Electronic Supplementary Information

Organogel-assisted topochemical synthesis of multivalent glycopolymer for high-affinity lectin binding

Baiju P. Krishnan, Sreedevi Raghu, Somnath Mukherjee and Kana M. Sureshan*

School of Chemistry, Indian Institute of Science Education and Research, Thiruvananthapuram,

CET Campus, Thiruvananthapuram-695016 (India).

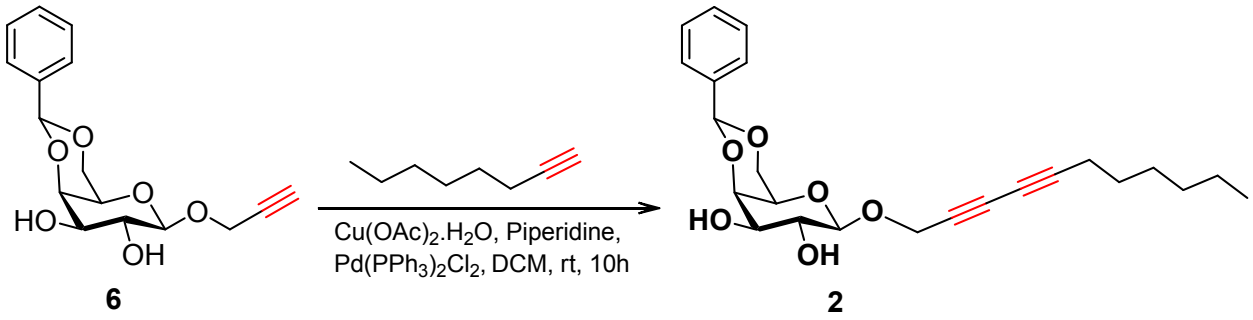
Email: email: kms@iisertvm.ac.in

1. Materials and methods:

All solvents were dried prior to use according to standard methods. All the reagents were purchased from Sigma-Aldrich and were used without further purification. TLC analyses were carried out using precoated TLC silica gel 60-F_{254} purchased from Merck. Chromatograms were visualized under UV light and by dipping the plates into ceric ammonium molybdate staining solution followed by heating. Purifications were done by column chromatography using 200-400 mesh silica gel. Melting point was determined using a Stuart, SMP 30 melting point apparatus. NMR spectra were reordered using a BRUKER AVANCE-II-500 NMR spectrometer. UV-Vis spectra were recorded on Shimadzu UV-3600 UV-VIS-NIR. Topochemical polymerization in gel state was studied using UV-Vis spectrometer. At first, a toluene gel of **2** was prepared in a test tube at its CGC. Then test tube was heated and hot solution was transferred immediately into a quartz cuvette (10 mm, 700 μL). It became gel inside the quartz cuvette upon cooling. UV-Visible spectra of the gel were taken at different times of intervals during irradiation. Elemental analyses were carried out by using Elementar, vario MICRO cube elemental analyzer. IR spectra were recorded using IR Prestige-21 (Shimadzu). IR spectra of (i) gel samples were recorded by taking the gel in demountable cell (with NaCl windows) , (ii) the dichloromethane solution of the gelators were recorded by taking in a fixed NaCl cell and (iii) the xerogel samples were recorded by mixing with KBr and making pellets. Morphologies of the toluene gels were investigated by using FEI LOVA NANOSEM 450 scanning electron microscope. A small piece of xerogel was placed on carbon-tape pasted to a steel grid, sputter coated with chromium and then directly imaged under the scanning electron microscope. UV irradiation was carried out in RAYONET UV irradiation chamber using S. N. E Ultraviolet Co 300 nm lamp. Photopolymerization of toluene gel of **2** was monitored by time-dependent Raman spectroscopy using a HR800 LabRAM confocal Raman spectrometer operating at 20 mW laser power using a peltier cooled CCD detector. Toluene gel of **2** (100 mg in 5 mL toluene) was irradiated with a UV-lamp of wavelength 300 nm at room temperature. At different times, small volume of the gel was withdrawn and freeze-dried to xerogels. The xerogel thus obtained was characterized with Raman spectroscopy. Very small amounts of solid sample were placed on a glass slide and spectra were recorded using a He-Ne laser

source having an excitation wavelength of 633 nm and with an acquisition time of 10 seconds using a 10x objective. DSC analyses of these xerogels were carried out using DSC Q20 differential scanning calorimeter, at a heating rate of 5 °C/min. MALDI-TOF mass spectra of samples (in DMSO) were recorded using Bruker Daltonics flex Analysis mass spectrometer. FITC conjugated plant lectins for binding study were purchased from Sigma Aldrich (Peanut Agglutinin) and Vector Labs (*Erythrina crystagalli* Lectin, 5mg/mL in HEPES buffer and *Ricinus communis* Lectin, 5mg/mL in HEPES). For making the stock solution of proteins, as well as for washing purposes 0.01 M PBS buffer (1 mM $CaCl_2$, 4 mM $MgCl_2$, pH 7.4) was used. Cell culture plates were purchased from Corning Inc. The glass cover slips (12 x 12 mm) were purchased from Himedia. Confocal fluorescence microscopy images were captured using a Leica SP5 laser confocal microscope.

2. Scheme S1



Synthesis of diol 6

Diol **2** was synthesized from D-galactose by following a procedure reported in the literature.[1]

Synthesis of diyne 2

A solution of diol **6** (1 g, 3.26 mmol), 1-octyne (2.40 ml, 16.33 mmol), piperidine (0.96 ml, 9.78 mmol), $Cu(OAc)_2.H_2O$ (60 mg) and $Pd(PPh_3)_2Cl_2$ (1 mg) in CH_2Cl_2 (15 ml) was stirred in open air atmosphere at room temperature. The reaction was monitored by TLC. After completion of the reaction (10 h), the reaction mixture was washed successively with

saturated NH_4Cl (2× 20 mL) solution and brine (20 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography using a mixture of petroleum ether–EtOAc (1:2.5 v/v) as eluent to afford diyne **2** (0.900 g, 67%) as a white solid. m.p. 160 ˚C, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.39-7.37 (m, 2H), 7.30-7.29 (m, 3H), 5.48 (s, 1H), 5.05 (d, *J* = 5.0 Hz, 1H), 4.88 (d, *J* = 6.0 Hz, 1H), 4.44-4.32 (m, 2H), 4.27 (d, *J* = 7.65 Hz, 1H), 4.00 (d, *J* = 3.6 Hz, 1H), 3.98 (s, 2H), 3.46 (s, 1H), 3.44-3.40 (m, 1H), 3.33-3.31 (m, 1H), 2.27 (t, *J* = 13.95, 2H), 1.42-1.37 (m, 2H), 1.29-1.16 (m, 6H), 0.79 (t, *J* = 13.9 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 138.6, 128.5, 127.8, 126.2, 101.4, 99.7, 81.9, 75.8, 72.5, 71.8, 70.6, 69.8, 68.4, 66.1, 64.4, 55.5, 30.6, 27.8, 27.5, 21.9, 18.3, 13.8. Anal Calcd for $C_{24}H_{30}O_6$ C = 69.54, H = 7.30. Found C = 69.70, H = 7.18.

3. Gelation studies

Gelation tests and determination of critical gelation concentration (CGC) were done as reported.[2] The CGC of diyne **2** in different solvents are tabulated in Table S1.

Table S1: Gelation abilities of diyne **2** in various solvents[a] and CGC

Solvents	**2**	**Solvents**	**2**
	CGC (wt%)		**CGC (wt%)**
Toluene	0.8 (TG)	Methanol	-- (S)
Benzene	1.8 (TG)	Ethanol	-- (S)
p-xylene	1.0 (TG)	Tetrahydrofuran	-- (S)
Mesitylene	1.1 (TG)	Hexane:EtOAc (1:1)	-- (S)
Hexadecane	0.50 (OG)	Water	-- (I)
Sunflower oil	1.70 (OG)	Silicon oil	-- (WG)
Diesel	0.25 (OG)	Petrol	1.9 (OG)
Kerosene	1.30 (TG)	DMSO	-- (S)

[a]Nature of the gels is in the parenthesis: TG = transparent gel; OG = opaque gel; WG = weak gel; S = soluble; I = insoluble; PS = partially soluble

4. Determination of gel-sol transition temperature (T_{gel})

Determination of T_{gel} was done as reported.[2] The concentration dependence of T_{gel} of toluene gels of **2** showed the enhancement in gel stability with gelator concentration as anticipated (Fig. S1).

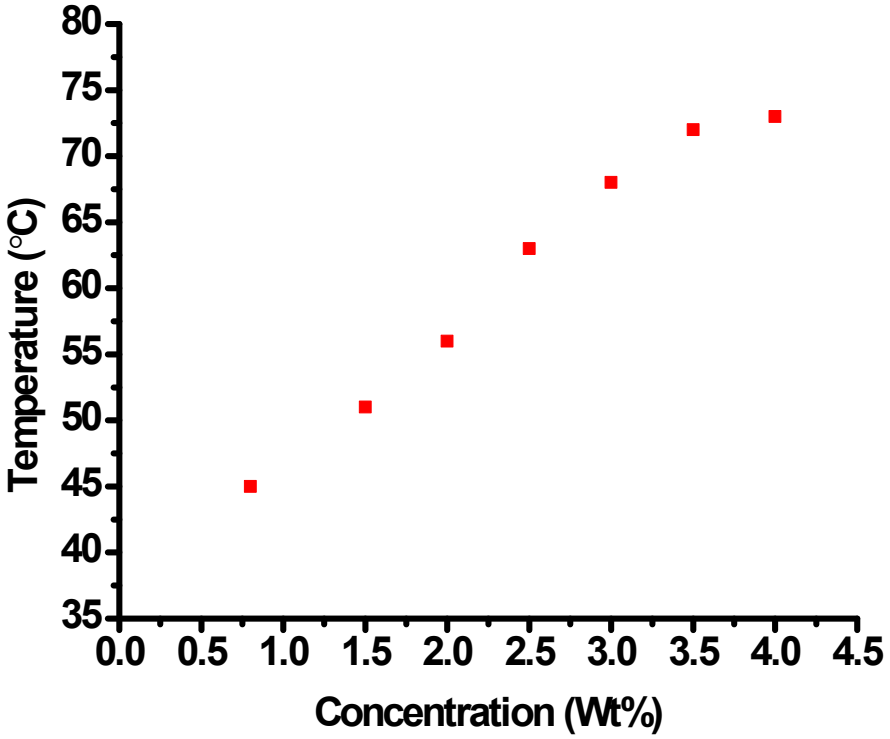


Fig. S1 Plot of gel-sol transition temperature (T_{gel}) of toluene gel of diyne **2** against gelator concentration.

5. Evidence for the involvement of hydrogen bonded assembly in gelation

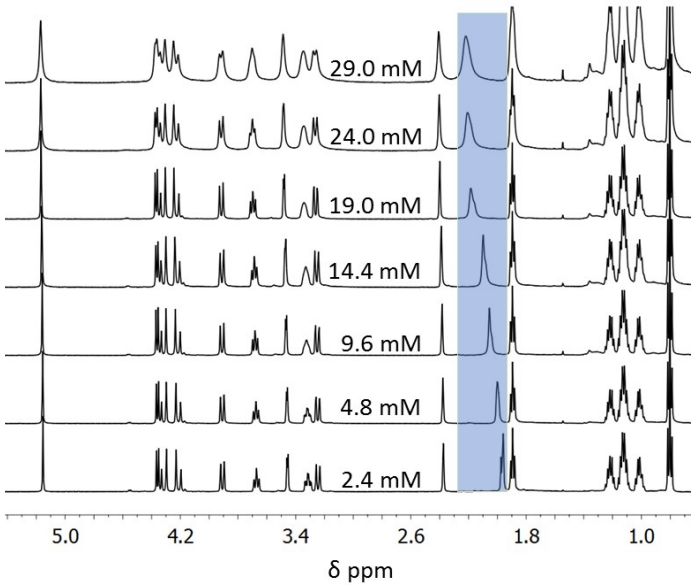


Fig. S2 Concentration dependent ^{1}H NMR of diyne **2** in C_6D_6 (a gelling solvent) at 25 °C. The change in chemical shift of OH signals and line broadening suggest the involvement of intermolecular hydrogen bonding in self-assembly and thus in gelation.

6. SEM analysis

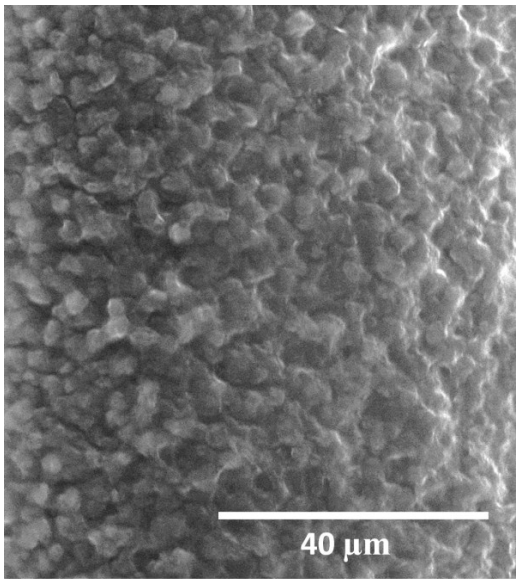


Fig. S3 SEM image of xerogel made from toluene gel of diyne **2**.

7. Comparison of PXRDs of xerogel of diyne 2 and crystals of diol 1

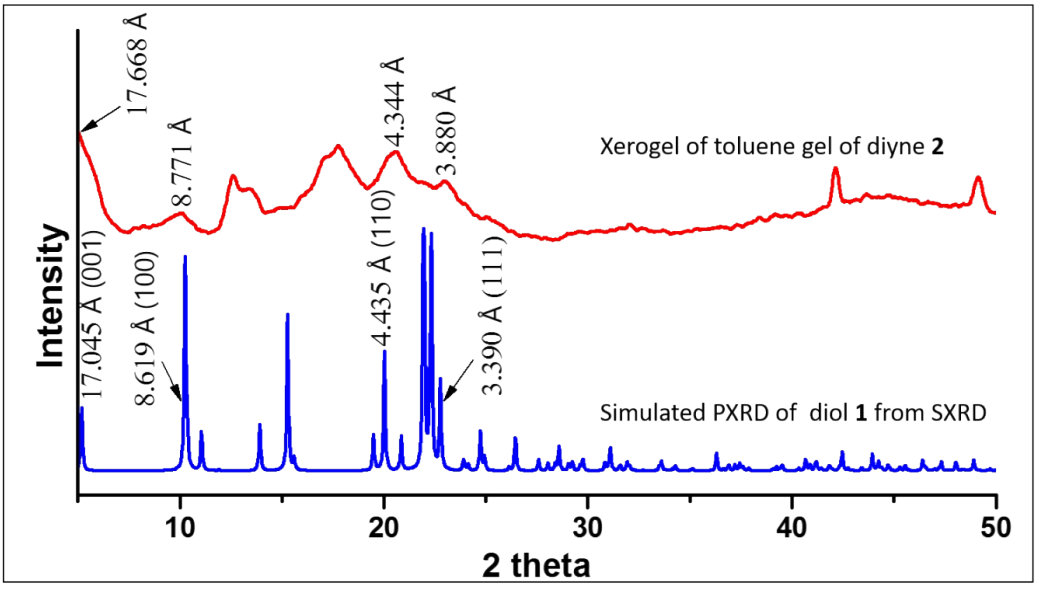


Fig. S4 Overlay of PXRD patterns of xerogels of **2** (made from toluene gel) with simulated PXRD of crystals of diol **1**.

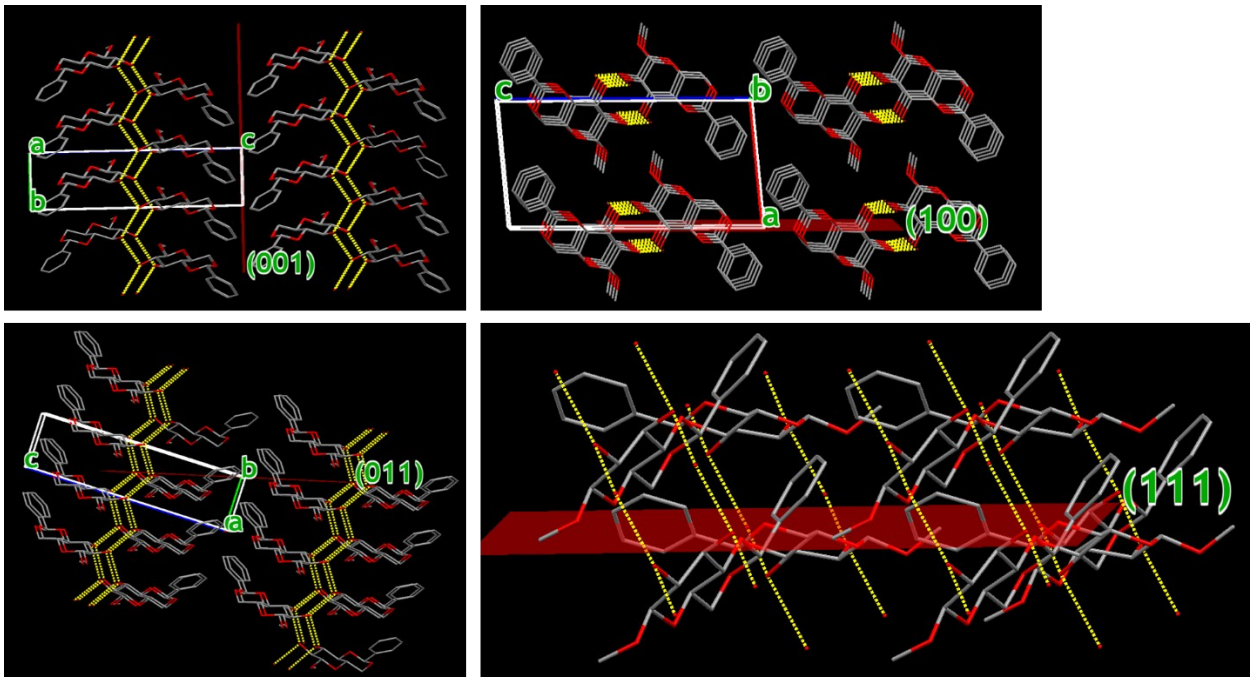


Fig. S5 Molecular packing of diol **1** along within the plane of indentation [(001), (100), (011) and (111) which are similar to the planes in xerogel of diyne **2**. Hydrogen bonds are shown as dotted yellow lines.

8. Topochemical photopolymerization of 2

(A) Procedure: Photopolymerization of **2** was conducted in its toluene gel by irradiating with a 120 W UV lamp (λ 300 nm) at 25 °C. For these experiments toluene gel of **2** was made at its CGC and irradiated for 2 days. The colorless transparent gel became orange red during irradiation (Fig. S6A & S6B). The gel was intact and was more stable than the gel before photoirradiation. After irradiation, T_{gel} of the gel has increased to 50 °C from 45 °C. The insoluble polymer could be separated by shaking photoirradiated gel with DCM (Fig. S6C). Interestingly, color of xerogel made from toluene gel of **2** also changed to orange red upon UV irradiation, suggestive of the formation of PDA even in the xerogel state.

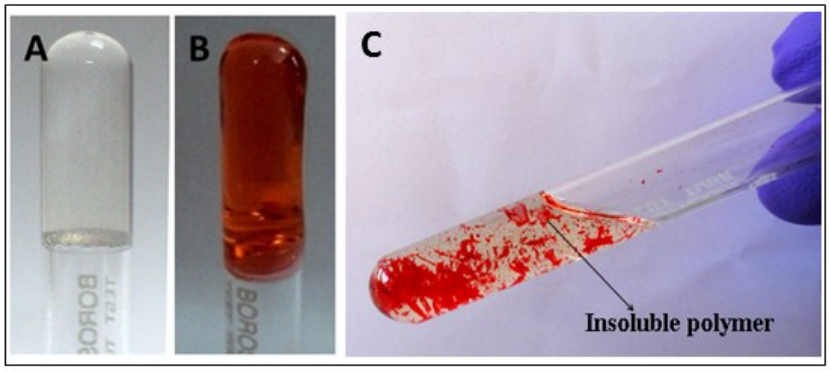


Fig. S6 Photographs of toluene gel of **2** before (A) and after (B) photoirradiation; (C) Photograph of the insoluble PDA obtained after dichloromethane treatment of polymerized gel.

(B) Time-dependent Raman spectra

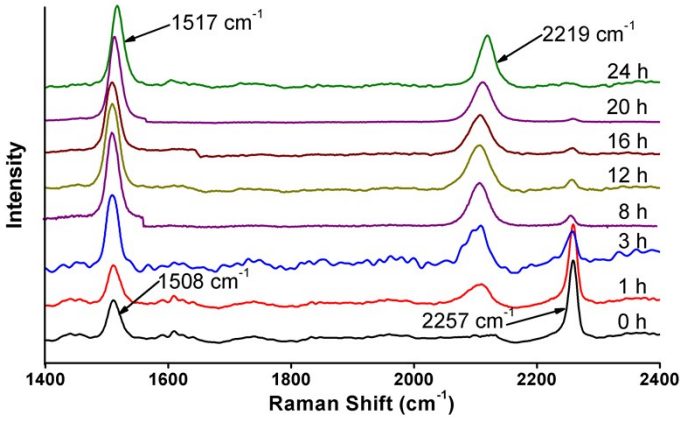


Fig. S7 Time-dependent Raman spectra of xerogels made from toluene gel of **2** irradiated with 300 nm UV light for different durations.

(C) SEM analysis

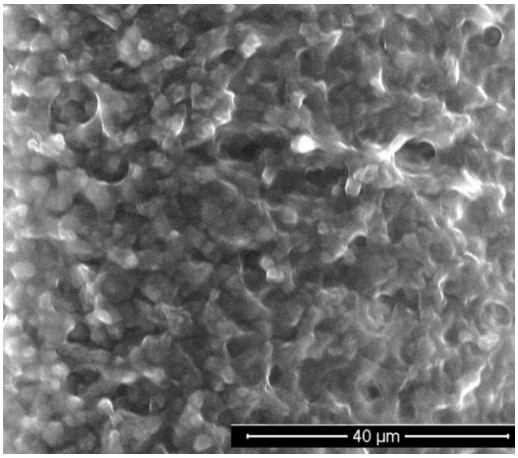


Fig. S8 SEM image of xerogel made from toluene gel of diyne **2** after UV irradiation (PDA-3).

(D) PXRD analysis

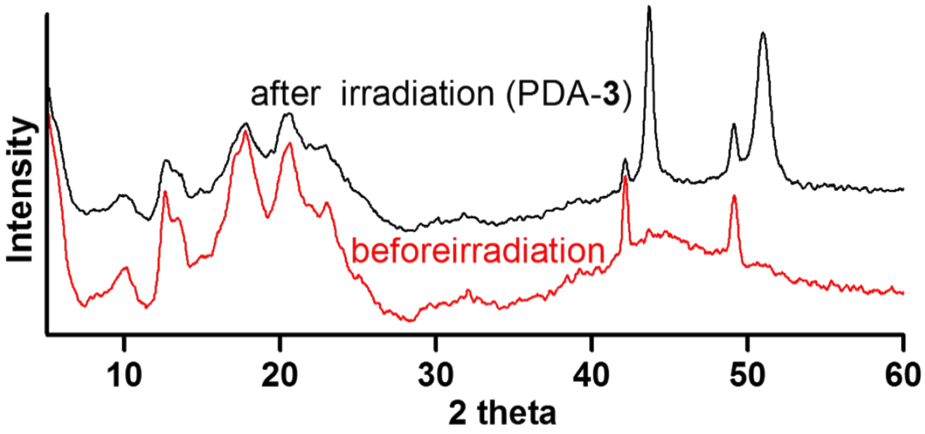


Fig. S9 Overlay of PXRD patterns of xerogels made from toluene gel of **2** before and after UV-irradiation (PDA-**3**).

9. Synthesis, characterization and binding studies of PDA-4 with lectin

(A) Synthesis: 10 µL each of monomer solution in toluene (0.0984 M) were drop-casted on glass slides and were allowed to congeal at rt. After formation of the gel, glass slides were irradiated under 300 nm UV light for 7 days for complete polymerization. These glass PDA-**3**-coated slides were kept in a beaker containing 2N HCl for 3 hours in order to remove benzylidene protecting groups (Fig. S10). PDA-**4**-coated glass slides thus obtained were washed with water to remove HCl and kept for drying under reduced pressure.

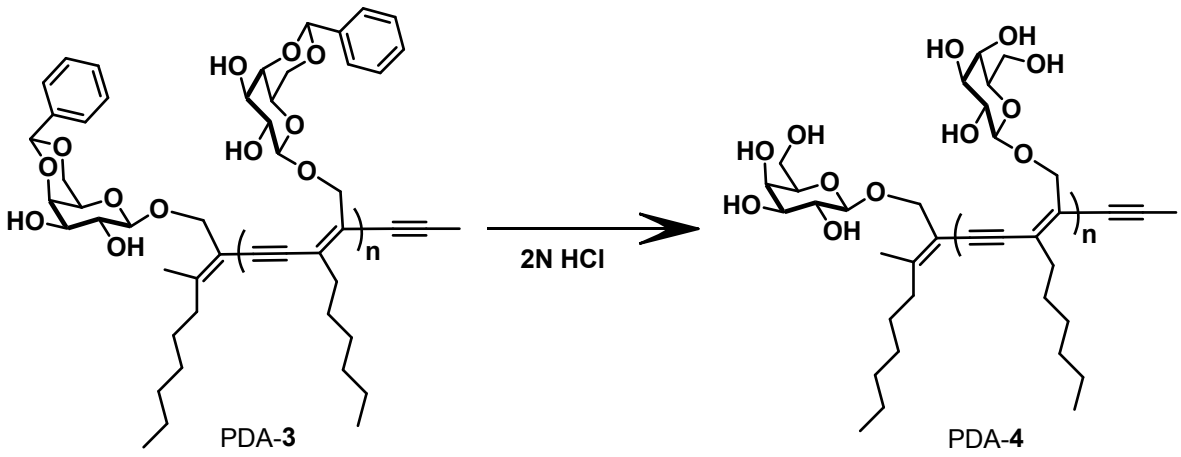


Fig. S10 Preparation of PDA-**4** from PDA-**3**.

(B) Characterization of PDA-4 using FTIR and Raman spectroscopy: PDA-**4** was scratched out of the glass slides and mixed with KBr and pelleted and recorded its IR spectrum. For Raman characterization, this PDA-**4**-glass slide was placed under the microscope and the spectrum was recorded using a He-Ne laser source having an excitation wavelength of 633 nm and with an acquisition time of 10 seconds using a 10x objective.

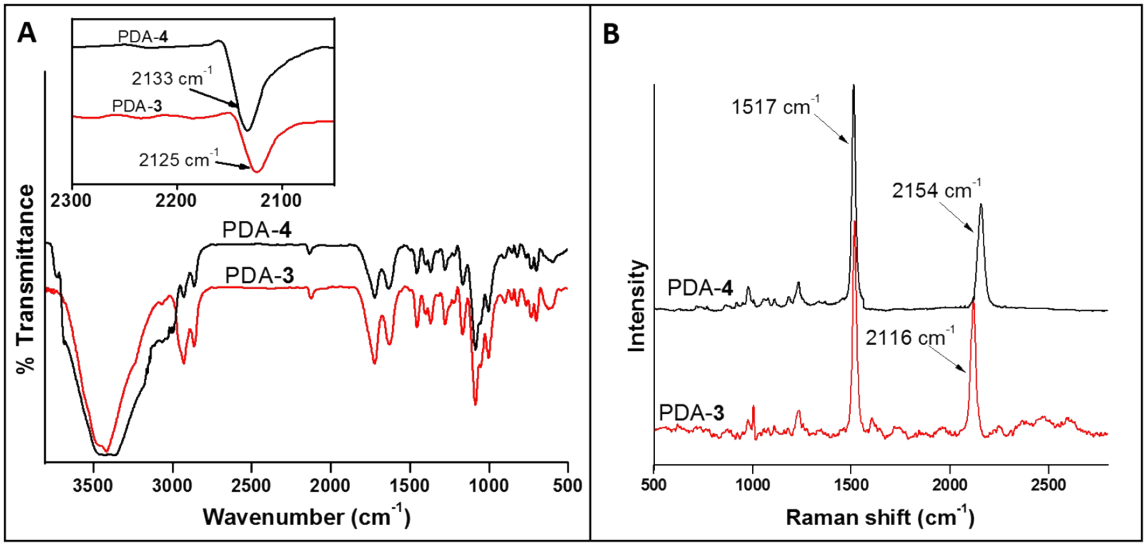


Fig. S11 (A) Overlay of FT-IR spectra of PDA-**3** and PDA-**4**; (B) Overlay of Raman spectra of PDA-**3** and PDA-**4**.

(C) Characterization of PDA-4 using solid-state UV-Vis spectroscopy

PDA-**3** and PDA-**4** were prepared on glass slides and solid-state UV-Vis absorption spectra were recorded using these glass slides on Shimadzu UV-3600 UV-VIS-NIR.

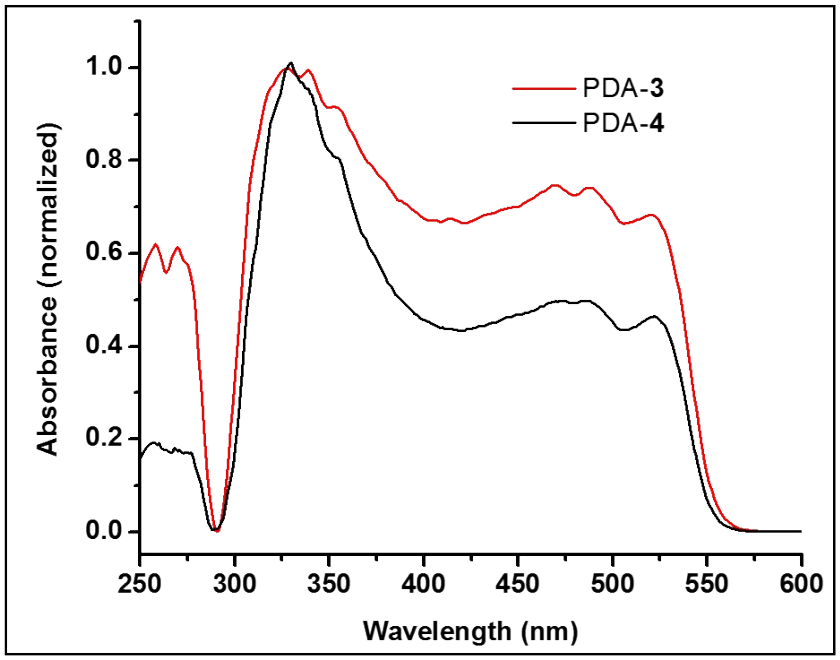


Fig. S12 Overlay of solid-state UV-Vis spectra of PDA-**3** and PDA-**4**

(D) binding studies of PDA-4 with lectins: A 24 (3 x 8) well cell culture plate was taken and a glass coverslip (1.2 cm x 1.2 cm) was placed in each well. 0.0984 M solution of the monomer **2** was prepared in hot toluene (204 mg/5 mL). 10 µL of this monomer solution was drop-casted on each glass slides and was allowed to congeal at rt. After formation of the gel, culture plates were irradiated under 300 nm UV light for 7 days for complete polymerization. After this, 500 µL of 2N HCl was added to each well and kept for 3 hours to cleave the benzylidene protecting groups. Each well was washed thoroughly with water and then with 500 µL of PBS (3 times) to remove HCl.

To six wells of each row, 20, 30, 40, 50, 60 and 70 µL of the protein stock solution (1 mg/mL in PBS) were added under dark condition. Each well was made up to 300 µL using PBS buffer (0.01 M, pH 7.4). The wells were covered with aluminum foil to prevent exposure to light and kept undisturbed for 3 h at room temperature. The foil was removed and the suspended solution in each well was pipetted out to 1 mL Eppendorf tubes. Each well was given 2 times PBS (pH 7.4, 200 µL each) wash and the washings were also transferred to respective Eppendorf tubes. Absorbance of solution obtained from each well was measured at 494 nm using UV spectrophotometer (Fig. S14, S16 & S18 & Table S2-S4).

Each protein binding assay was done in triplicate. The average of absorbance for each concentration was calculated from three parallel experiments. From a standard plot of absorbance *vs* concentration of the respective protein (Fig. S13, S15 & S17), concentration of the unbound fraction was estimated in each well. Scatchard plot, a plot of [Bound]/[Free] vs [Bound], was made and it was found that Scatchard plots were linear for binding of PDA-**4** with all the three proteins tested. The association constant of PDA-protein interaction for each proteins was calculated from the slope of the straight line of respective Scatchard plot. The maximum amount of lectin that can be bound on PDA was measured from X-intercept (Y = 0).

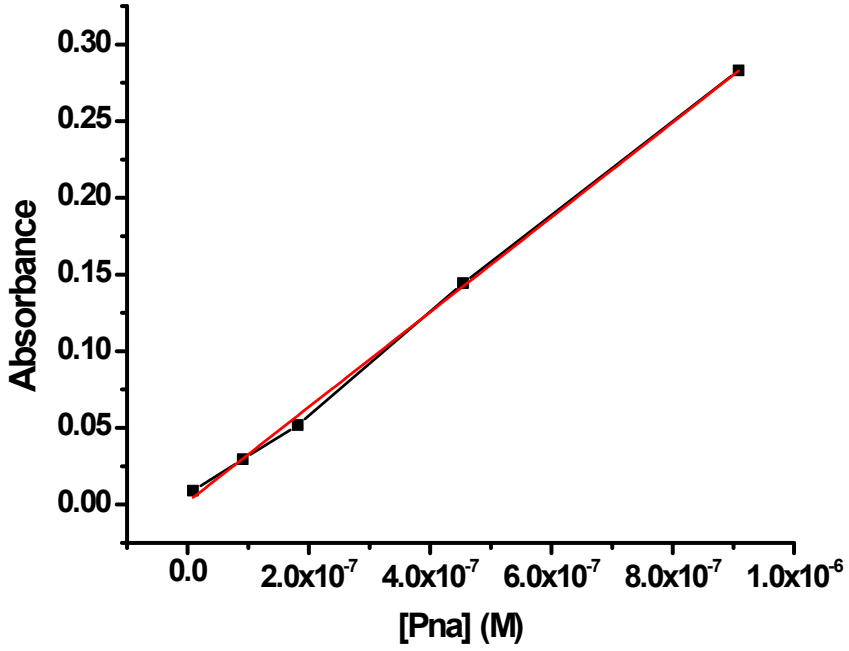


Fig. 13 Standard plot of absorbance *vs* concentration of the protein (Pna).

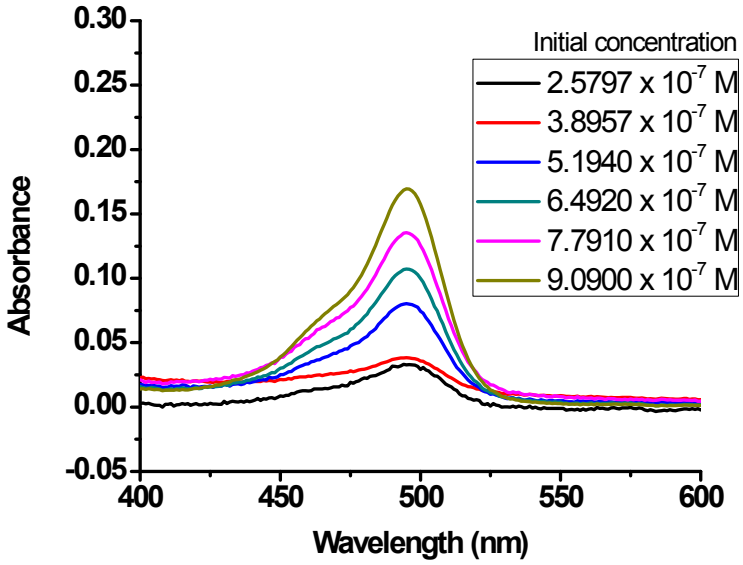


Fig. S14 Absorption spectra of solution after incubation of PDA-**4** with different concentrations of Pna. The absorbance is a measure of unbound protein in the respective well.

Table S2: Details of concentration of bound and free proteins in the binding study of PDA-**4** with peanut agglutinin.

Concentration of lectin	Volume used for binding studies [Protein (µL) + PBS buffer (µL)]	Initial concentration of protein in the well	Absorbance of free protein	[Free protein] (M)	[Bound protein] (M)	[Bound protein]/[Free protein]
9.09 µM Peanut agglutinin	20 + 680	2.5797×10^{-7}	0.03316	1.0821×10^{-7}	1.4976×10^{-7}	1.38398
	30 + 670	3.8957×10^{-7}	0.03836	1.8120×10^{-7}	2.0837×10^{-7}	1.14994
	40 + 660	5.1940×10^{-7}	0.08033	2.5337×10^{-7}	2.6603×10^{-7}	1.04997
	50 + 650	6.4920×10^{-7}	0.10759	3.4168×10^{-7}	3.0752×10^{-7}	0.90002
	60 + 640	7.7910×10^{-7}	0.13581	4.2910×10^{-7}	3.5000×10^{-7}	0.81566

	70 + 630	9.0900 x 10^{-7}	0.17010	5.3900 x 10^{-7}	3.7000 x 10^{-7}	0.68646

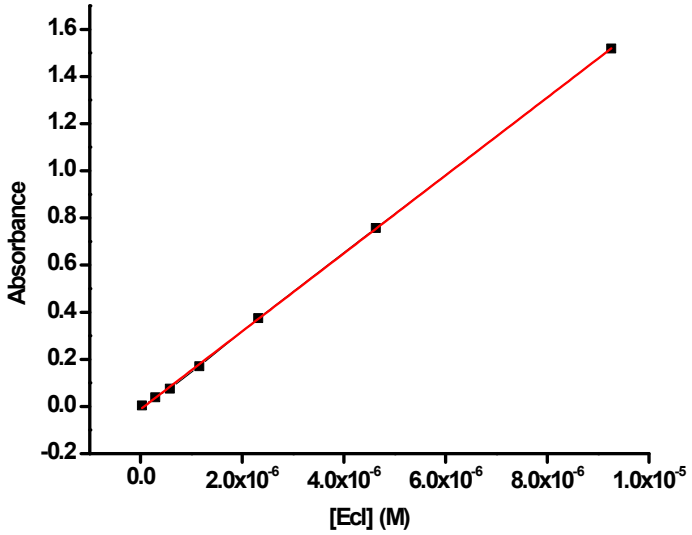


Fig. S15 Standard plot of absorbance *vs* concentration of the protein (Ecl).

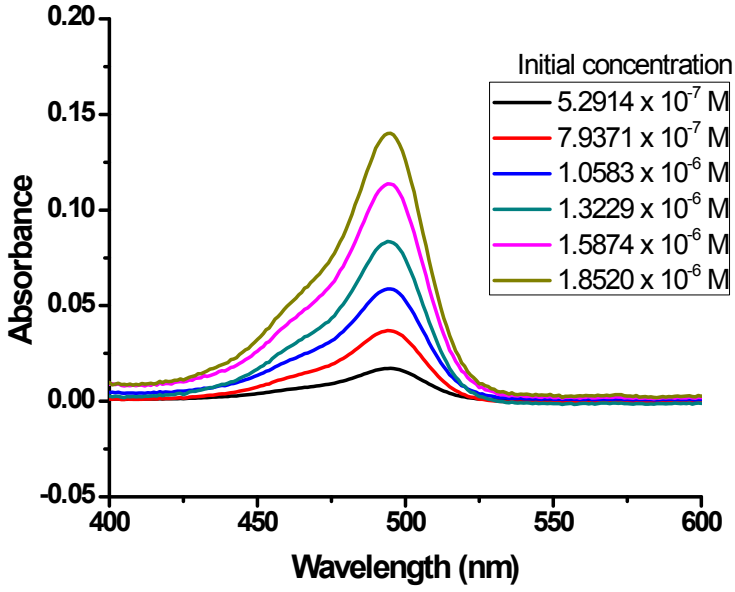


Fig. S16 Absorption spectra of solution after incubation of PDA-**4** with different concentrations of Ecl. The absorbance is a measure of unbound protein in the respective well.

Table S3: Details of concentration of bound and free proteins in the binding study of PDA-**4** with *Erythrina crystagalli* lectin

Concentration of lectin	Volume used for binding studies [Protein (µL) + PBS buffer (µL)]	Initial concentration of protein in the well	Absorbance of free protein	[Free protein] (M)	[Bound protein] (M)	[Bound protein]/[Free protein]
18.52 µM µM *Erythrina crystagalli* lectin	20 + 680	5.2914×10^{-7}	0.01721	1.7069×10^{-7}	3.5845×10^{-7}	2.10001
	30 + 670	7.9371×10^{-7}	0.03690	2.9397×10^{-7}	4.9974×10^{-7}	1.69997
	40 + 660	1.0583×10^{-6}	0.05881	4.2332×10^{-7}	6.3498×10^{-7}	1.50000
	50 + 650	1.3229×10^{-6}	0.08392	5.7516×10^{-7}	7.4774×10^{-7}	1.30006
	60 + 640	1.5874×10^{-6}	0.11381	7.5592×10^{-7}	8.3148×10^{-7}	1.09996
	70 + 630	1.8520×10^{-6}	0.14113	9.2151×10^{-7}	9.3049×10^{-7}	1.00974

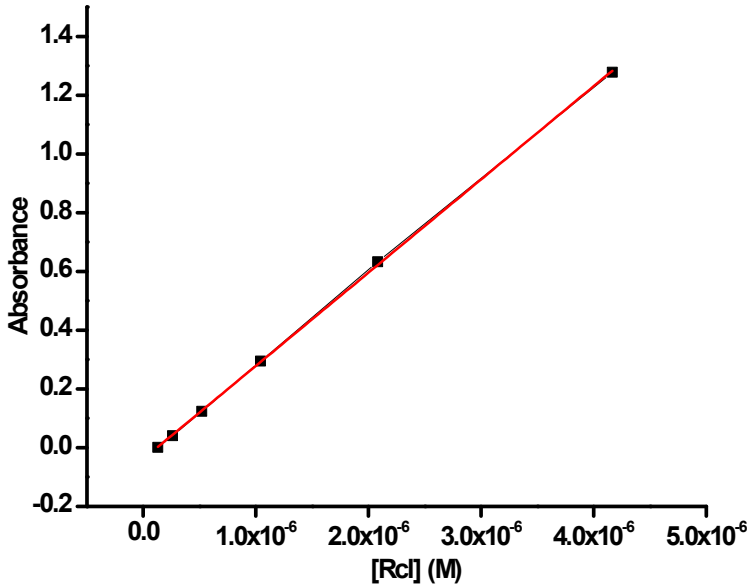


Fig. S17 Standard plot of absorbance *vs* concentration of the protein (Rcl).

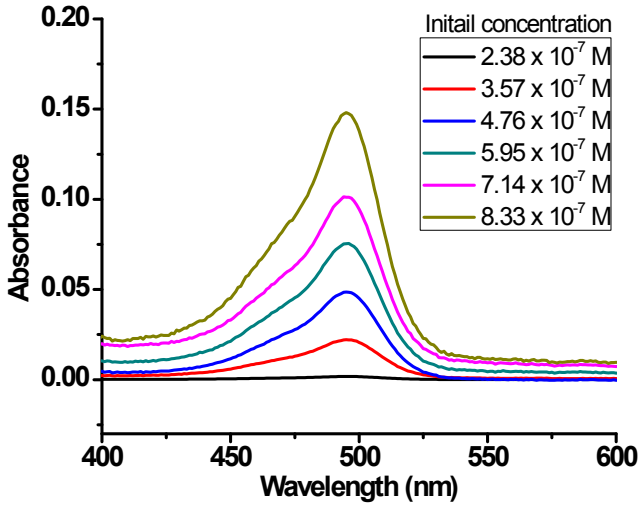


Fig. S18. Absorption spectra of solution after incubation of PDA-**4** with different concentrations of Rcl. The absorbance is a measure of unbound protein in the respective well.

Table S4: Details of concentration of bound and free proteins in the binding study of PDA-**4** with *Ricinus communis* lectin.

Concentration of lectin	Volume used for binding studies [Protein (µL) + PBS buffer (µL)]	Initial concentration of protein in the well	Absorbance of free protein	[Free protein] (M)	[Bound protein] (M)	[Bound protein]/[Free protein]
8.33 µM µM *Ricinus communis* lectin	20 + 680	2.38×10^{-7}	0.0018	1.2526×10^{-7}	1.1274×10^{-7}	0.90005
	30 + 670	3.57×10^{-7}	0.0222	1.9833×10^{-7}	1.5867×10^{-7}	0.80003
	40 + 660	4.76×10^{-7}	0.0488	2.8000×10^{-7}	1.9600×10^{-7}	0.70000
	50 + 650	5.95×10^{-7}	0.0756	3.7188×10^{-7}	2.2312×10^{-7}	0.59998
	60 + 640	7.14×10^{-7}	0.1022	4.6065×10^{-7}	2.5335×10^{-7}	0.54998

	70 + 630	8.33×10^{-7}	0.1490	5.6667×10^{-7}	2.6633×10^{-7}	0.47010

10. Preparation and characterization of PDA-5 and its protein binding study to rule out non-specific binding

To confirm the role of carbohydrate units of PDA-**4** in binding with lectins, we have removed galactose from PDA-**4** by treating PDA-**4**-coated glass plates with conc. HCl for 2h (Fig. S19). The PDA-**5** thus obtained was washed many times with water to remove galactose and HCl. PDA-**5** was characterized with FTIR spectroscopy and Raman spectroscopy.

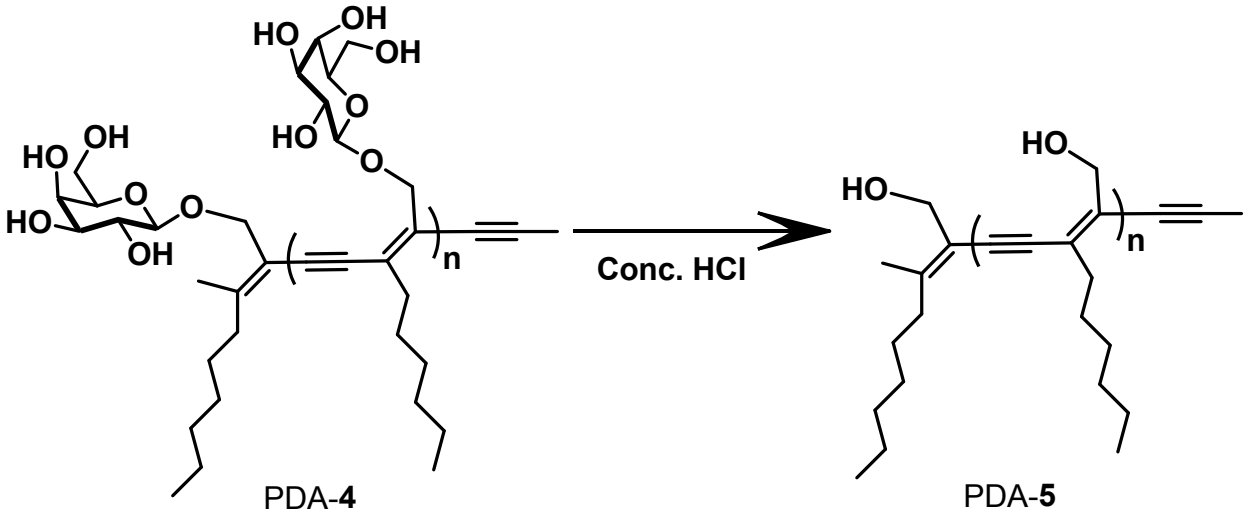


Fig. S19 Preparation of PDA-**5** from PDA-**4**.

(A) FT-IR spectroscopy study: For FTIR experiment, PDA-**5** was scratched out of the glass slide, mixed with KBr and made a pellet. FTIR spectrum of PDA-**5** showed drop in intensities of the peaks due to OH stretching and also the shifting of peak due to C≡C bond stretching from 2133 to 2091 cm^{-1}, suggesting the removal of pendant galactose units from PDA-4 (Fig. S20).

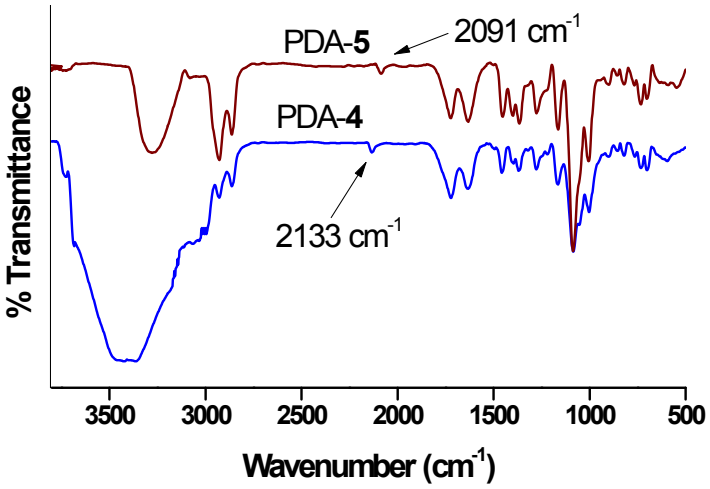


Fig. S20 Overlay of FT-IR spectra of PDA-**4** and PDA-**5**.

(B) Raman spectroscopy study: For Raman studies, PDA-**5**-coated glass plate was placed under the microscope and the spectrum was recorded using a He-Ne laser source having an excitation wavelength of 633 nm and with an acquisition time of 10 seconds using a 10x objective. A comparison of Raman spectra of PDA-**4**-sugar and after conc. HCl washing (PDA-**5**) showed a shift of C≡C bond stretching band from 2154 cm^{-1} to 2109 cm^{-1} (Fig. S21), suggesting the removal of galactose units from PDA-**4**.

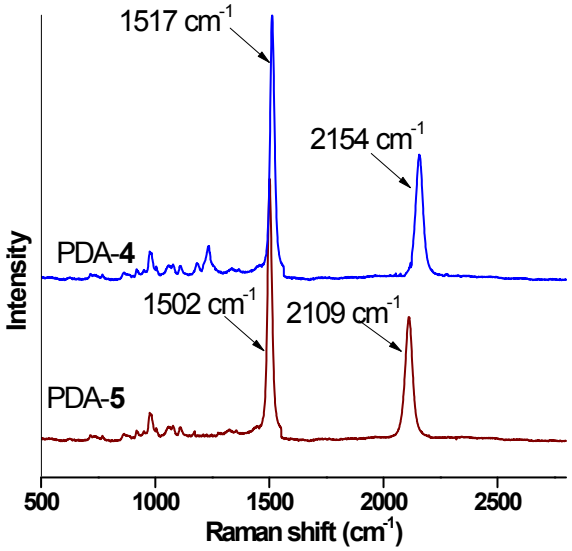


Fig. S21 Overlay of Raman spectra of PDA-**4** and PDA-**5**.

(C) Solid-state UV-Vis spectroscopy study: PDA-**5** was prepared on glass slide and solid-state UV-Vis absorption spectrum was recorded using this glass slide on Shimadzu UV-3600 UV-VIS-NIR.

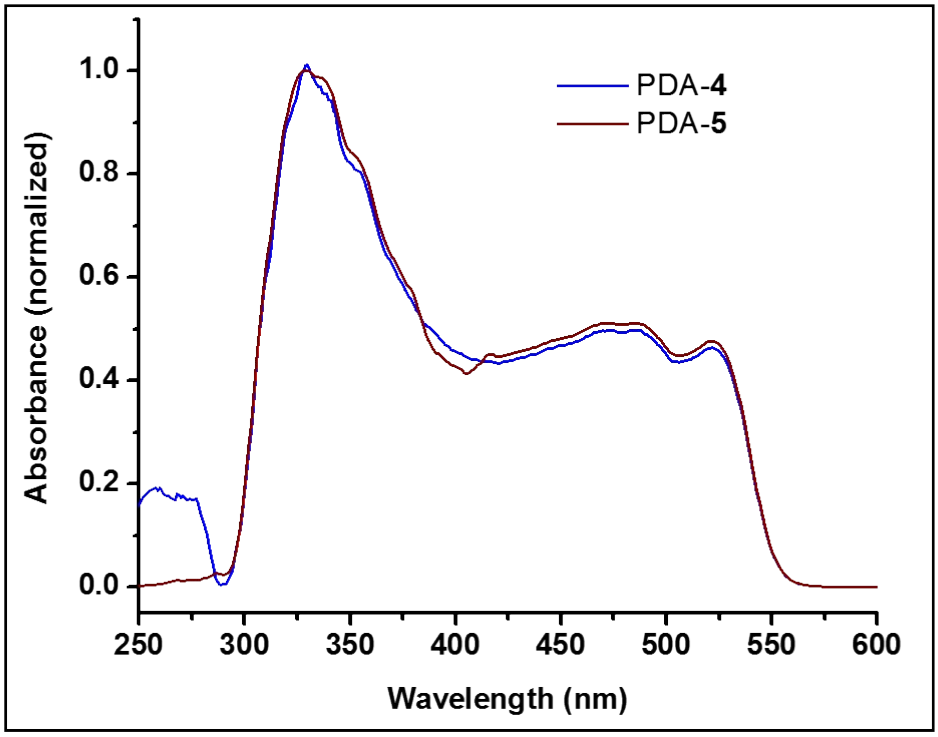


Fig. S22 Overlay of solid-state absorption spectra of PDA-**4** and PDA-**5.**

(D) PDA-5-protein binding study to rule out non-specific binding: After removal of galactose from PDA-**4**, PDA-**5**-coated glass plates were kept in three wells of the culture plate. To each of these wells, 70 µL of the proteins were added under dark condition. Each well was made up to 300 µL using PBS buffer (0.01 M, pH 7.4). The wells were covered with aluminum foil to prevent exposure to light and kept undisturbed for 3 h at room temperature. The foil was removed and the suspended solution in each well was pipetted out to 1 mL Eppendorf tubes. Each well was given 2 times PBS (pH 7.4, 200 µL each) wash and the washings were also transferred to respective Eppendorf tubes. The concentration of protein solutions before and after incubation was same, suggesting that there were no binding with PDA-**5**. These experiments suggest that carbohydrate units are necessary for binding of these proteins.

(E) Confocal Fluorescence microscopy: Since these proteins are FITC conjugated, binding to the polymer can easily be visualized using confocal fluorescence microscopy. After incubation of PDA-**5**-coated glass slides with proteins, we have taken confocal fluorescence microscopic images of these PDA-coated plates by exciting at 493 nm and collecting the emission in the range of 510-525 nm. We found that PDA-**5**-coated glass slide did not show any emission (Fig. S23) suggesting that proteins are not immobilized on the polymer surfaces due to the lack of carbohydrate-protein interactions.

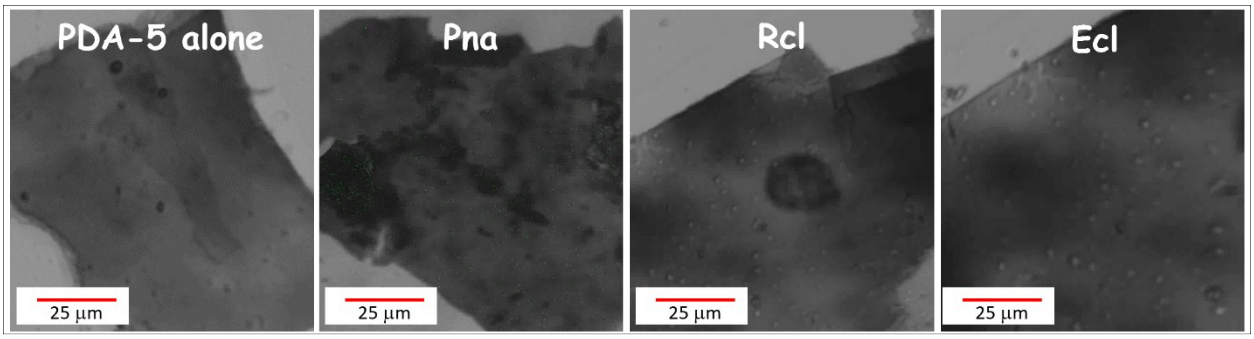


Fig. S23 Confocal fluorescence microscopy images of PDA-**5** before (A) and after incubating with (B) Pna, (C) Rcl and (D) Ecl.

.

11. NMR spectra

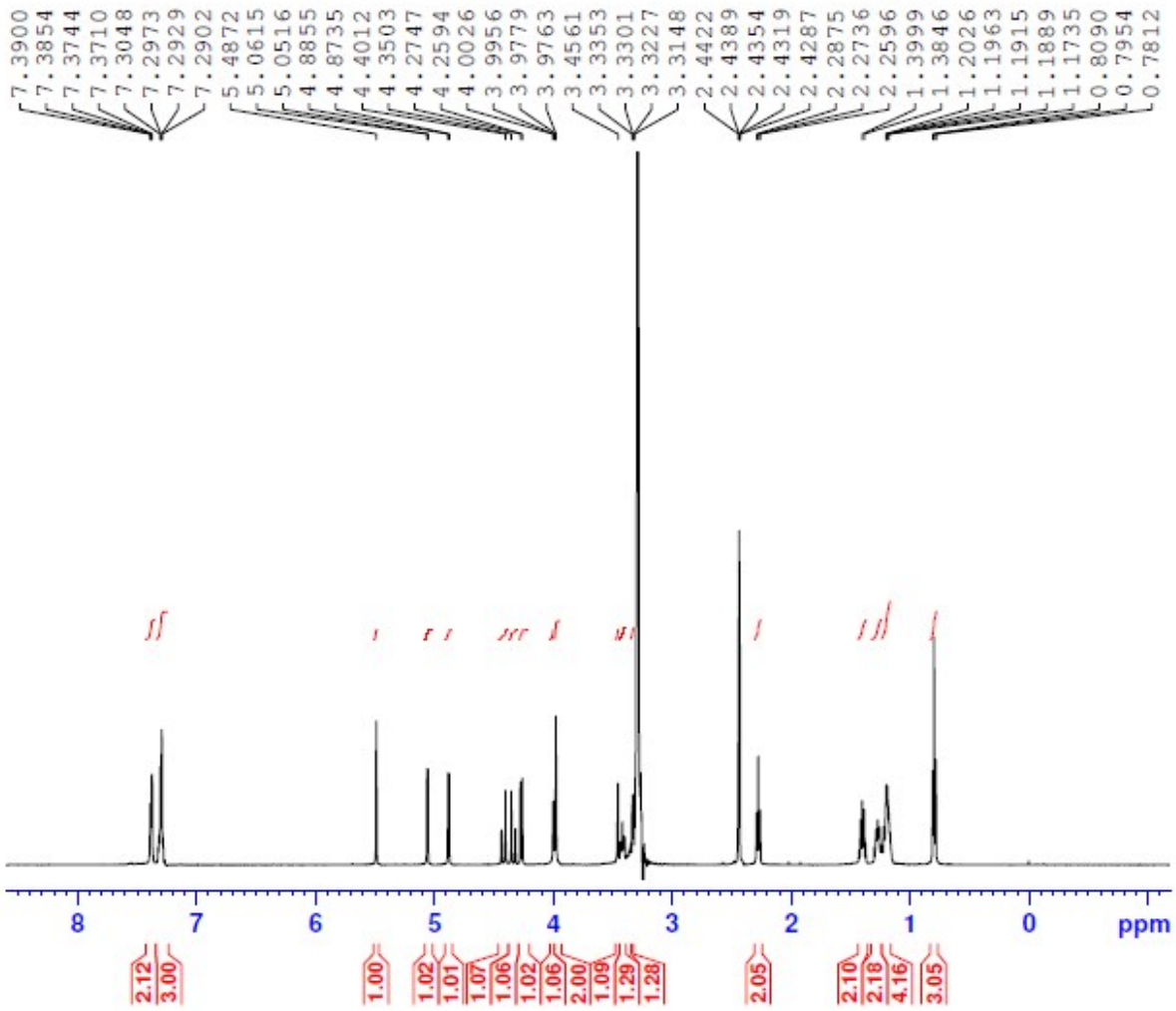


Fig. S24^{1}H NMR spectrum of compound **2**.

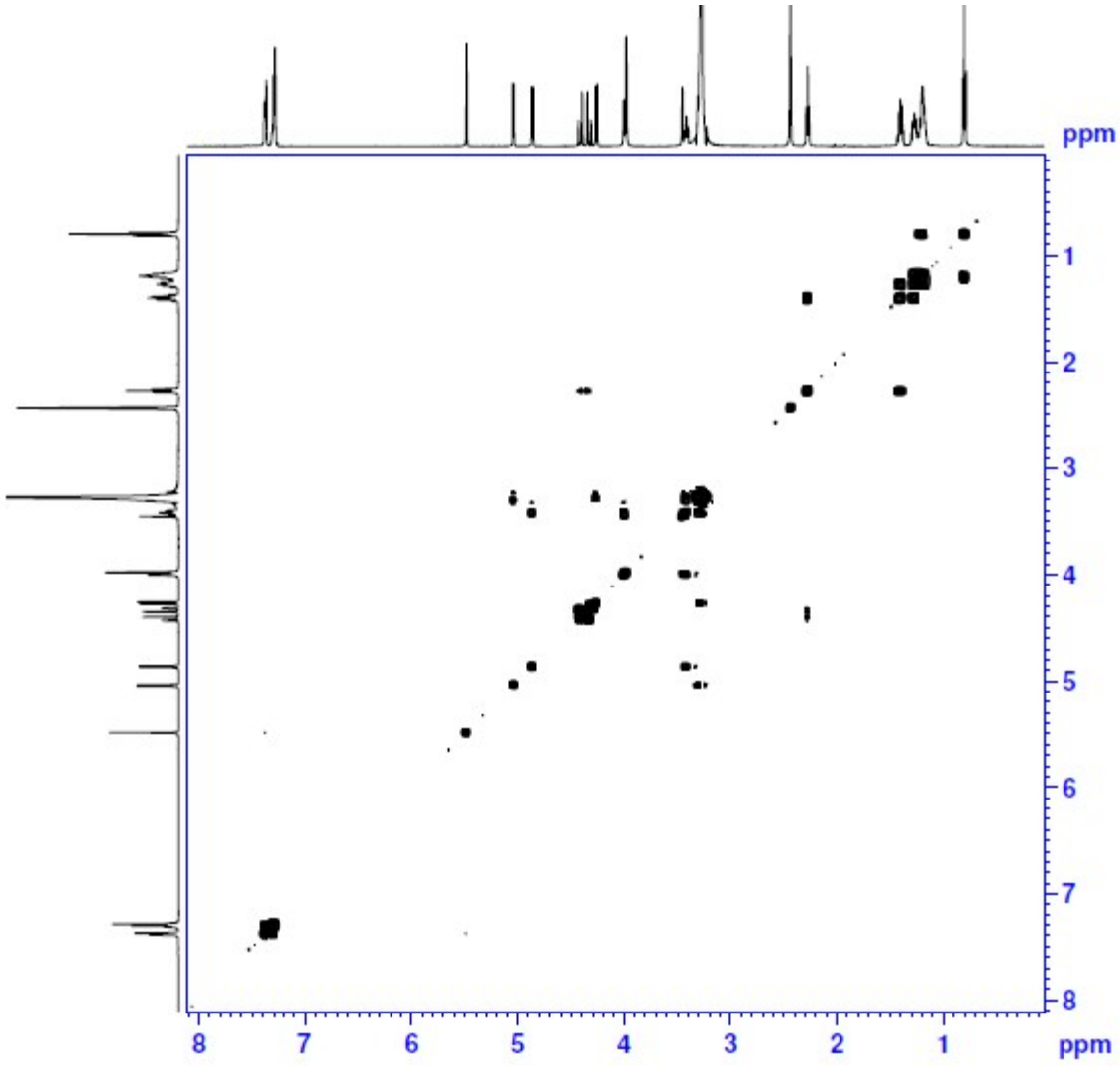


Fig. S25 COSY spectrum of compound **2**.

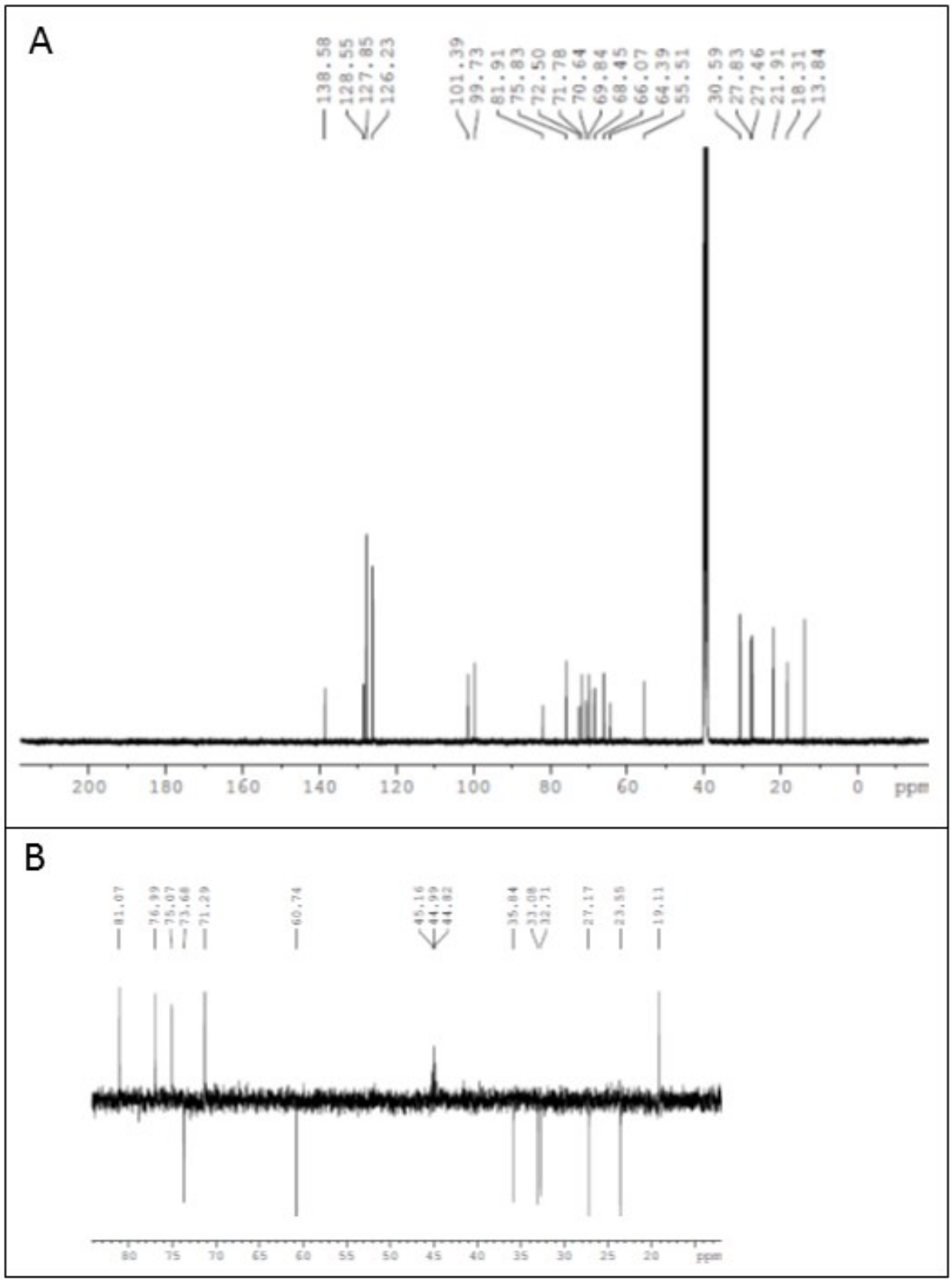


Fig. S26 ^{13}C NMR (A) and DEPT spectrum (B) of compound **2**.

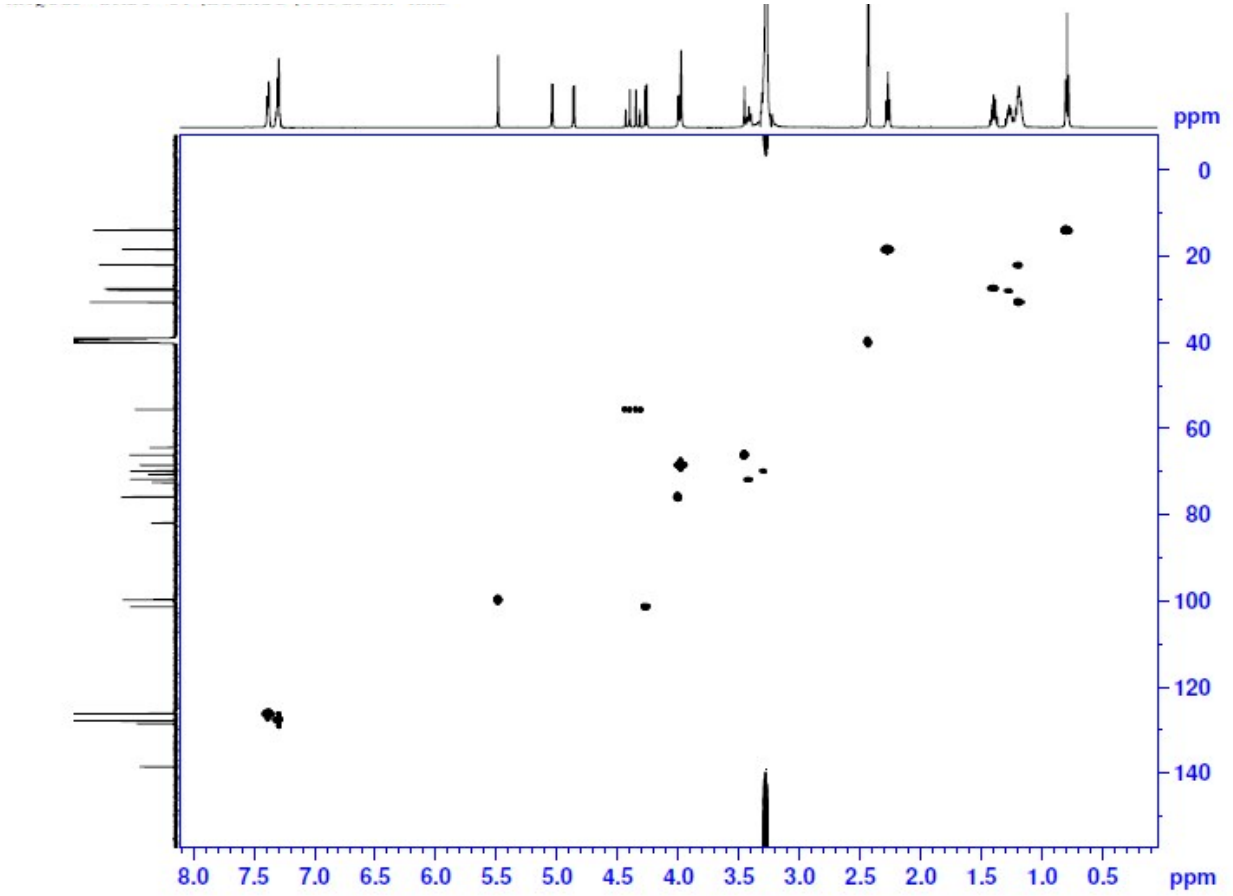


Fig. S27 HMQC spectrum of compound **2**.

12. References:

(1) B. P. Krishnan, S. Ramakrishnan and K. M. Sureshan, *Chem. Commun*., 2013, **49**, 1494.

(2) (*a*) K. M. Sureshan, K. Yamaguchi, Y. Sei and Y. Watanabe, *Eur. J. Org. Chem*., 2004, 4703. (*b*) B. P. Krishnan, S. Mukherjee, P. M. Aneesh, M. A. G. Namboothiry and K. M. Sureshan, *Angew. Chem. Int. Ed.,* 2016, **55**, 2345.