cl₂ (50.0 mL). *p*-Toluenesulfonyl chloride (1.13 g, 5.90 mmol)



and triethyl amine (1.1 mL, 7.87 mmol) were added and the reaction mixture was stirred overnight at ambient temperature. After completion of the reaction (18 h and complete conversion was observed in ¹H-NMR), the reaction mixture was concentrated under reduced pressure. The obtained residue was diluted with CH₂Cl₂ (200.0 mL) and washed with an aqueous saturated NaHCO₃ solution, brine. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure yielding the desired compound (1.93 g, quantitative). ¹H-NMR (400 MHz, CDCl₃): $\delta = 9.85$ (s, 1H, CHO), 7.80 – 7.77 (m, 4H, ArH), 7.31 (s, 2H, ArH), 7.00 (s, 2H, ArH), 4.18–4.13 (m, 4H, CH₂), 3.84 (s, 2H, CH₂), 3.66–3.60 (m, 6H, CH₂), 2.41 (s, CH₃). ¹³C NMR (100 MHz, CDCl₃): $\delta = 190.8$ (CHO), 163.8 (C_{Ar}), 144.8 (C_{Ar}), 132.9 (C_{Ar}), 131.9 (2xC_{Ar}), 130.0 (C_{Ar}), 129.8 (2xC_{Ar}), 127.9 (2xC_{Ar}), 114.8 (2xC_{Ar}), 70.8 (2xCH₂), 69.5 (CH₂), 69.2 (CH₂), 68.7 (CH₂), 67.7 (CH₂), 21.6 (CH₃).

4-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)benzaldehyde (**A23**):

2-(2-(2-(4-Formylphenoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (8.03 g, 19.7 mmol) was dissolved in DMF (100 mL). Sodium azide (6.39 g, 98.3



mmol) was added and the reaction was stirred while heating to 60 °C. After stirring for 18 h the reaction mixture was allowed to cool down and concentrated under reduced pressure. The residue was dissolved in an aqueous 1M HCl solution (200 mL) and extracted with EtOAc (200 mL). The organic layer was washed with a saturated aqueous NaHCO₃ solution (200 mL) and brine (200 mL). The organic layer was dried over MgSO₄, filtered off and concentrated under reduced pressure. Purification using a flash column chromatography (silica gel, 0 % → 10 % MeOH in CH₂Cl₂) yielded the desired compound (5.04 g, 92 %) as a light-brown oil. **¹H NMR** (400 MHz, CDCl₃): δ = 9.86 (s, 1H, CHO), 7.82 (d, *J* = 7.7 Hz, 2H, Ar-*H*), 7.02 (d, *J* = 7.8 Hz, 2H, Ar-*H*), 4.21 (m, 2H), 3.89 (m, 2H), 3.73 - 3.67 (m, 6H), 3.37 (m, 2H). **¹³C NMR** (100 MHz, CDCl₃): δ = 190.8 (CHO), 163.8 (*C*_{Ar}), 131.9 (2x*C*_{Ar}), 130.0 (*C*_{Ar}), 114.9 (2x*C*_{Ar}), 70.9 (CH₂), 70.7 (CH₂), 70.1 (CH₂), 69.5 (CH₂), 67.7 (CH₂), 50.6 (CH₂). **MS** (ESI Pos.) *m/z*: 280.4 [(M+H)⁺].

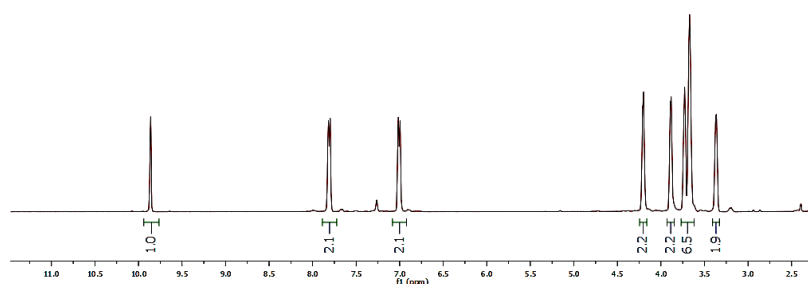


Figure S28: ¹H NMR spectrum of 2-(2-(2-(4-Formylphenoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**A23**) in CDCl₃ at 25 °C.

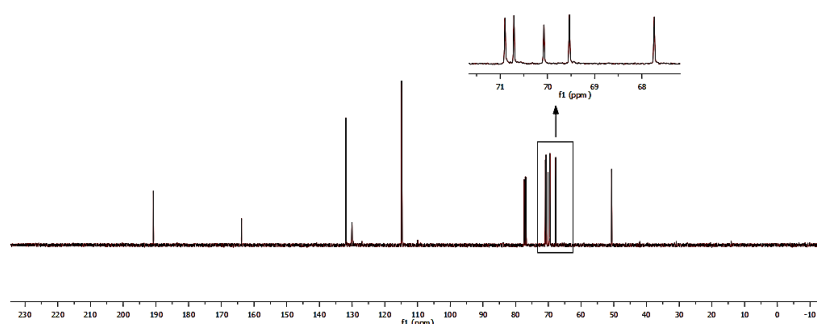
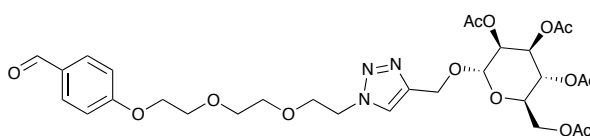


Figure S29: ¹³C NMR spectrum of 2-(2-(2-(4-Formylphenoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**A23**) in CDCl₃ at 25 °C.

Aldehyde Labelled Mannose Acetate Derivative (**A24**):

4-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)benzaldehyde (**A23**) (0.73 g, 2.62 mmol), 2,3,4,6-tetraacetylpropargylmannoside (1.01 g, 2.62 mmol) and triethyl amine (0.15



mL, 1.05 mmol) were added to a degassed mixture of CuI (0.05 g, 0.26 mmol) in CH₂Cl₂. The reaction mixture was stirred at ambient temperature for 3h. After completion of the reaction, it was concentrated under reduced pressure and the obtained residue was diluted with EtOAc. The organic layer was washed with satd. NaHCO₃ solution and brine, dried over MgSO₄. After concentration under reduced pressure, the obtained crude compound was purified by column chromatography (Silica gel, EtOAc) to obtain the desired compound as viscous oil (1.65 g, 95 %). *R_f* (MeOH/CH₂Cl₂ 1/4) = 0.8. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) =

9.86 (s, 1H, CHO), 7.82 (m, 3H, triazole-H and Ar-H), 7.01 (d, $J = 7.9$ Hz, 2H, ArH), 5.29 (s, 2H, OCH₂), 5.21 (s, 1H, mannose-H), 4.92 (s, 1H, mannose-H), 4.79 (bs, 2H, mannose-H), 4.55 (m, 2H, OCH₂), 4.28 (m, 1H, mannose-H), 4.20 (t, 2H, OCH₂), 4.08 (m, 2H, OCH₂), 3.89 – 3.83 (m, 4H, OCH₂), 3.68 – 3.64 (m, 4H, OCH₂), 2.12 (s, 3H, OCOCH₃), 2.09 (s, 3H, OCOCH₃), 2.01 (s, 3H, OCOCH₃), 1.95 (s, 3H, OCOCH₃). **MS** (ESI Pos.) m/z : 688.4 [(M+Na)⁺].

Aldehyde Labelled Mannose Derivative (**A25**):

Sodium methoxide solution (0.01 mL, 0.01 mmol, 30 mol % in CH₃OH) was added dropwise to a solution of the acetate derivative (0.70 g, 1.05 mmol) in CH₃OH (5.0 mL). After complete addition, it was stirred at room temperature while the solution turned greenish gradually. After completion of the reaction, it was concentrated under reduced pressure and then acidified with an ion exchange resin (eluent: CH₂Cl₂/MeOH 1/1) to obtain the desired compound as a pale yellow viscous liquid (0.50 g, 96 %). R_f (MeOH/CH₂Cl₂ 1/4) = 0.4. **¹H NMR** (400 MHz, CD₃OD): δ (ppm) = 9.84 (s, 1H, CHO), 8.05 (s, 1H, Triazole-H), 7.88 (d, $J = 7.9$ Hz, 2H, ArH), 7.11 (d, $J = 7.1$ Hz, 2H, ArH), 4.83 (s, 1H, mannose-H), 4.77 (d, 1H, mannose-H), 4.62 (s, 1H, mannose-H), 4.57 (t, 2H, OCH₂), 4.22 (t, 2H, OCH₂), 3.89 (t, 2H, OCH₂), 3.83 (m, 3H, OCH₂ & mannose-H), 3.77 – 3.72 (m, 2H, OCH₂), 3.67 – 3.54 (m, 7H, OCH₂ & mannose-H). **¹³C NMR** (100 MHz, CD₃OD): δ (ppm) = 192.80 (CHO), 165.58 (C_{Ar}), 145.10 (C_{Triazole}), 133.14 (C_{Ar}), 131.46 (C_{Triazole}), 126.15 (C_{Ar}), 116.07 (C_{Ar}), 100.74 (C_{Mannose}), 74.99 (C_{Mannose}), 72.53 (C_{Mannose}), 72.03 (C_{Mannose}), 71.73 (C_{Mannose}), 71.47 (OCH₂), 70.58 (OCH₂), 70.28 (OCH₂), 69.09 (OCH₂), 68.60 (OCH₂), 62.98 (OCH₂), 60.64 (OCH₂), 51.45 (NCH₂). **MS** (ESI Pos.) m/z : 520.4 [(M+K)⁺].

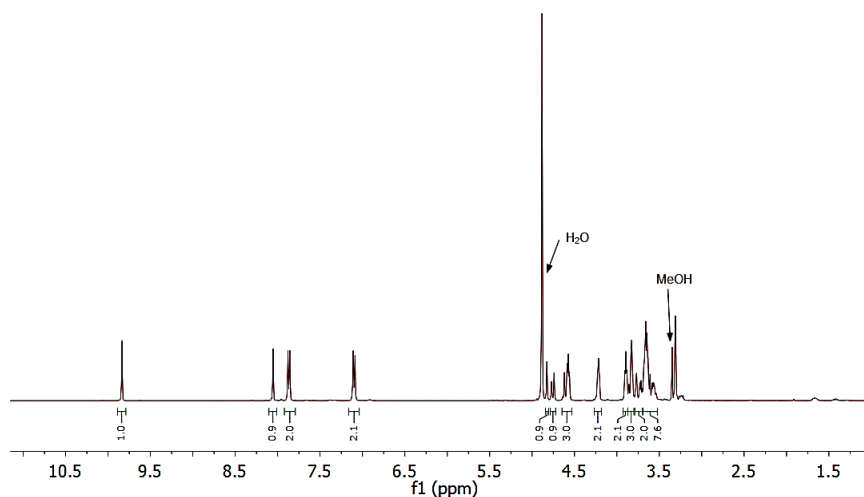
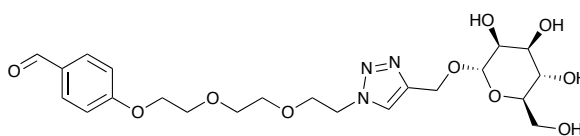


Figure S30: ¹H NMR spectrum of aldehyde labelled mannose derivative (**A25**) in CD₃OD at 25 °C.

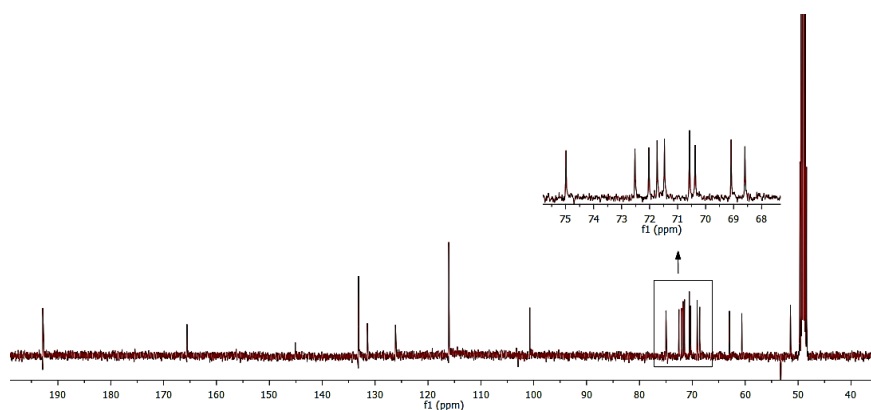


Figure S31: ^{13}C NMR spectrum of aldehyde labelled mannose derivative (**A25**) in CD_3OD at 25 °C.

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