Supplementary Information

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1) Experimental Section

Materials: 2, 7-dibromofluorene, 6-bromo-1-hexanol, 4-dimethyl amino pyridine dicyclohexyl carbo diimide (DCC), Tetrabutyl ammonium bromide, o-phthaldialdehyde, Phosphate buffered saline, trifluoro acetic acid, $Pd(PPh_3)_4$, and 1, 4-benzene diboronic bis(pinacolatoester) were purchased from sigma Aldrich. boc-L-glutamic acid-1-tert butyl ester was purchased from Alfa Aesar chemical Ltd & co. NaOH, Na_2CO_3, K_2CO_3 and 2-mercaptoethanol were purchased from Merck chemicals. Toluene, THF, methanol, DCM, ethylacetate and pet ether were purchased locally and dried by the standard drying procedures. HPLC grade acetonitrile, hexane, 2-propanol and methanol were purchased from Merck chemicals.

Methods: NMR spectrum was analyzed using Bruker-AVENS 400 MHz spectrometer. Chemical shifts are reported in ppm at 25 ^oC using $CDCl_3$ and DMSO-d6 solvents containing trace quantity of tetramethylsilane (TMS) as internal standard. The MALDI-TOF analysis was done on Voyager-De-STR MALDI-TOF (Applied Biosystems, Framingham, MA, USA) equipped with 337-nm pulsed nitrogen laser used for desorption and ionization. 1 μM solution of sample was premixed with DHB (2,5 dihydroxy benzoic acid) matrix in THF and mixed well before spotting on 96-well stainless steel MALDI plate by dried droplet method for MALDI analysis. The molecular weights of the polymer was determined by Gel Permeation Chromatography (GPC), equipped with a Viscotek VE 1122 pump, Viscotek VE 3580 RI detector and Viscotek VE 3210 UV/vis detector in tetrahydrofuran (THF) using polystyrene as standards. Scanning Electron Microscopy (SEM) images were recorded using a FEI, QUANTA 200 3D scanning electron microscope with tungsten filament as electron source. Polymer powders were directly mounted on the carbon tape. Before recording the morphology, films were coated with a 5 nm thick gold film by spluttering method. The thermal stability and uptake of enantiomers by the polymer was analyzed using a PerkinElmer:STA 6000 thermogravimetric analyzer (TGA) under nitrogen atmosphere from 50 to 800 °C at 10 °C/min. Differential scanning calorimetric (DSC) analysis was performed using a TA Q10 model. 2−3 mg of the sample was taken in aluminum pan, sealed and scanned at 10 °C/min. The instrument was calibrated with indium standards before measurements.

Circular Dichroism (CD) studies.

Solution state CD measurements were recorded using JASCO-815 CD spectrometer equipped with a Jasco PTC-424S/15 peltier system. 2 mm path-length quartz cuvettes were used for a sample volume of 1 mL in distilled water at 25 ^{0}C. Three scans were averaged for each sample. The polymer powder was ground with KBr and made into a thin transparent pellet and used for the solid state CD measurement.

HPLC Measurements.

Chiral HPLC measurements were performed in an Agilent technology (1200 infinity series USA) instrument using CHIRALCEL OJ-H columns (150 x 4.6 mm, particle size 5 μm) maintained at 35 ^{0}C using UV detector (λ at 257 nm). The mobile phase used was 2-propanol:n-hexane = 10:90 with 0.1 % trifluoroacetic acid. The flow rate of the mobile phase was 0.8 ml/min and the injected volume was 10 μl. Analytical quantification was performed using 2 different columns. For amino acids the following method was adopted; HPLC – Agilent technologies (1200 infinity series USA) equipped with Eclipse Plus-C18, (4.6 x 100mm) column maintained at 35 ^{o}C, detector – UV detector (λ at 334 & 350 nm). Mobile phase- A (PBS buffer), B (acetonitrile/methanol/water-45/40/15). Composition A and B were varied for each amino acids. For glutamic acid the composition was A (90%) and B (10%), for tyrosine A (60%) and B (40%) and for phenylalanine A (40%) and B (60%). For leucine and proline the mobile phase was changed to A (25%) and B (75%). The flow rate of the mobile phase was 0.5 ml/min. The quantification of mannitol sugar was performed using Agilent technologies HPLC (1200 infinity series USA) equipped with HC – 75 Pb^{2+} (Hamiltaon, 7.8 mm x 300 mm) column maintained at 80 ^{o}C, detector – refractive index detector. During analysis the temperature was maintained at 40 ^{o}C. Mobile phase: H_2O; Flow rate: 0.5 ml /min and the injected volume was 10 μl.

Heterogeneous enantioselective separation (HES)

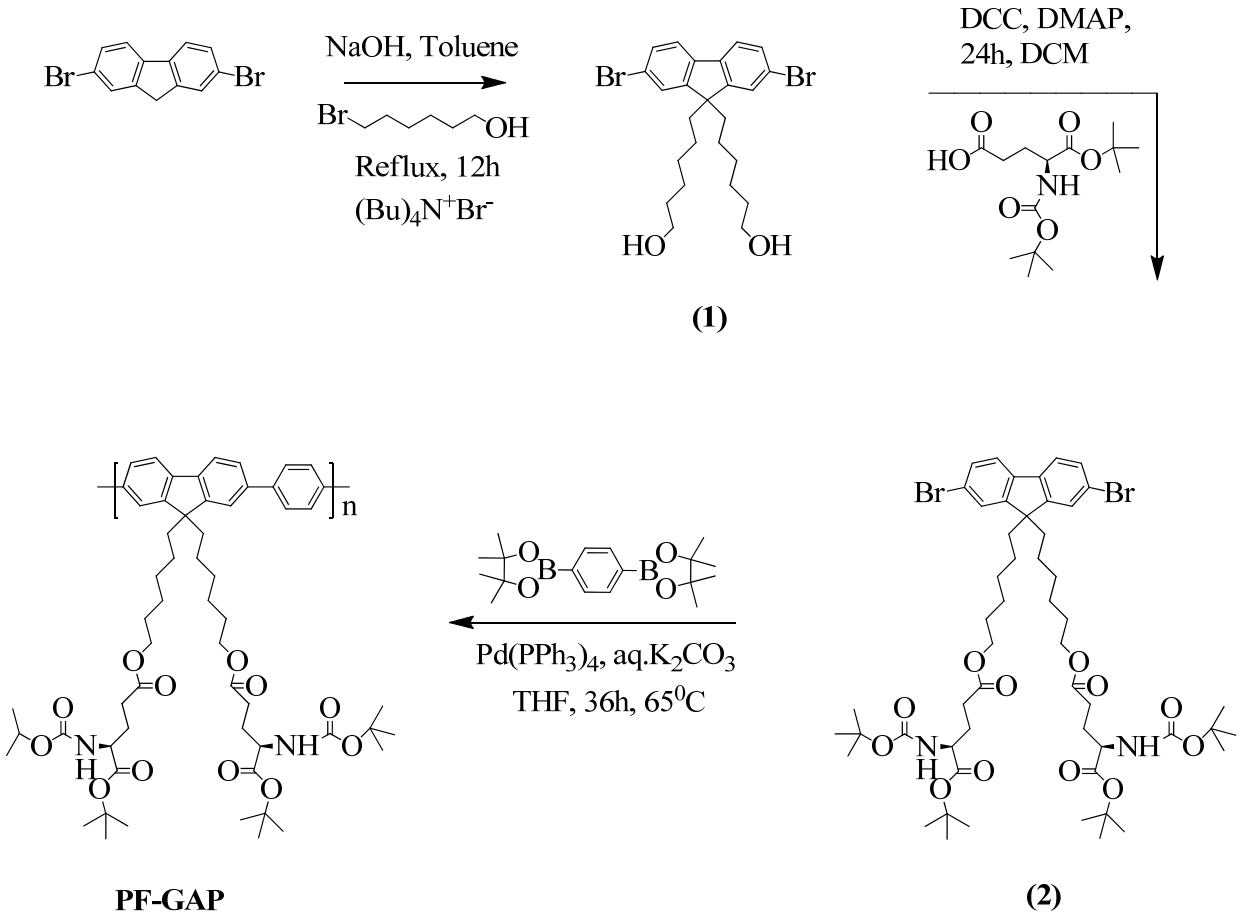
HES experiments were carried out in water. Racemic mixture (10 mg of (D):10 mg of (L)) of enantiomers was dissolved in 10 ml of distilled water. The Fine powdered (5 mg) polymer particles were suspended in water and stirred for 48 hours. At the end of 48 hours the mixture was filtered using whatmann filter paper to separate out the polymer. The polymer powder was used to measure the solid state CD measurement. The decanted aqueous solution was used for

quantifying the enantiomer uptake of the polymer. The enhancement of solid state CD of polymers was calculated from the area under the curve of the CD spectra of the polymer before and after HES process. The ratio between the areas gave the % enhancement of chiral amplification.

The percentage enantiomer uptake of polymer was determined using solution state CD spectra of filtered solutions and pure enantiomers (10 mg/10 ml) in water. The area under the curve was calculated for each reference enantiomer and filtered solutions. The ratio between the areas gave the % uptake of enantiomer by the polymer.

Sample preparation for HPLC: The amino acids were quantified in HPLC using a derivatization procedure. o-Phthalaldehyde (OPA) reacts with primary amines in the presence of 2-mercaptothiol to form highly fluorescent isoindole products (D. Fekkes, *J. Chromatogr. B: Bomed. Sci. Appl.* 1996, **682**, 3-22). In a typical experiment, o-phthaldialdehyde (OPA) (1.34 g), 2-mercaptoethanol (6 ml) was dissolved in borate buffer. The pH of the borate buffer was maintained at 6.9. This derivatizing reagent was kept overnight at 4 ^{0}C and filtered through 0.45 μm PTFE filter. The amino acid was dissolved in water. The derivatizing reagent (OPA+thiol) was added to free amino acids to form isoindole products. This fluorescent isoindole product is characteristic of each amino acid and has different characteristic retention times. The concentration of the isoindole derivative directly indicates the concentration of amino acids in solution. To calculate enantiomeric excess (ee) of amino acids adsorbed on polymer, the amino acids were separated from the polymer and quantified using HPLC. Known concentrations (10, 7, 5, 3 mg/ml) of the derivatized amino acids were injected in HPLC to quantify the unknown amount of adsorbed enantiomer in the PF-GAP polymer. The area under the peak in HPLC was measured using the software for all the enantiomers from which the amount of unknown enantiomer was calculated.

2) Scheme of polymer synthesis (PF-GAP).



(1)

PF-GAP

(2)

3) Synthetic procedures for monomers and polymer

1. Synthesis of 2, 7-dibromo-9, 9-di-n-hexanolfluorene (1)

2, 7-dibromofluorene (6 g, 18.52 mmol), 6-bromohexan-1-ol (8.3 g, 46.3 mmol) and tetrabutyl ammonium chloride (3 g, 9.26 mmol) were taken in two neck round bottom flask and dissolved in toluene (120ml). Then 60g of 50 wt% of aqueous NaOH solution was added to the reaction mixture and heated to 120^0 C under argon atmosphere for 18h. After cooling to room temperature, water was added and the aqueous layer was extracted with diethyl ether. The toluene layer was extracted with water until the color of the solution turned yellow. The aqueous layer was again extracted with diethyl ether. The ether layer was dried over sodium sulphate and evaporated under reduced pressure. The crude product was purified by column chromatography with hexane:ethyl acetate (97:3). Yield-92%. ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 7.6-7.3 (m, 6H), δ 3.50 (t, 4H), δ 1.93-1.87 (m, 4H), δ 1.66-1.56 (m, 4H), δ 1.35 (m, 4H), δ1.08 (m, 4H), 0.56 (m, 4H).

2. Esterification of 2, 7-dibromo-9, 9-di-n-hexanolfluorene (2)

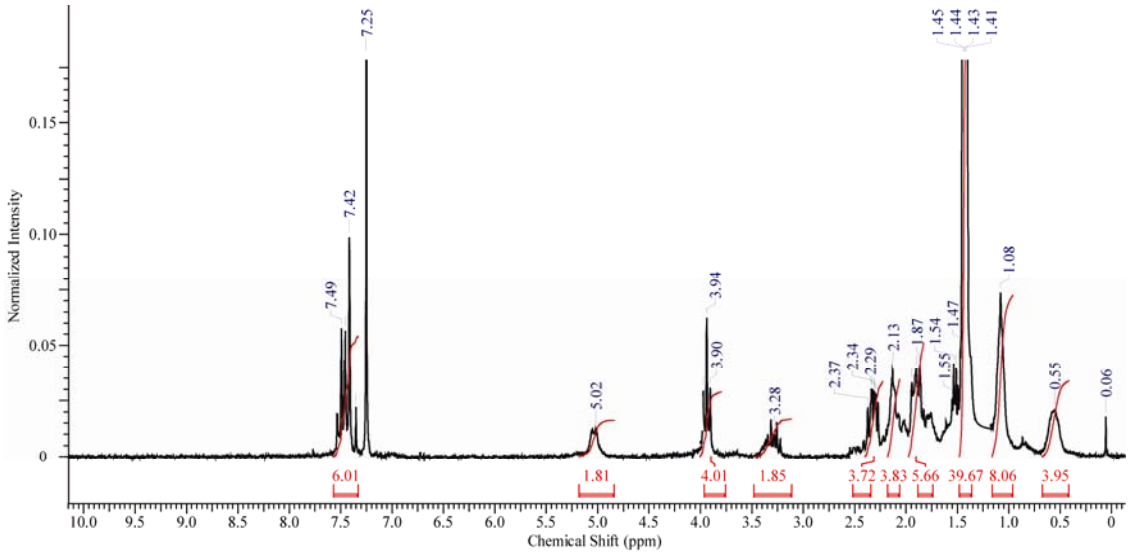
4-dimethyl amino pyridine (2.56 g, 21 mmol) and boc-L-glutamic acid-1-tert butyl ester (7.24 g, 23.85 mmol) were taken in two neck round bottom flask under argon atmosphere. Dry DCM was added to the reaction mixture and the RB was cooled to 0 ^{0}C. After 5 minutes dicyclohexyl carbo diimide (DCC) was added and the whole mixture was stirred for 1h at the same temperature. 2, 7-dibromo-9, 9-di-n-hexanol fluorene was added to reaction mixture at 0 ^{0}C and RB was warmed to room temperature and stirred for 16h. Reaction mixture was diluted with DCM and the organic layer was extracted twice with 0.02 M NaOH. The organic layer was extracted twice with saturated $NaHCO_3$ followed by washing with brine, water and finally it was evaporated under reduced pressure. The product was purified by column chromatography using pet ether: ethyl acetate (55:45). ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 7.6-7.3 (m, 6H), δ 5.02 (d, 2H), δ 3.94 (t, 2H), δ 3.28 (q, 2H), δ 2.34 (q, 4H), δ 2.13 (m, 4H), δ 1.93-1.87 (m, 4H), δ 1.43 (s, 18H), δ 1.41 (s, 18H), δ1.08 (m, 8H), 0.55 (m, 4H). MALDI-TOF analysis; Calculated mass-1131.472; observed-1131.469; FT-IR stretching frequency (υ) in cm^{-1}; 3362, 2977, 2931, 2859, 1716, 1505, 1450, 1365, 1250, 1149, 1058 and 752.

3. Polymerization of (2)

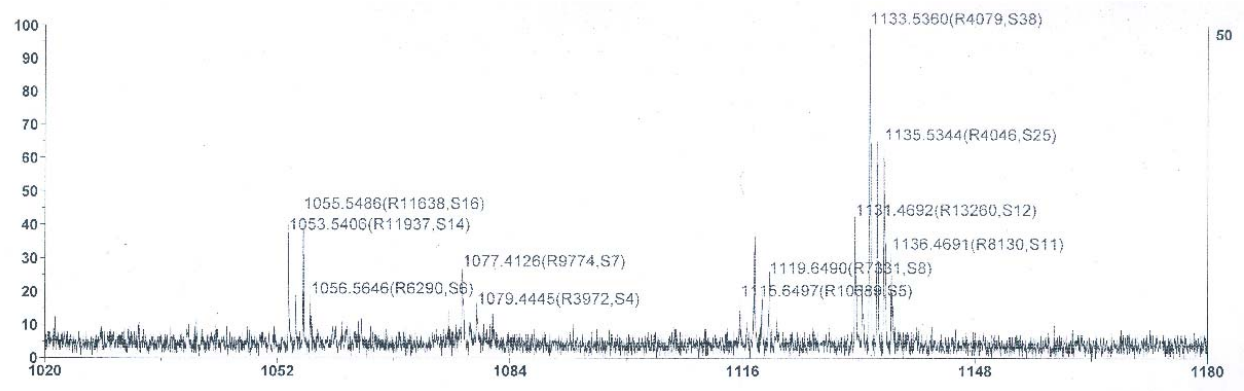
Monomer (1 g, 0.91 mmol), 1,4-benzene diboronic ester (0.3 g, 0.91mmol) and $Pd(PPh_3)_4$ (40 mg, 12 μmol) were taken in a two neck round bottom flask fitted with reflux condenser and connected with argon atmosphere. Dry THF (12 ml) was added to the reaction mixture which was then subjected to a sequence of three freeze-pump-thaw cycles. Degassed aqueous K_2CO_3 (0.503 g, 3.64 mmol) was then added to the reaction mixture and the contents were refluxed at 65 ^{0}C for 48h. The polymerization solution was evaporated under reduced pressure and dissolved in THF and filtered through whatmann filter paper to remove the Pd catalyst. The solvent was concentrated to 1 ml and the polymer was precipitated in methanol. The methanol precipitation was repeated 3 times. Finally, the polymer powder was dried under vacuum. The crude yield of the polymer (PF-GAP) was 1.15g (89%). ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 7.9-7.3 (m, 6H), δ 5.08 (b, 2H), δ 3.94 (b, 4H), δ 3.30 (b, 2H), δ 2.32 (b, 4H), δ 2.06 (m, 4H), δ 1.93-1.86 (m, 4H), δ 1.41 (bs, 18H), δ 1.40 (b, 18H), δ1.12 (b, 8H), 0.74 (b, 4H). M_n=25400; M_w=42800; PDI=1.7. ^{13}C NMR spectrum (400MHz, $CDCl_3$) δ 172.76, 171.23, 155.25, 152.11, 138.95, 130.18, 125.95, 121.42, 121.42, 81.96, 79.57, 70.7, 67.82, 64.51, 55.46, 53.3, 40, 29.38, 28.34, 28.19, 27.86, 25.47, 23.43.

4) Characterization of monomers and polymer

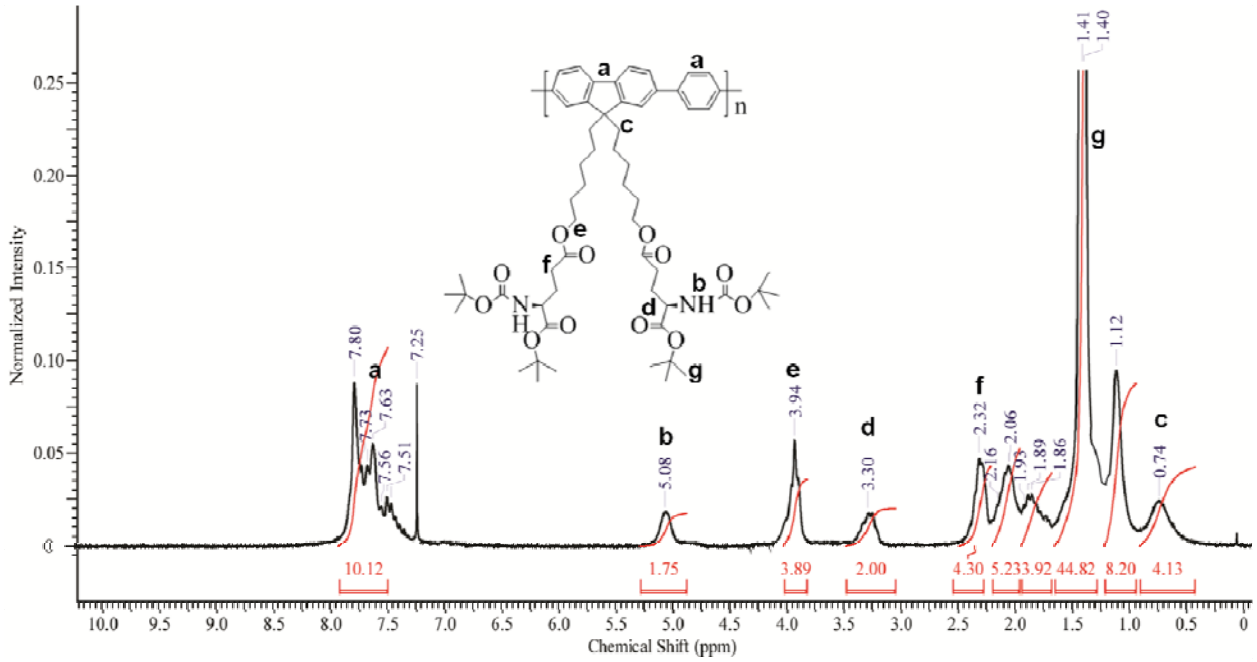
a)^{1}H NMR spectrum of monomer (2) in $CDCl_3$.



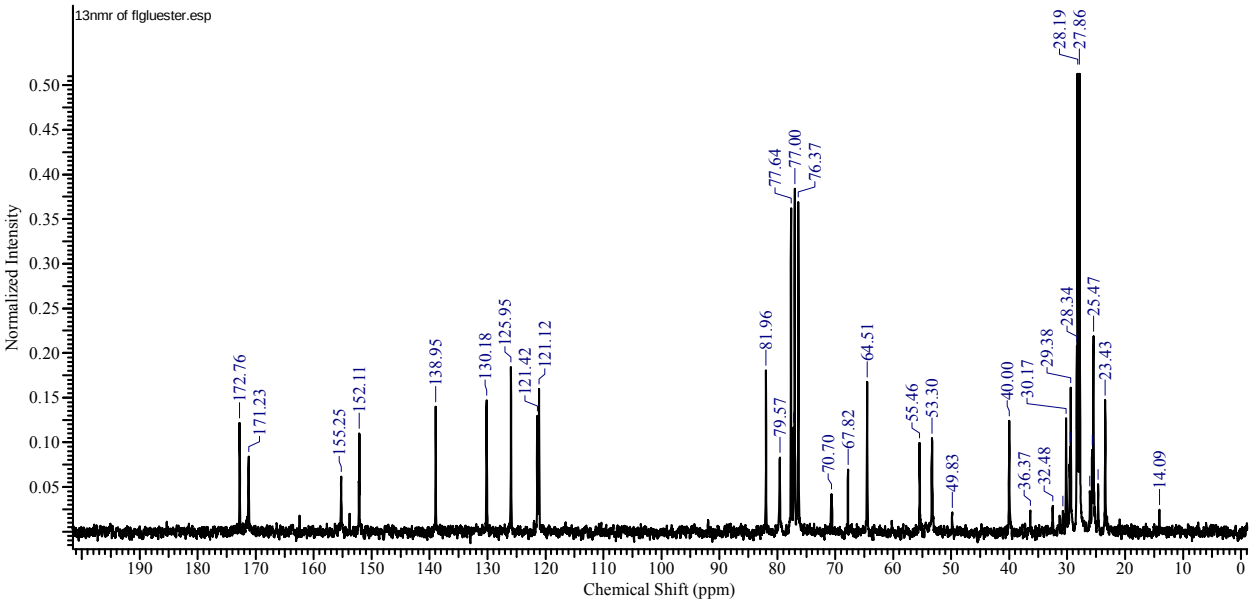
b) MALDI-TOF spectrum of monomer (2)



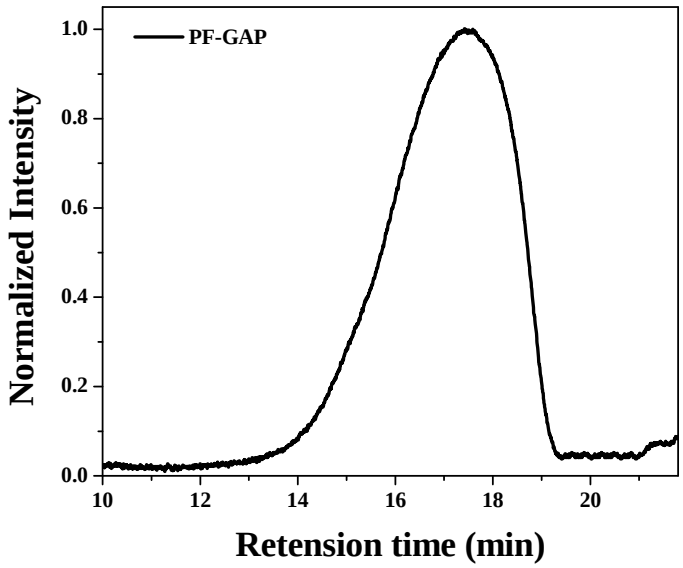
c) ^{1}H NMR spectrum of polymer (PF-GAP) in $CDCl_3$.



d) ^{13}C NMR spectrum of PF-GAP in $CDCl_3$.



e) Size exclusion chromatogram (SEC) of PF-GAP using THF as eluent and polystryrene as standard.



The molecular weight of the polymer was analyzed using size exclusion chromatography (SEC) using THF as eluent. The molecular weights of the polymer obtained from SEC were M_n = 25,400; M_w = 43200; Polydispersity (Đ) = 1.7 using polystyrene standards.

Figure S1) Solution state CD spectra of PF-GAP and protected glutamic acid in THF.

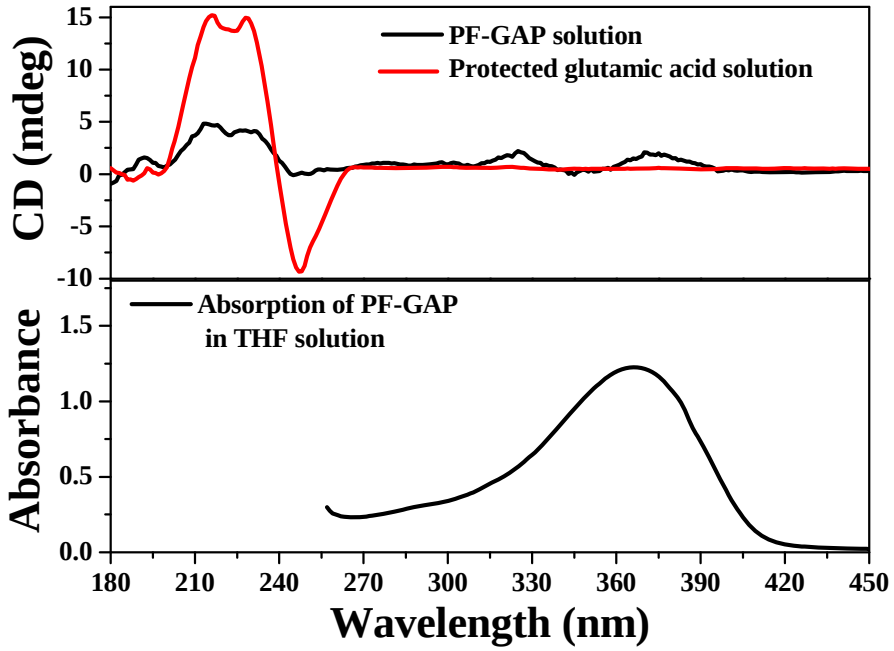
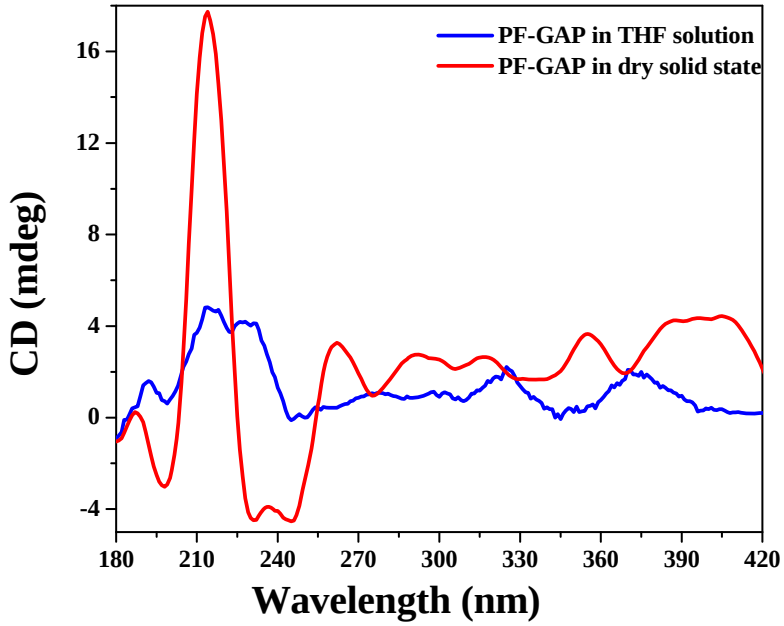


Figure S1 compares the normalized CD spectra of the protected L-glutamic acid (GAP) with that of the polymer PF-GAP in THF solvent. The absorption spectra of PF-GAP in THF is also given for comparison. L-GAP showed positive dichroic maxima at 215 and 228 nm with well defined negative maxima at 247 nm in its CD spectrum. The CD spectrum of the polymer was similar in shape to that of GAP with positive dichroic maxima at 215 and 228 nm, but the negative extreme had double inflection points at 244 and 250 nm. More importantly, CD effects were observed covering the entire absorption range of polyfluorene (300-400 nm, absorption maximum: 368 nm), where GAP did not have any dichroic activity. The CD spectrum of PF-GAP in the 300-400 nm region consisted of positive bands at 325 and 375 nm with a negative broad hump at 343 nm. The observation of the CD signal in the absorption range of the polymer confirmed the transfer of chirality from the side chain appended amino acid to the polymer backbone.

Figure S2) Comparison of the CD spectra of PF-GAP in solution and solid state.



The CD spectrum of the powder sample was characterized by an intense positive cotton effect with peak maximum around 210 nm along with negative cotton effects around 230 nm and 245 nm. These features of the CD spectrum are characteristic of α-helix conformation. Compared to the CD spectrum in THF (see figure S2), the intensity of the signal in the < 275 nm region was high in the powder form. However, the CD signal beyond 300 nm in the range of the polymer absorption was not very significant.

Figure S3) FT-IR spectra of the polymer PF-GAP in dry form (a) and after two days of treatment with water (b).

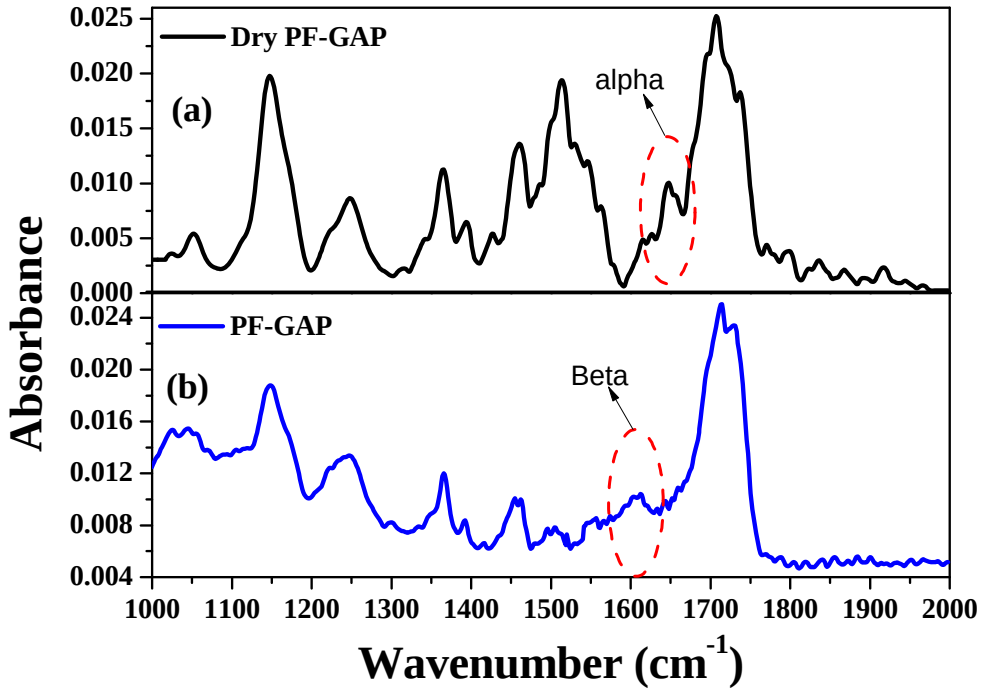
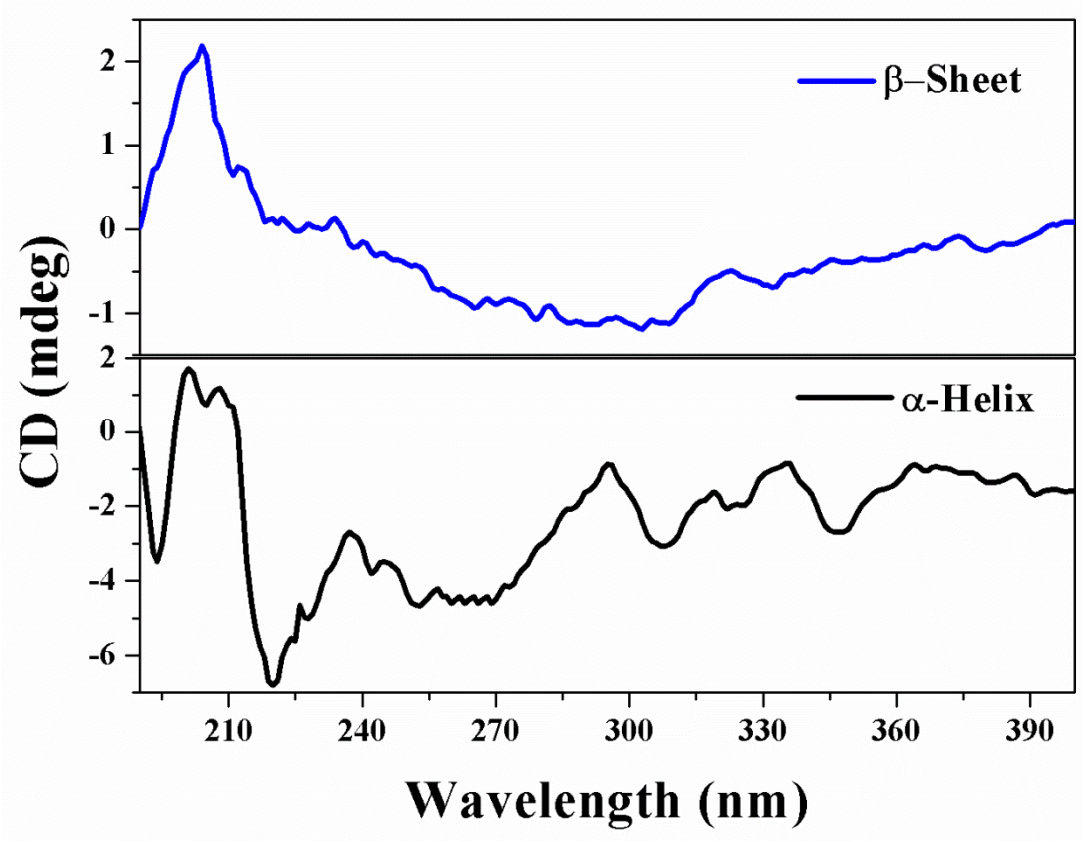


Figure S3 compares the expanded region in the FTIR spectra of the polymer powder before and after treatment with water. The dry polymer powder exhibited vibration band at 1648 cm^{-1}, which was typical for α-helix conformation. The plot in the bottom showed a complete absence of this vibration; instead a band appeared ~ 1610 cm^{-1}, which is attributed to the beta sheet structure.

Figure S4) CD spectra of PF-GAP confirming reversibility.



The conformation of the polymer was reversible upon complete drying of the polymer under the vacuum for 24h. CD spectra taken for the powder after the drying process showed the characteristic α-helix conformation.

Figure S5) Scanning electron microscopy images of PF-GAP particles on carbon tape.

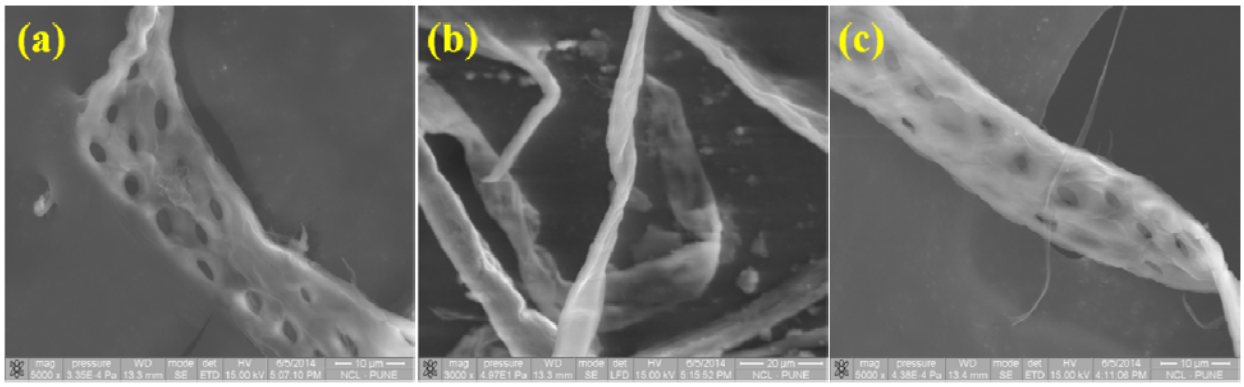
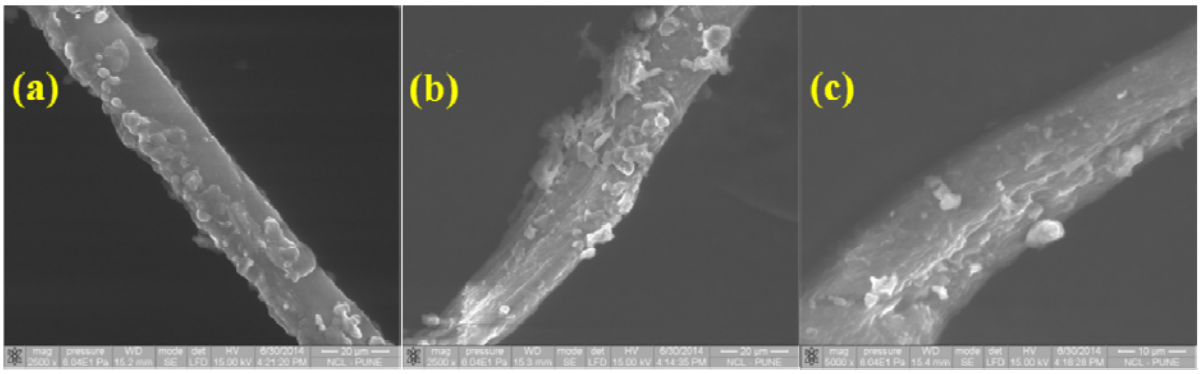


Figure S6) SEM images of PF-GAP after two days stirring in aqueous solution containing racemic mixture of enantiomers.



The scanning electron microscopy (SEM) images of the dry PF-GAP and PF-GAP after treatment with water containing racemic mixture of various substrates are given in figure S5 and figure S6 respectively. Dry PF-GAP revealed fibrous filaments with pores on the surface. This higher ordered hierarchical morphology is expected to mimic protein super structures with potential to exhibit specific molecular adsorption. The SEM images of PF-GAP after treatment with water containing racemic mixture (figure S6) became swollen fibers indicating the uptake of the substrate.

Figure S7) The chemical structure of all substrates screened in the present study.

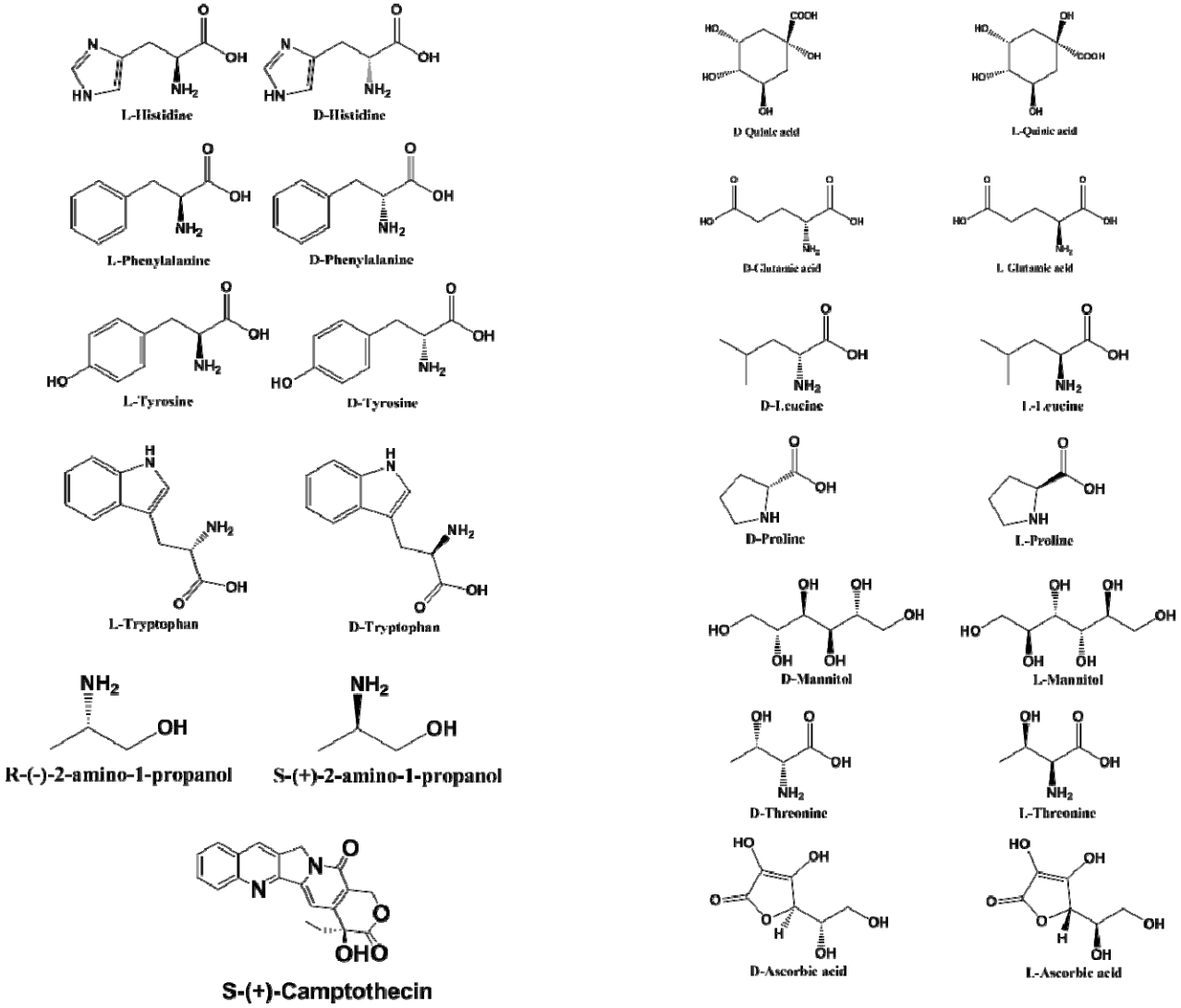
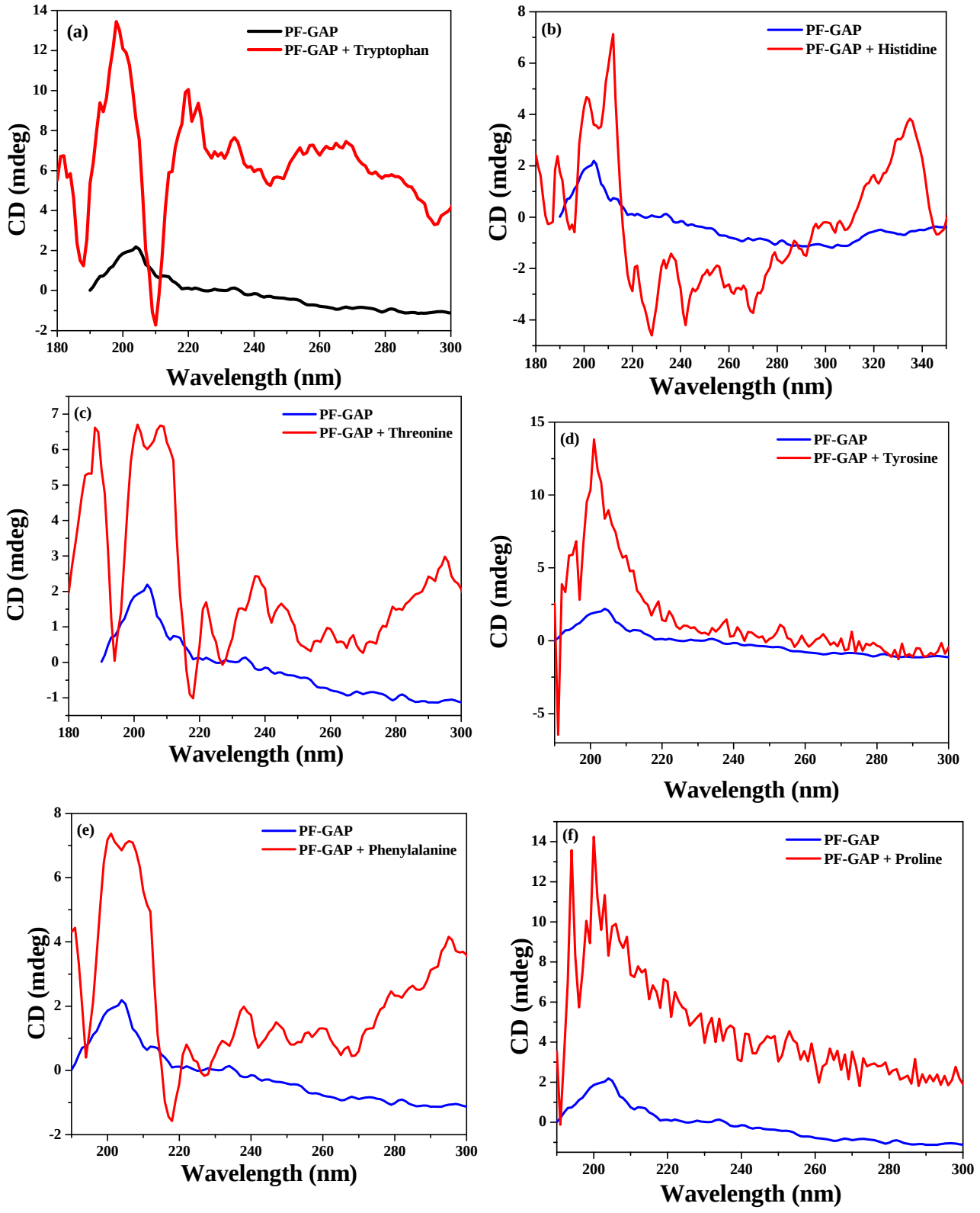


Figure S8) Solid state CD spectra of PF-GAP and PF-GAP adsorbed with various substrates.



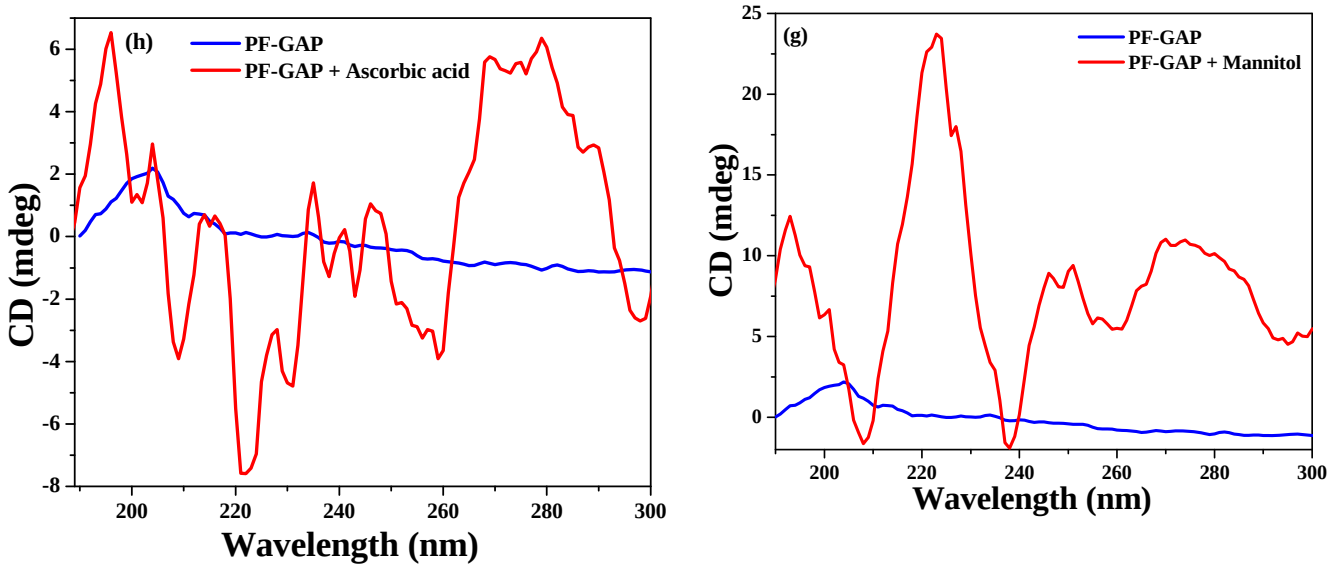
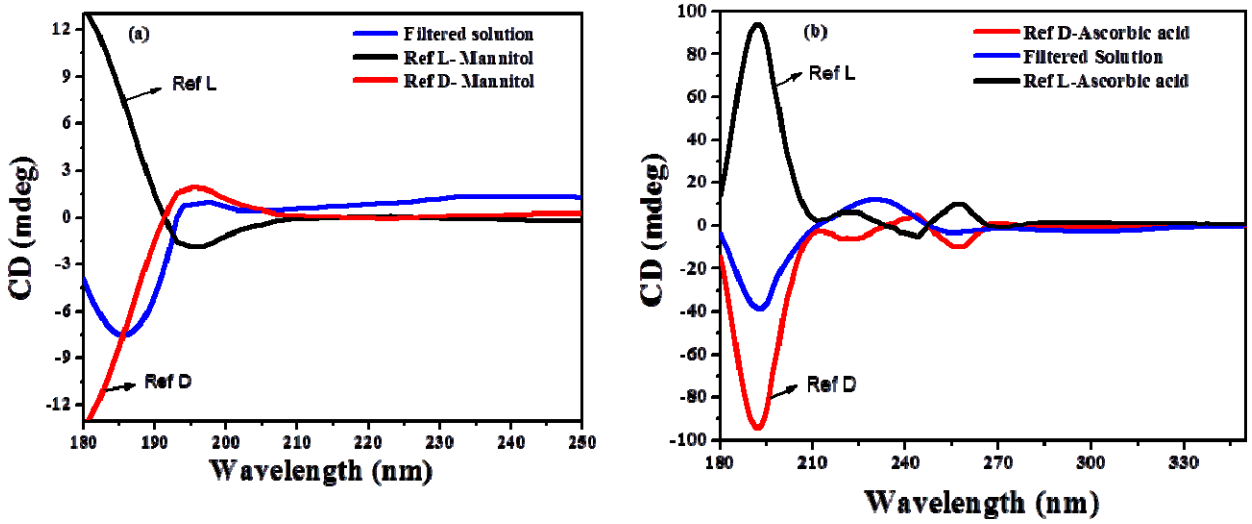
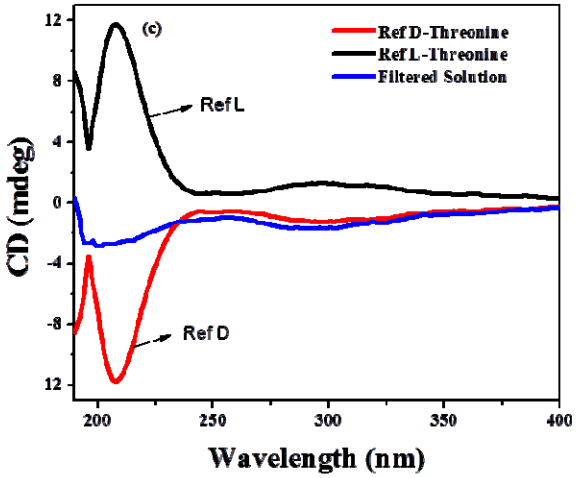
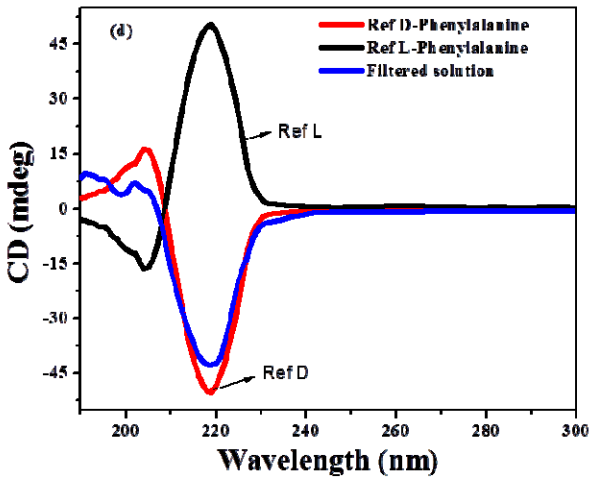
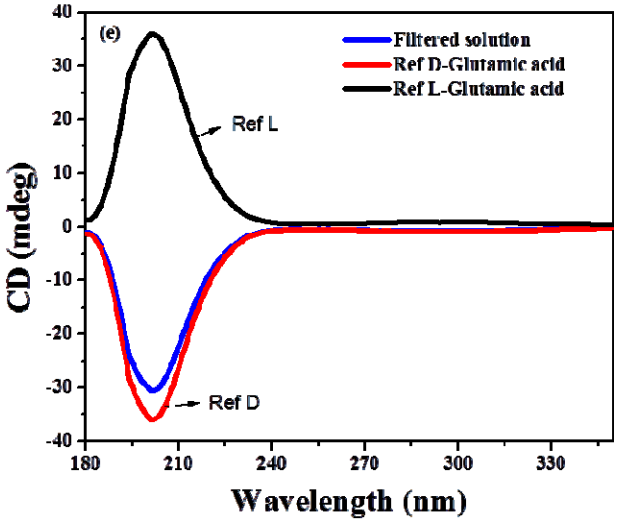


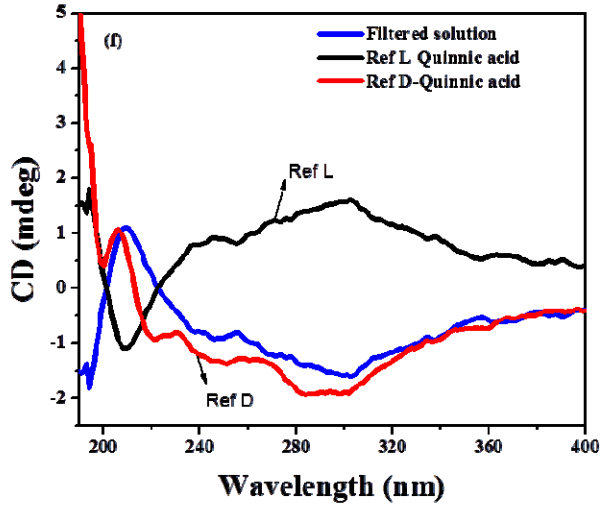
Figure S9) Circular dichroism spectra of the water solution obtained from heterogeneous enatioselective separation for various racemic mixtures. The ratio of the area under the CD curve for pure D- enantiomer and filtered solution was used to determine the ee which are listed in table-1.

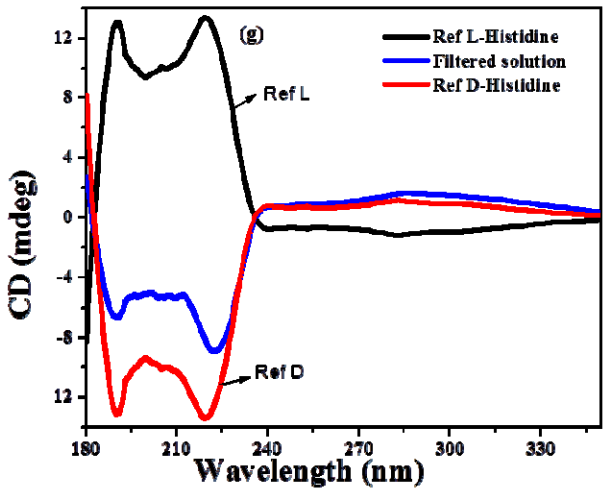


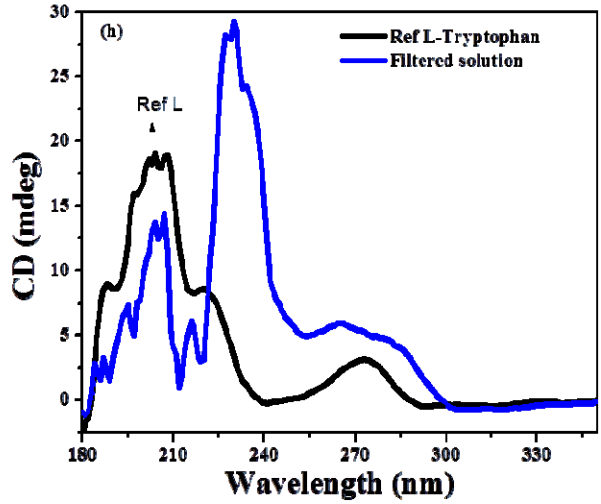

(c)
Ref D-Threonine
Ref L-Threonine
Filtered Solution
Ref L
Ref D
CD (mdeg)
Wavelength (nm)

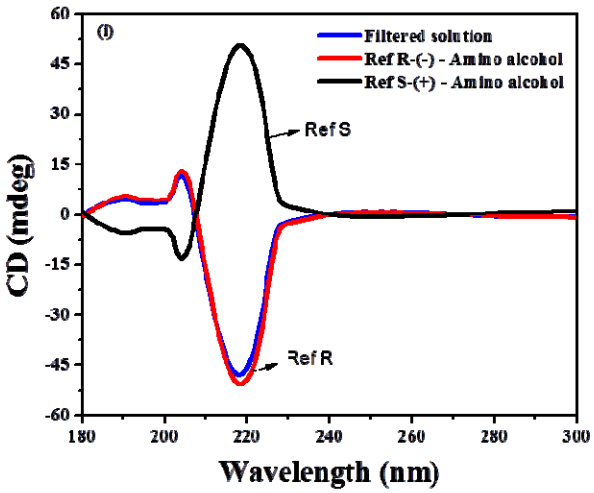

(d)
Ref D-Phenylalanine
Ref L-Phenylalanine
Filtered solution
Ref L
Ref D
CD (mdeg)
Wavelength (nm)

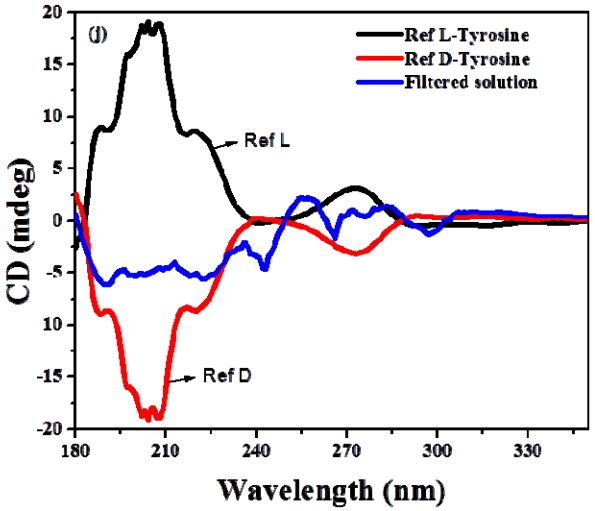

(e)
Filtered solution
Ref D-Glutamic acid
Ref L-Glutamic acid
Ref L
Ref D
CD (mdeg)
Wavelength (nm)

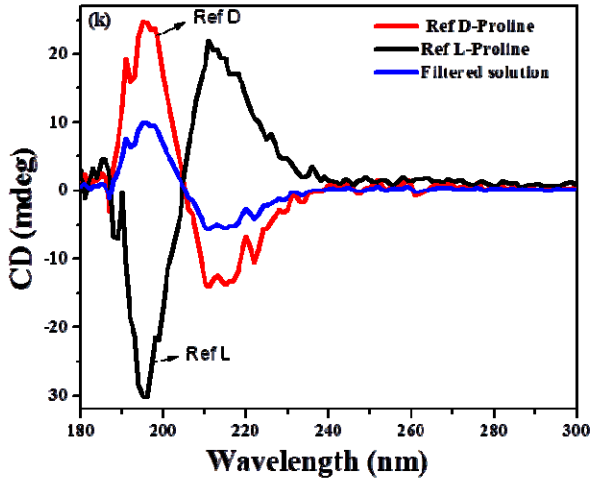

(f)
Filtered solution
Ref L Quinnic acid
Ref D-Quinnic acid
Ref L
Ref D
CD (mdeg)
Wavelength (nm)

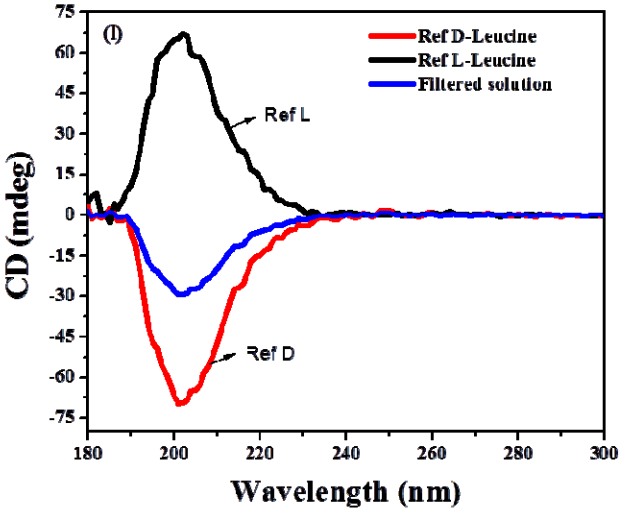

(g)
Ref L-Histidine
Filtered solution
Ref D-Histidine
Ref L
Ref D
CD (mdeg)
Wavelength (nm)

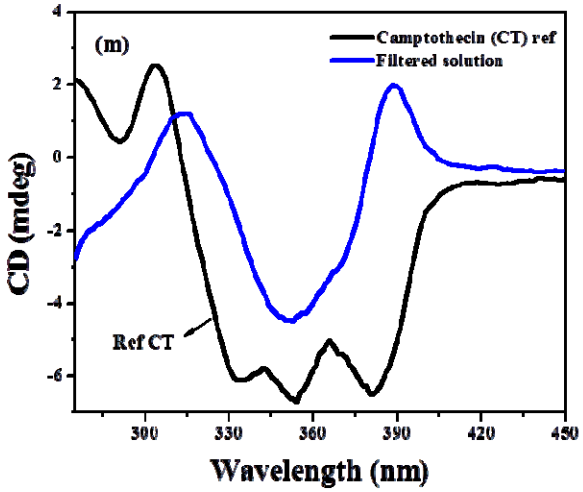

(h)
Ref L-Tryptophan
Filtered solution
Ref L
CD (mdeg)
Wavelength (nm)


(i)
Filtered solution
Ref R-(-) - Amino alcohol
Ref S-(+) - Amino alcohol
Ref S
Ref R
CD (mdeg)
Wavelength (nm)

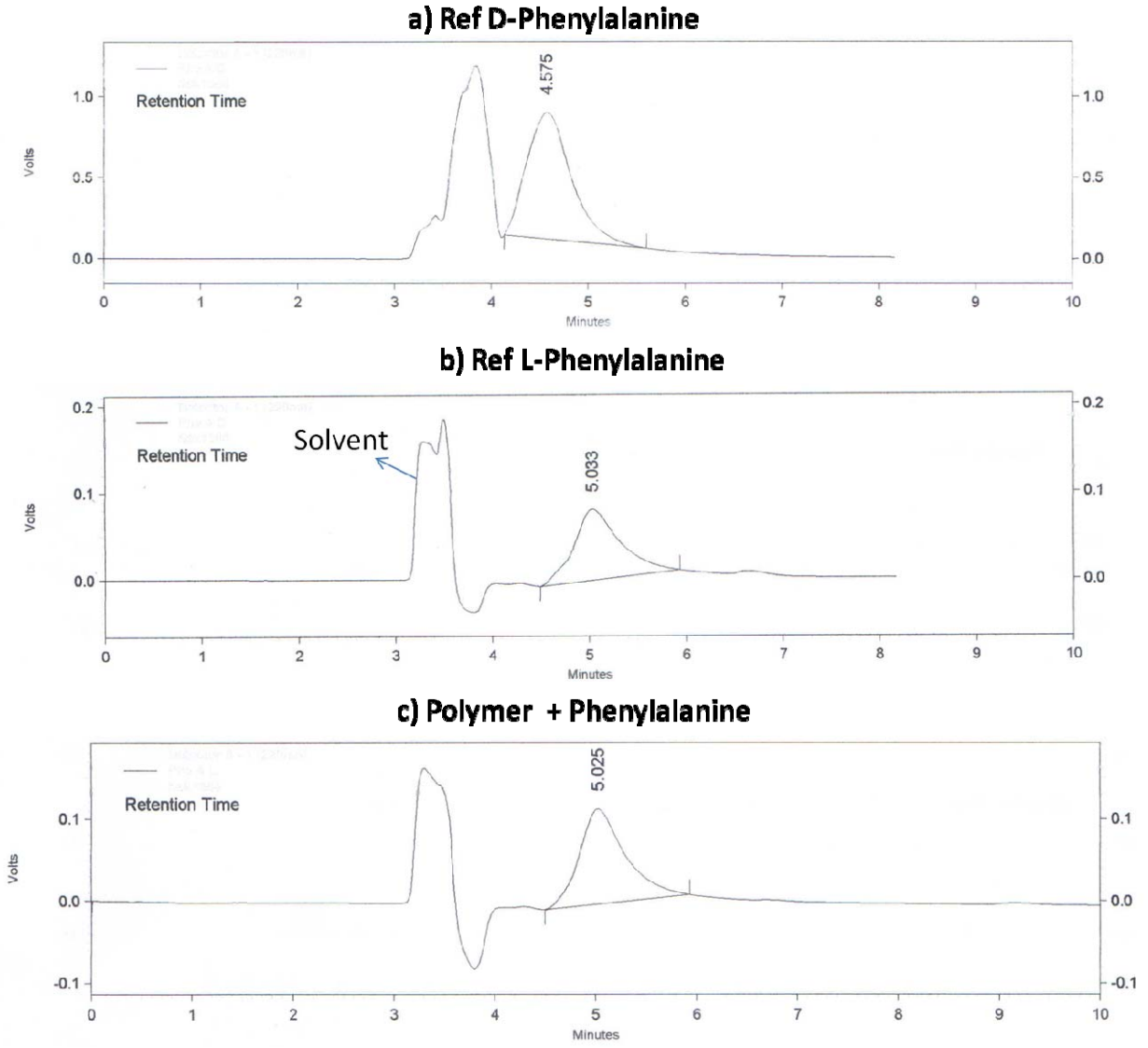

(j)
Ref L-Tyrosine
Ref D-Tyrosine
Filtered solution
Ref L
Ref D
CD (mdeg)
Wavelength (nm)


(k)
Ref D
Ref D-Proline
Ref L-Proline
Filtered solution
Ref L
CD (mdeg)
Wavelength (nm)


(l)
Ref D-Leucine
Ref L-Leucine
Filtered solution
Ref L
Ref D
CD (mdeg)
Wavelength (nm)

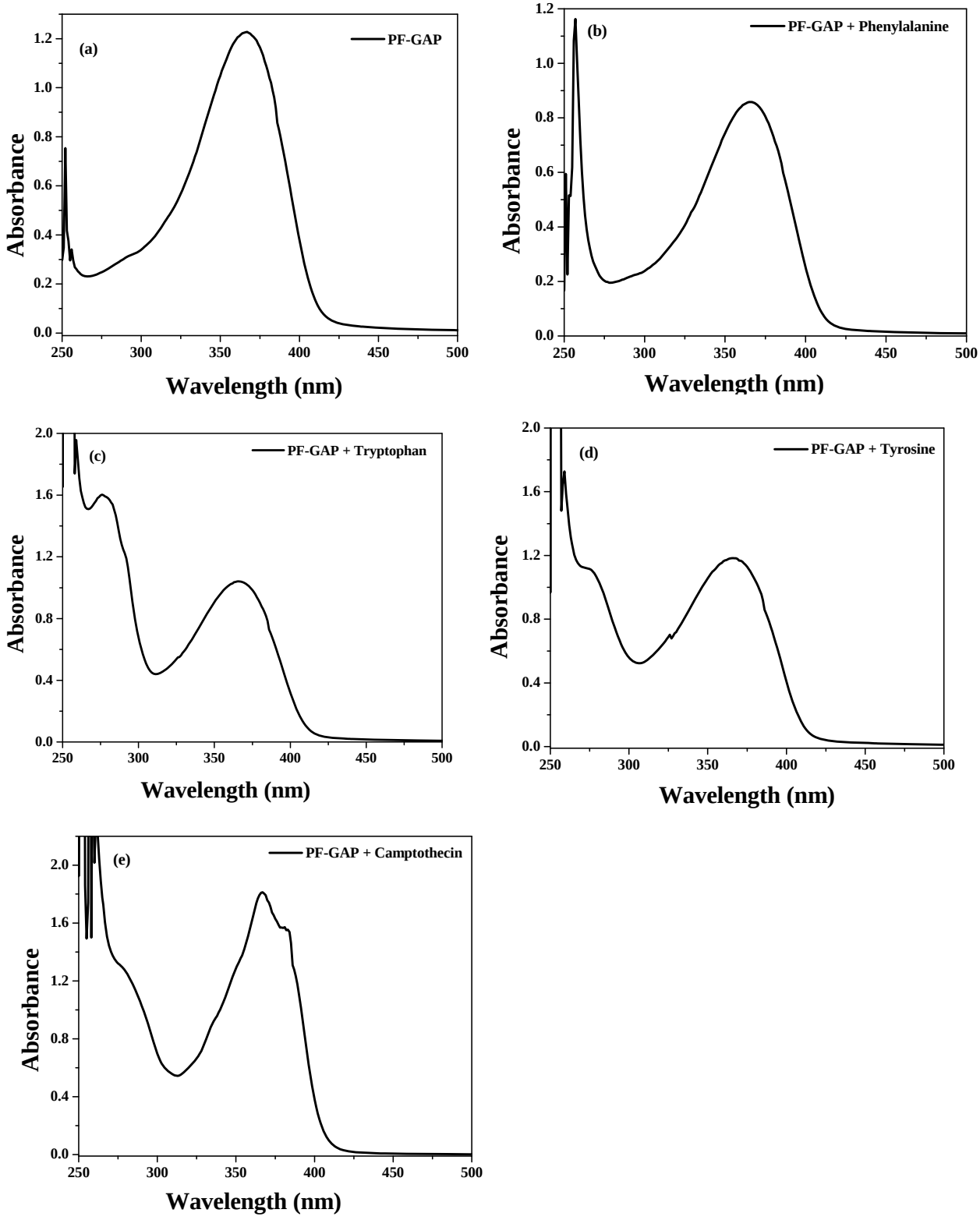

(m)
Camptothecin (CT) ref
Filtered solution
Ref CT
CD (mdeg)
Wavelength (nm)

S10) Chiral HPLC chromatogram of D- & L- Phenylalanine and adsorbed Phenylalanine that was precipitated from the polymer.



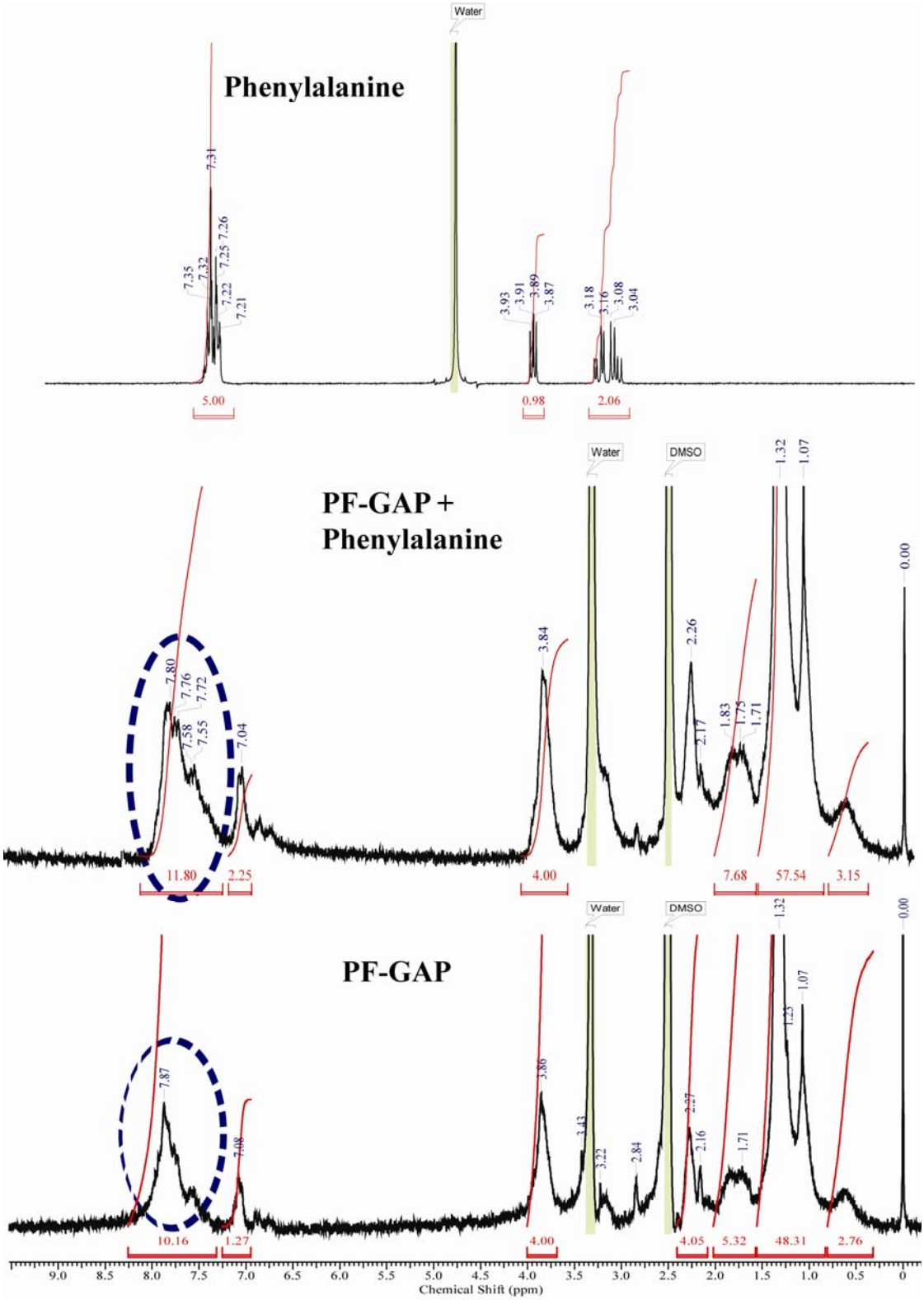
Chiral HPLC was performed for one sample to show PF-GAP polymer selectively uptakes only one type of enantiomer from the racemic mixture. Pure L- and D-phenylalanine samples injected in chiral HPLC (see method section for column and other details) column showed retention times of 5.033 and 4.575 minutes respectively. The polymer adsorbed phenylalanine that was precipitated from the polymer showed a retention time of 5.025 minutes. There was no peak at 4.575 minutes clearly indicating the selective uptake of L-phenylalanine by PF-GAP polymer from their racemic mixture.

Figure S11) Absorption spectra recorded in DMSO for PF-GAP, PF-GAP treated with phenylalanine, tyrosine, tryptophan and camptothecin.



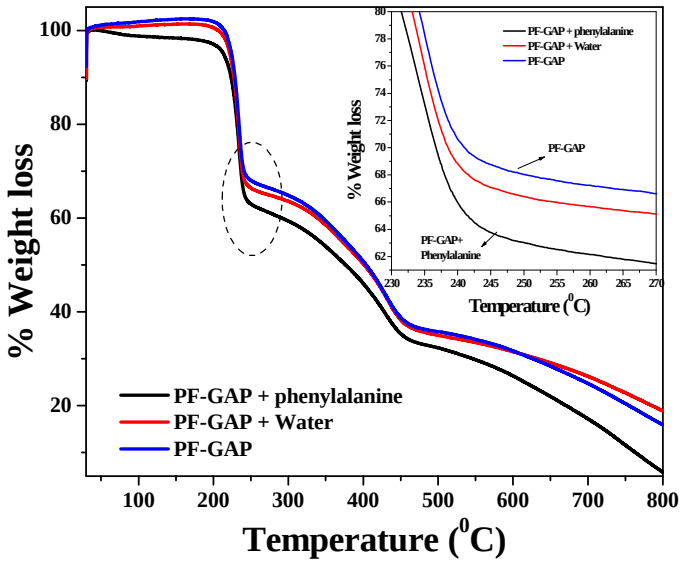
The enantioselective uptake by the polymer could be confirmed by dissolving the polymer with the adsorbed substrate in DMSO and recording the absorption spectra. Figure S11 shows the absorption spectra for PF-GAP + Phenylalanine, PF-GAP + Tryptophan, PF-GAP + Tyrosine and PF-GAP + Camptothecin along with the absorption spectrum of PF-GAP alone. The absorption spectra had peaks indicating presence of the adsorbed substrates confirming their uptake by the polymer.

Figure S12) ^{1}H NMR spectra of PF-GAP, PF-GAP+Phenylalanine in DMSO-d6, and Phenylalanine recorded in D_2O.



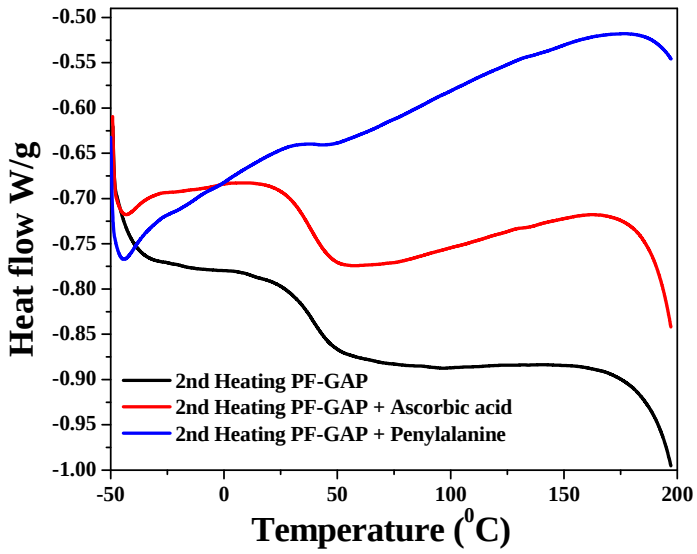
One of the adsorbed polymer – PF-GAP + Phenylalanine was taken as a representative example and its proton NMR spectrum was recorded in DMSO-d6. Figure S12 compares the proton NMR spectra of PF-GAP and PF-GAP + Phenylalanine in DMSO-d6. The aromatic protons of phenylalanine appeared in the range 7.2 – 7.3 ppm (Figure S12 also gives the proton NMR spectrum of phenylalanine recorded in D_2O), which merged with that of the aromatic protons of the polymer resulting in broadening of the entire aromatic region. The aliphatic proton signals of phenylalanine were also broadened which indicated interaction between the polymer and substrate, unlike a simple physical mixture where the peak shape would not be affected.

Figure S13) Thermogravimetric analysis (TGA). TGA analysis of PF-GAP in dry form, 2 days water treated, 2 days stirred in aqueous solution containing racemic mixture of phenylalanine. The inset shows the enlarged portion of the circle.



The thermal characteristics of the substrate loaded polymer were also investigated using theromogravimetric analysis (TGA). Figure S13 compares the percentage weight loss data for PF-GAP + Phenylalanine along with that for dry PF-GAP and PF-GAP powder after stirring in water for 48 hours and drying. Although the decomposition pattern was the same, PF-GAP + Phenylalanine showed a higher (> 5 wt %) weight loss indicating loss of adsorbed material. In fact, PF-GAP stirred in water without any substrates also showed slightly different % weight loss indicating adsorption of water.

Figure S14) Differential Scanning calorimetry (DSC) analysis. DSC thermograms showing 2nd heating cycles of PF-GAP, PF-GAP treated with racemic mixtures of ascorbic acid and phenylalanine.



The DSC thermogram (Figure **S14**) also exhibited a slight lowering of the glass transition temperature from 39 °C for pristine polymer to 37 °C for Phenylalanine loaded polymer.