

†Electronic Supplementary Information (ESI)

S.1 Synthesis of 3-aminopropyl isonicotinate

Isonicotinic acid was conjugated to 3-amino-1-propanol via EDC/NHS coupling. Briefly, 3-amino-1-propanol (1g, 13.3 mM) was reacted with Di-tert-butyl dicarbonate (3.15 g, 14.43 mM) in the presence of triethyl amine (2.66g, 26.6 mM), using dichloromethane (30 mL) as solvent (Scheme I a). The reaction mixture was stirred at room temperature for 12 hours, followed by evaporating under vacuum using rotary flash evaporator (IKA 50, Germany). The residue was diluted with dichloromethane (DCM), and extracted with water, brine solution and 0.1 M H₂SO₄. The combined organic layer was concentrated to obtain 3-(Boc-amino)-1-propanol. In the next step, isonicotinic acid (1g, 8.1 mM) was dissolved in dichloromethane (20 mL) followed by addition of N-hydroxy succinimide (1.12 g, 9.7 mM). After stirring the reaction mixture for 15 minutes at room temperature, 3-(Boc-amino)-1-propanol (1.7 g, 9.7 mM), EDC.HCl (1.866g, 9.7 mM) and triethylamine (2 g, 20mM) were added, and the reaction was continued to proceed for overnight (Scheme I). The crude mixture was diluted with brine solution, and extracted with dichloromethane. Further, the combined organic layer was washed with saturated sodium bicarbonate solution. The obtained product viz. 3-[(tert-butoxycarbonyl) amino]propyl isonicotinate was purified through silica gel column (60-120 mesh size), using chloroform : methanol (ratio ranging from 90:10 to 55:10) as mobile phase. The purified product was then dissolved in dichloromethane:trifluoroacetic acid mixture at 1: 1 ratio for 3 hours, to remove boc. The compound formed viz. 3-aminopropyl isonicotinate was dried under high vacuum and characterized using mass spectrometry (MALDI – TOF-ABI 4700).

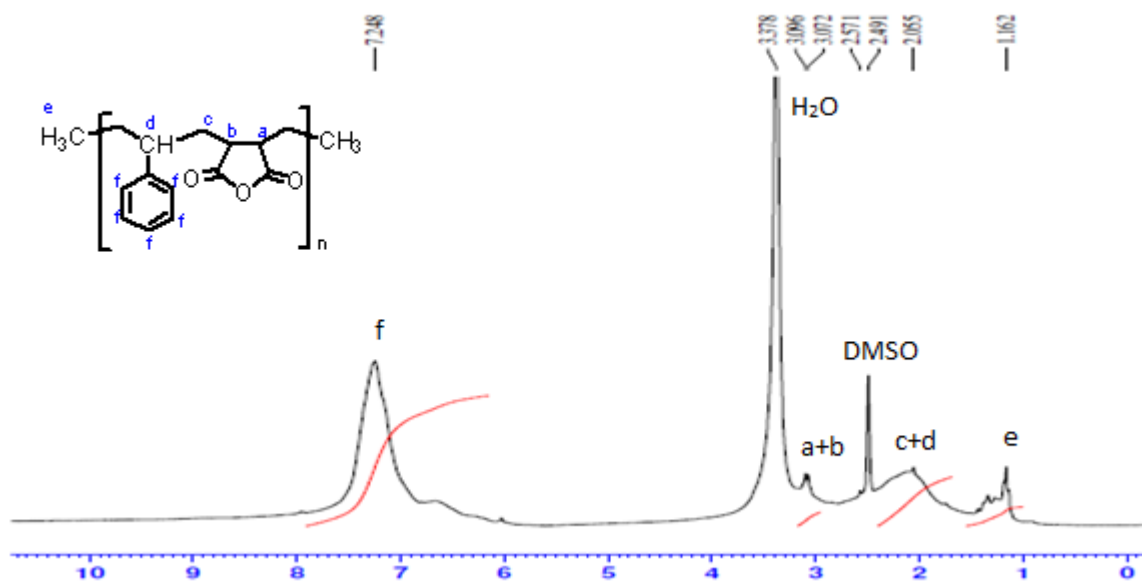


Figure S.1: ^1H NMR spectra (300 MHz, $\text{DMSO-}d_6$) of PSMA: δ 1.162 (br m, $-\text{CH}_3$), 2.07 (br, aliphatic $\text{CH}+\text{CH}_2$), 3.08 (m, CH anhydride), 7.25 (br m, Ar CH)

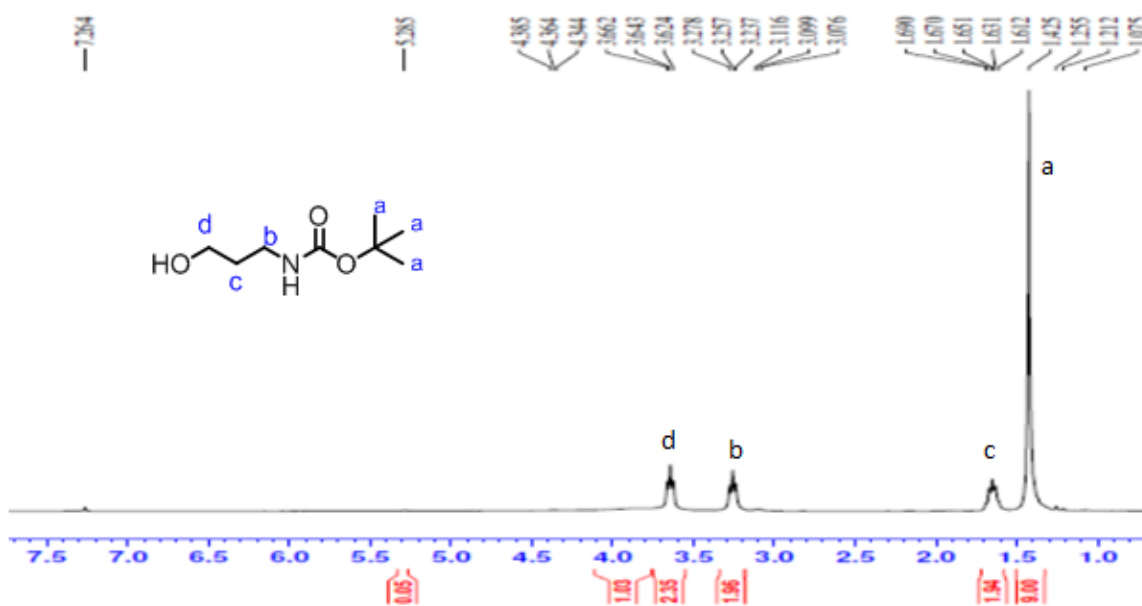


Figure S.2: ^1H NMR spectra (300 MHz, CDCl_3) of N-Boc-3-amino-1-propanol. δ 1.43 (s, 9H, Boc CH_3), 1.63 (m, 2H, $\text{HO-CH}_2-\text{CH}_2$), 3.26 (t, $J = 6$ Hz, 2H, HO-CH_2), 3.64 (t, $J = 5.7$ Hz, 2H, HN-CH_2)

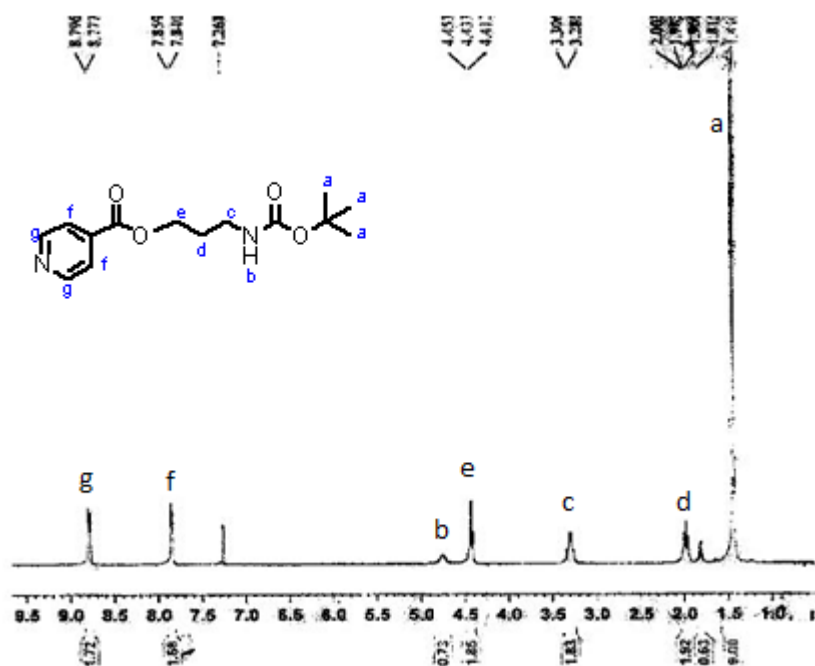


Figure S.3: ^1H NMR spectra (300 MHz, CDCl_3) of 3-(tert-butoxycarbonylamino)propyl isonicotinate. δ 1.44 (s, 9H, Boc CH_3), 1.9 (m, 2H, -O- CH_2 - CH_2), 3.3 (d, $J = 6$ Hz, 2H, HN- CH_2), 4.4 (t, $J = 6$ Hz, 2H, -O- CH_2), 4.75 (br s, 1H, Boc NH), 7.85 (d, $J = 5.8$ Hz, 2H, Ar CH), 8.8 (d, $J = 6$ Hz, 2H, Ar CH)

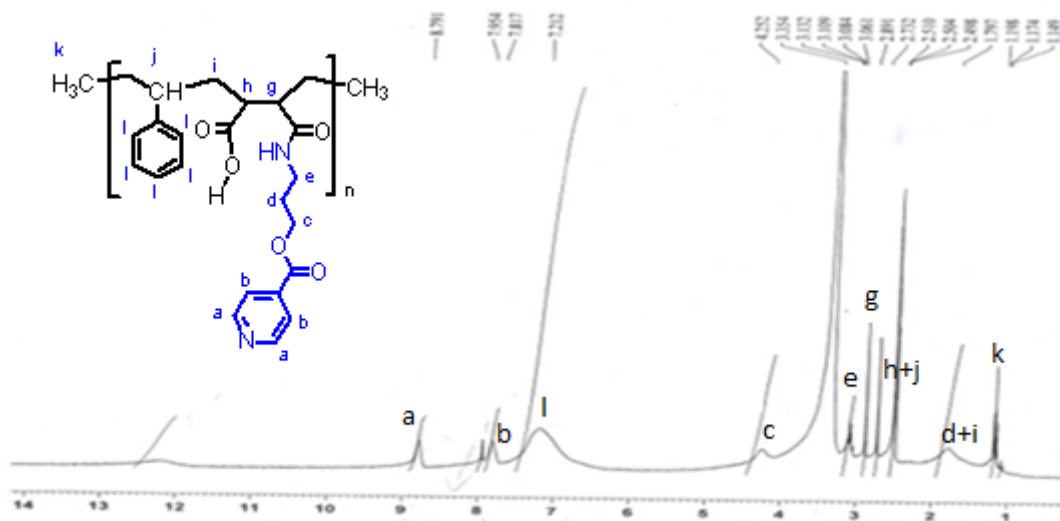


Figure S.4: ^1H NMR spectra (300 MHz, $\text{DMSO-}d_6$) of PSMA – isonicotinic acid conjugate. δ 1.18 (br m, - CH_3), 1.8 (br, aliphatic CH_2), 2.73 (m, aliphatic CH), 2.8 (m, aliphatic CH), 3.1 (m, aliphatic CH_2), 4.2 (br m, aliphatic CH_2), 7.2 (br m, Ar CH), 7.8 (br d, Ar CH), 7.9 (m, NH), 8.8 (br d, Ar CH).

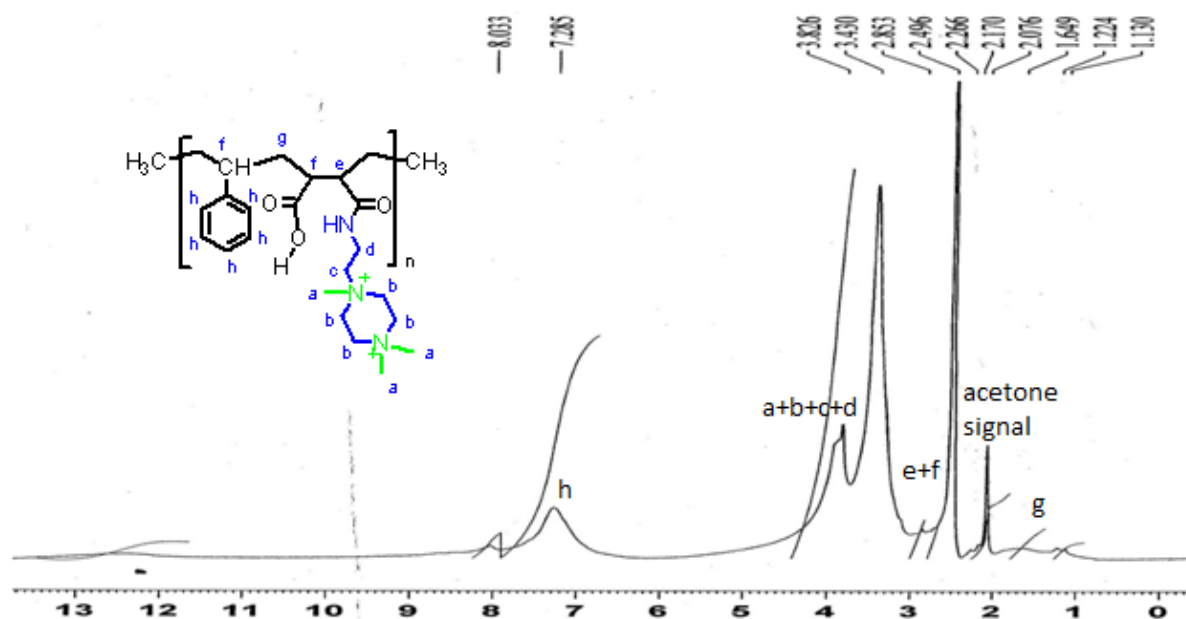


Figure S.7: ^1H NMR spectra (300 MHz, $\text{DMSO-}d_6$) of **P2**. δ 1.13 (br m, $-\text{CH}_3$), 1.65 (br, aliphatic CH_2), 2.85 (m, aliphatic CH), 3.83 (br m, aliphatic $\text{CH}_2 + \text{CH}_3$), 7.29 (br m, Ar CH).

S.2 Synthesis of 3-amino-1-propanol grafted PSMA

For the grafting of 3-amino-1-propanol to PSMA, 3-(Boc-amino)-1-propanol (0.572 g, 3.26 mM) was conjugated to the polymer (0.5 g, 1.6 mM), in the presence of triethyl amine (0.326 g, 3.26 mM), using dichloromethane as solvent (scheme III). The reaction mixture was concentrated under high vacuum to remove solvent and triethylamine, and the resulting polymeric derivative was washed with acidic water (pH 5) and dichloromethane, successively. Boc was then removed by treating with DCM: TFA (1:1 ratio), for 2 hours.

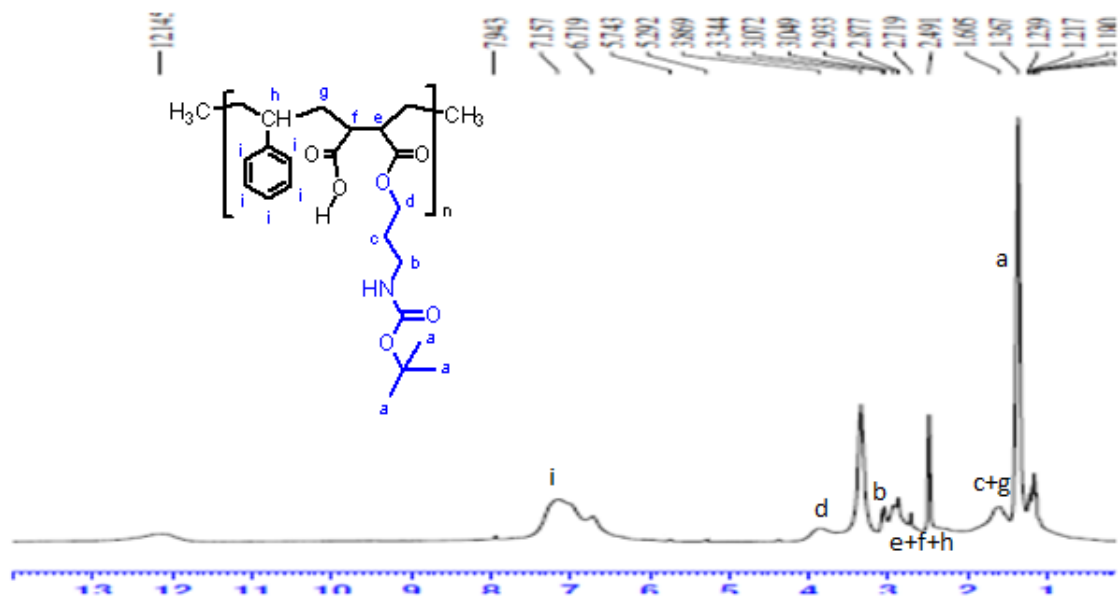


Figure S.8: ¹H NMR spectra (300 MHz, DMSO-*d*₆) of N-boc -3-amino-1-propanol grafted PSMA. δ 1.1(br m, -CH₃), 1.36 (br s, Boc CH₃), 1.6 (br m, aliphatic CH+ CH₂), 2.9 (br m, aliphatic CH+ CH₂), 3.8 (br m, aliphatic CH₂), 7.2 (br m, Ar CH).

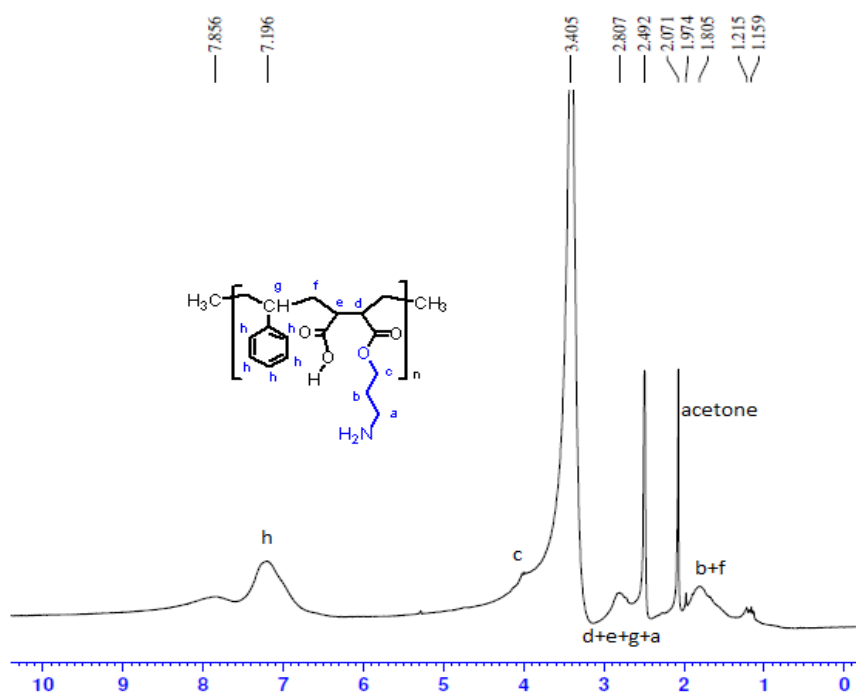


Figure S.9: ¹H NMR spectra (300 MHz, DMSO-*d*₆) of 3-amino-1-propanol grafted PSMA. δ 1.16 (br m, -CH₃), 1.8 (br m, aliphatic CH₂), 2.8 (br m, aliphatic CH+ CH₂), 4 (br m, aliphatic CH₂), 7.2 (br m, Ar CH).

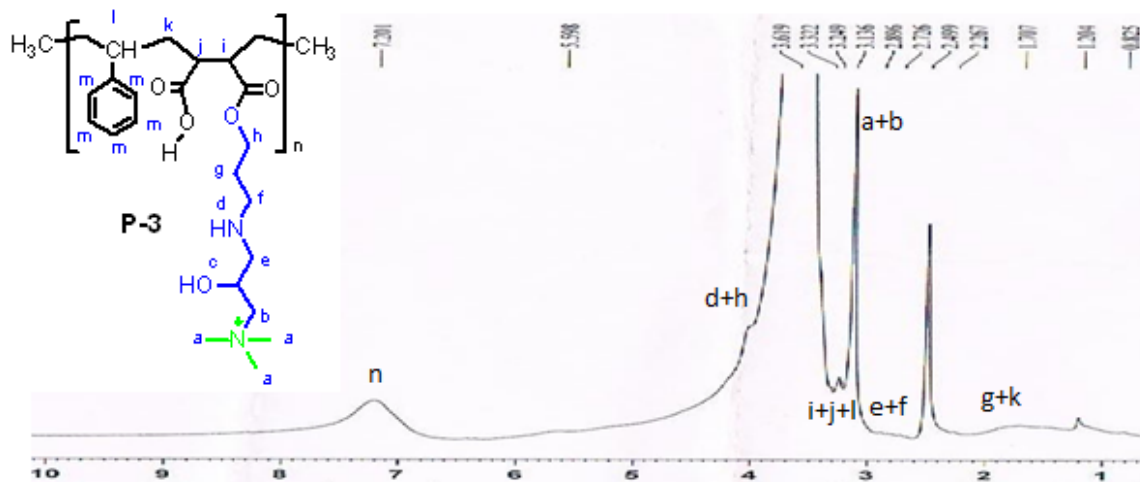


Figure S.10: ^1H NMR spectra (300 MHz, $\text{DMSO-}d_6$) of **P3**. δ 1.2 (br m, $-\text{CH}_3$), 1.7 (br m, aliphatic CH_2), 2.7 (br, aliphatic CH_2), 3.14 (br s, aliphatic N-CH_3), 3.3 (br m, aliphatic $\text{CH} + \text{CH}_2$), 4 (br m, aliphatic CH_2), 7.2 (br m, Ar CH).

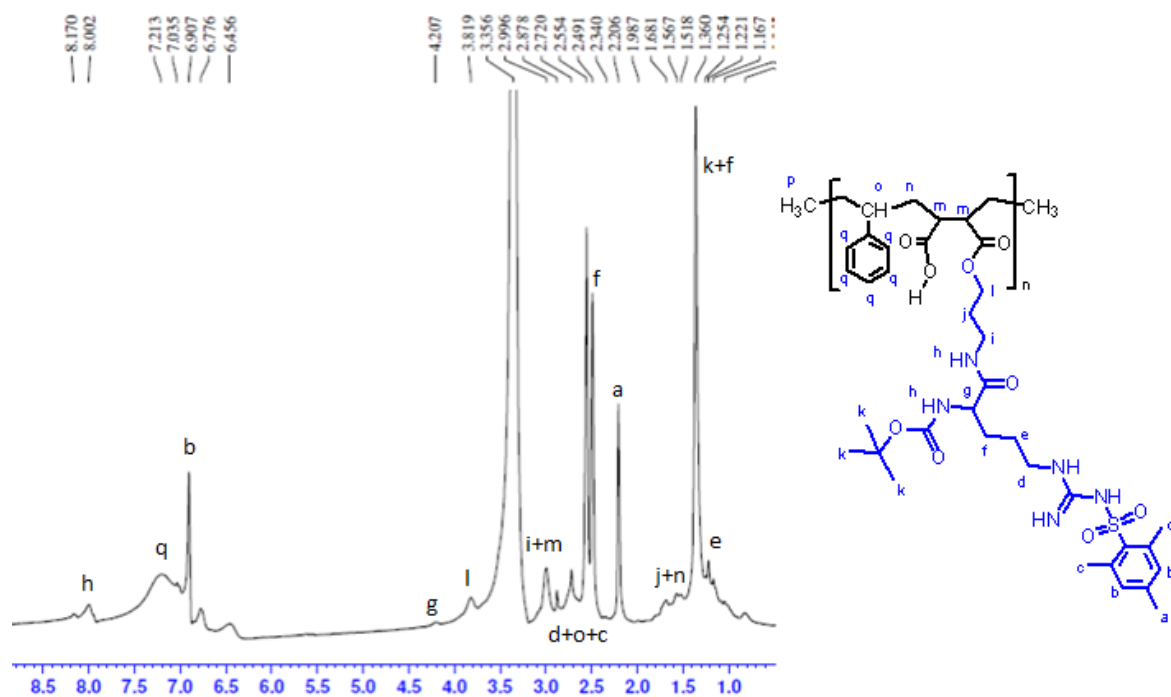


Figure S.11: ^1H NMR spectra (300 MHz, $\text{DMSO-}d_6$) of Boc-Arginine (Mts)-OH grafted PSMA. δ 1.17 (br m, aliphatic $\text{CH}_2 + \text{CH}_3$), 1.36 (br s, Boc CH_3), 1.68 (br m, aliphatic CH_2), 2.2 (br d, Ar CH_3), 2.55 (br d, Ar CH_3), 2.72 (br m, aliphatic $\text{CH} + \text{CH}_2$), 3 (br m, aliphatic $\text{CH} + \text{CH}_2$), 3.82 (br m, aliphatic CH_2), 4.2 (m, aliphatic CH), 6.9 (br s, Ar CH), 7.2 (br m, Ar CH), 8 (br m, NH).

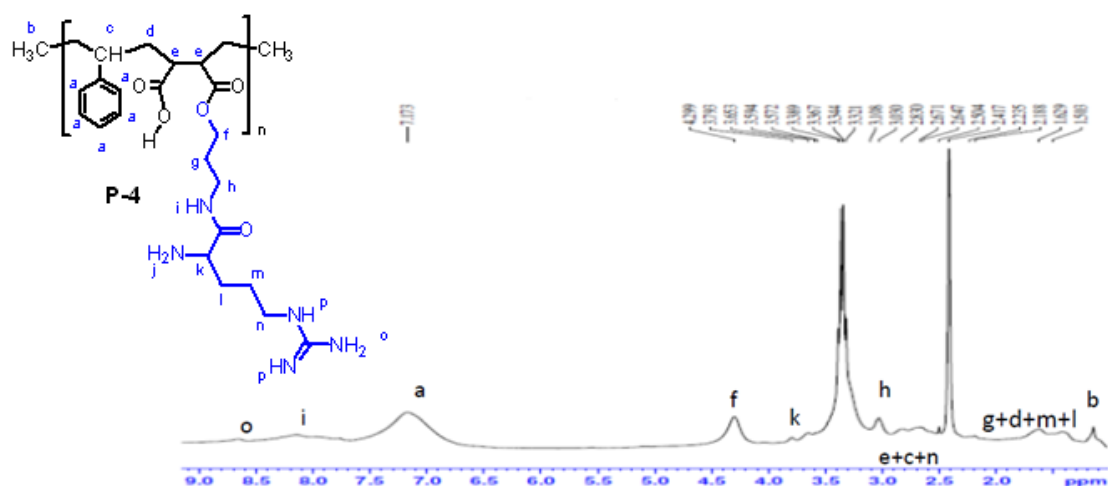


Figure S.12: ^1H NMR spectra (300 MHz, $\text{DMSO-}d_6$) of **P4**. δ 1.17 (br m, aliphatic CH_3), 1.7 (br, aliphatic CH_2), 2.7 (br, aliphatic $\text{CH} + \text{CH}_2$), 3.1 (br m, aliphatic CH_2), 3.7 (br m, aliphatic CH), 4.3 (br m, aliphatic CH_2), 7.2 (br m, Ar CH).

S.3 Synthesis of Mono N-Boc protected spermine

Spermine (2 g, 9.9 mM) was dissolved in methanol: DMF mixture at 1:1 ratio (35 mL), in the presence of triethyl amine (1.98 g, 19.8 mM). Then, Boc anhydride (0.54g, 2.47 mM) was dissolved in dry DMF (5 mL) and added slowly to the reaction mixture over one hour, under continuous stirring. Reaction was carried out overnight, and the solvents were removed under high vacuum. The residue was then mixed with saturated sodium bicarbonate solution, and Boc-protected spermine was extracted with ethyl acetate and dichloromethane, in succession. Extract was then concentrated, dried under high vacuum and characterized using mass spectrometry (MALDI – TOF-ABI-4700). To the obtained viscous product, 1 N HCl was added, and further extracted with ethyl acetate. The pH of the aqueous layer was increased to 9.5 with 1 N NaOH, and again extracted with ethyl acetate followed by dichloromethane.

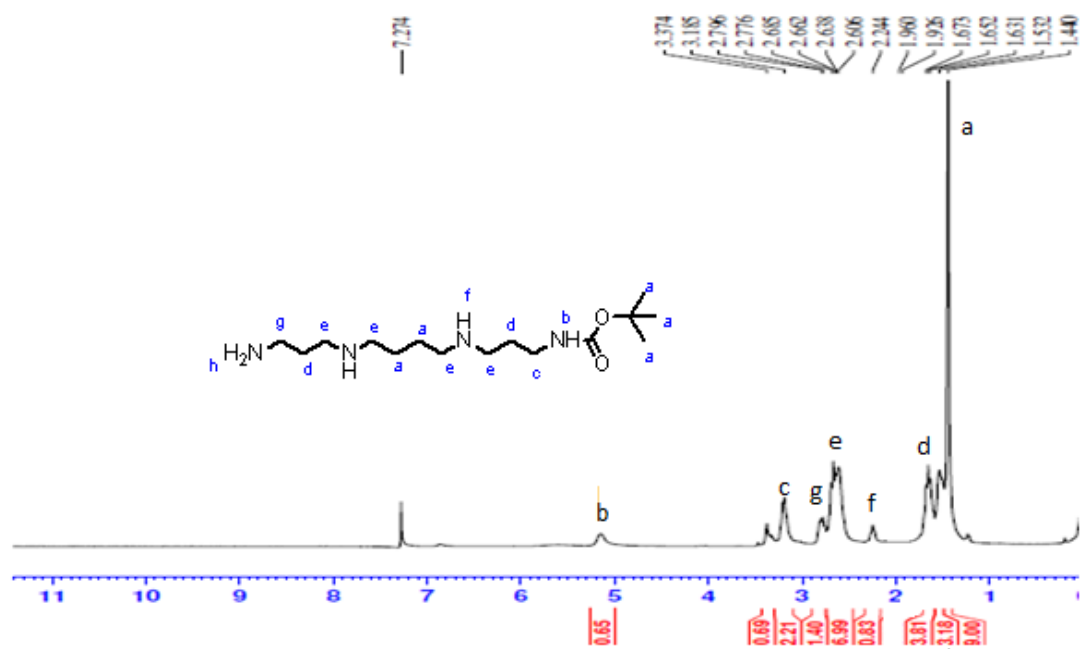


Figure S.13: ¹H NMR spectra (300 MHz, CDCl₃) of mono-N-Boc protected spermine. δ 1.44 (s, 9H, Boc CH₃), 1.53 (m, 4H, CH₂), 1.67 (m, 4H, CH₂), 2.24 (br s, 1H, CH₂-NH-CH₂), 2.64 (m, 8H, HN-CH₂-CH₂-), 2.8 (m, 2H, CH₂), 3.2 (m, 2H, HN-CH₂), 3.38 (br s, 1H, NH), 5.1 (br s, 1H, Boc NH).

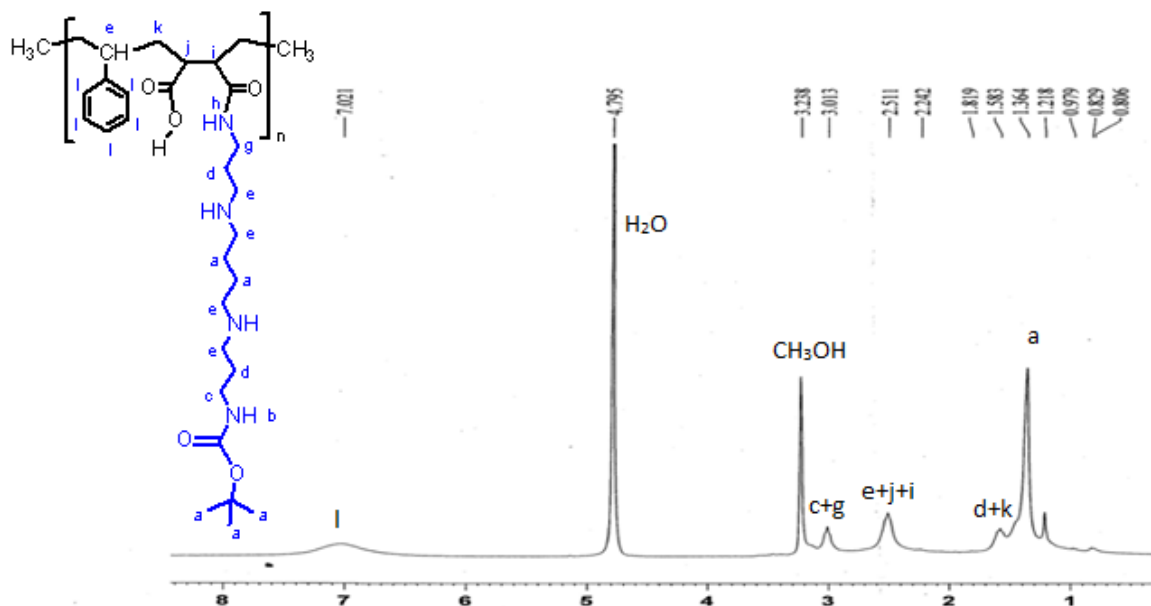


Figure S.14: ¹H NMR spectra (300 MHz, CD₃OD) of mono-N-Boc-protected spermine grafted PSMA. δ 1.2 (br m, aliphatic CH₃), 1.36 (br s, Boc CH₃), 1.6 (br m, aliphatic CH₂), 2.5 (br m, aliphatic CH + CH₂), 3 (br m, aliphatic CH₂), 7 (br m, Ar CH).

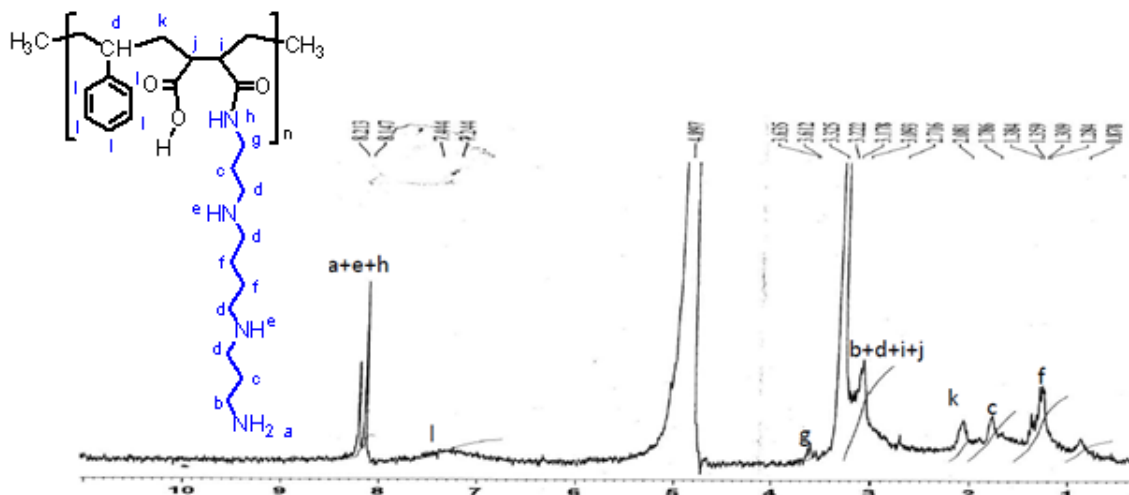


Figure S.15: ^1H NMR spectra (300 MHz, $\text{DMSO-}d_6$) of **P5**. δ 1.2 (br m, aliphatic $\text{CH}_2 + \text{CH}_3$), 1.8 (br m, aliphatic CH_2), 2.1 (br m, aliphatic CH_2), 3.1 (br, aliphatic $\text{CH} + \text{CH}_2$), 3.7 (br m, aliphatic CH_2), 7.4 (br m, Ar CH).

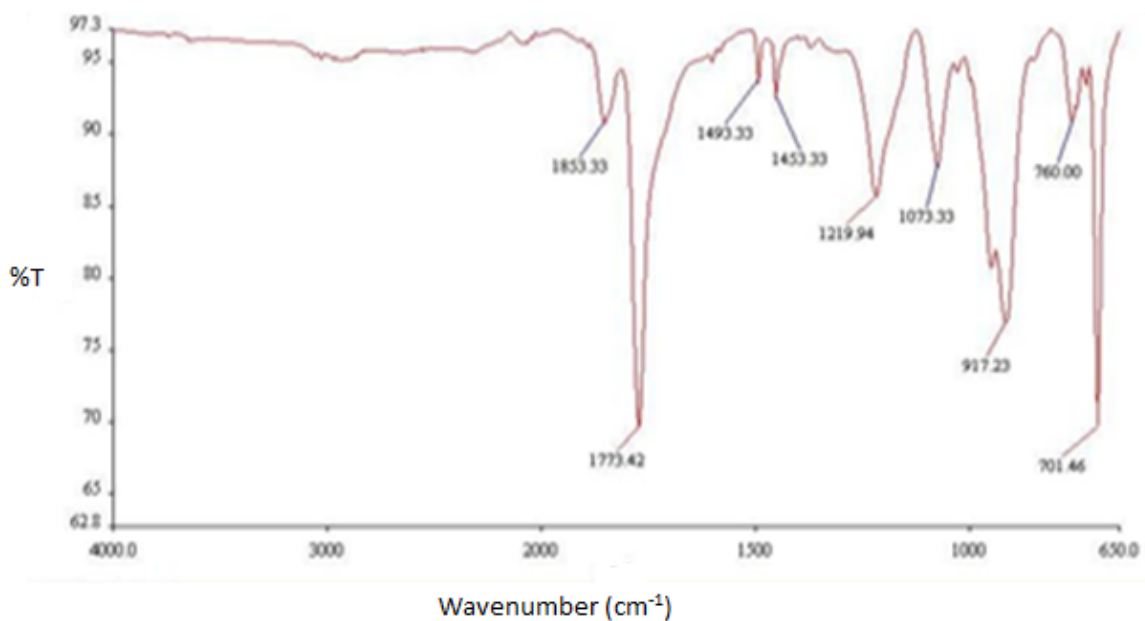


Figure S.16: ATR-FTIR spectra of PSMA.

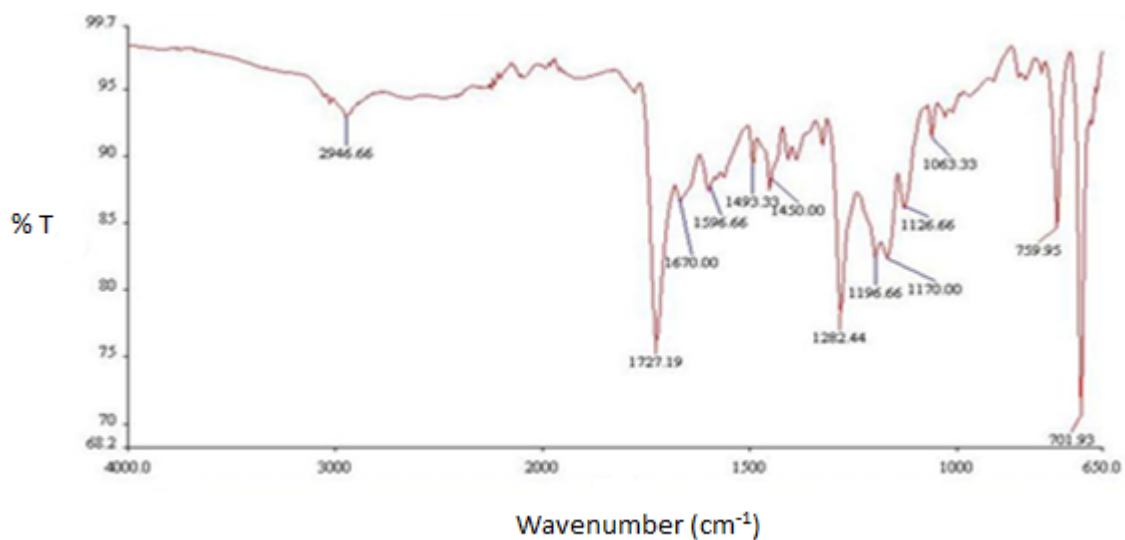


Figure S.17: ATR-FTIR spectra of P1.

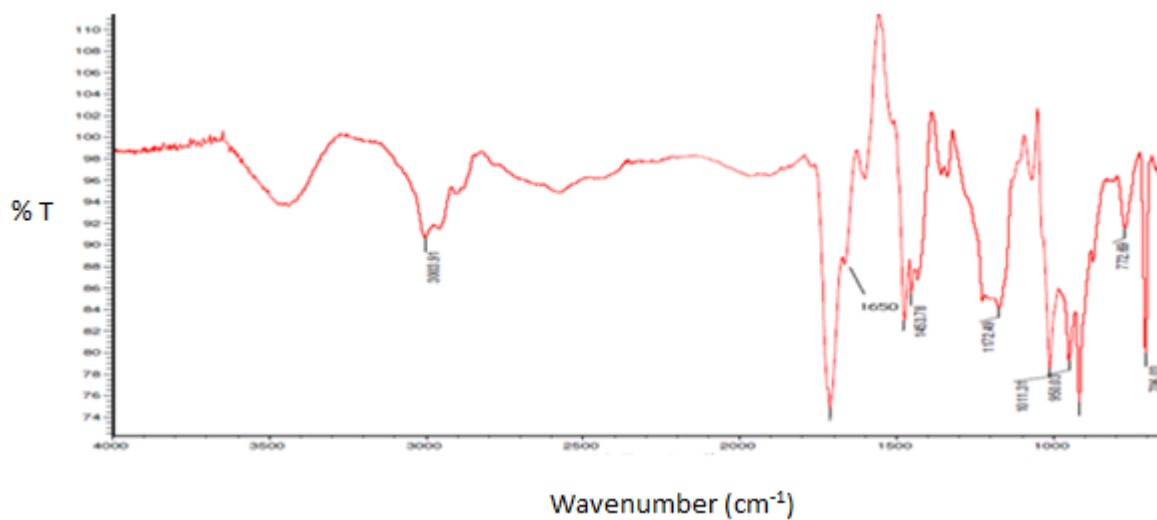


Figure S.18: ATR-FTIR spectra of P2.

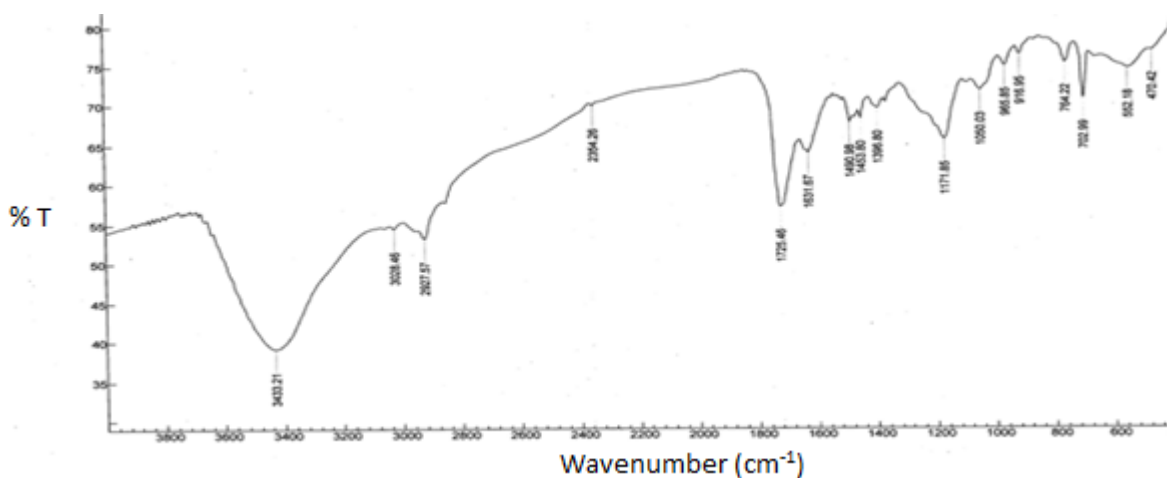


Figure S.19: ATR-FTIR spectra of P3.

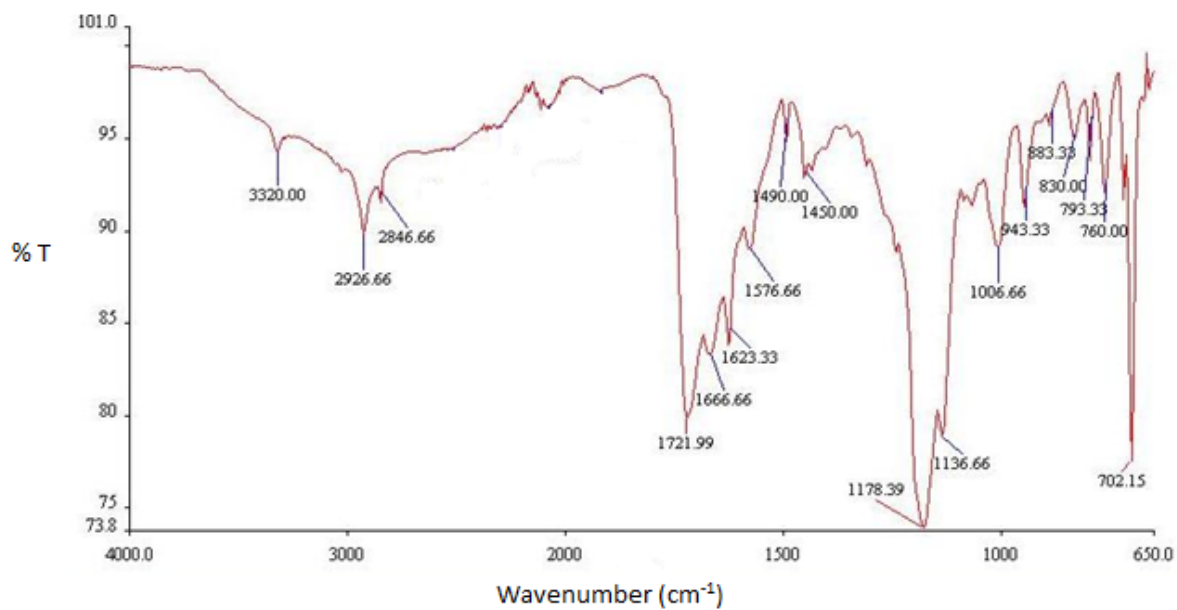


Figure S.20: ATR-FTIR spectra of P4.

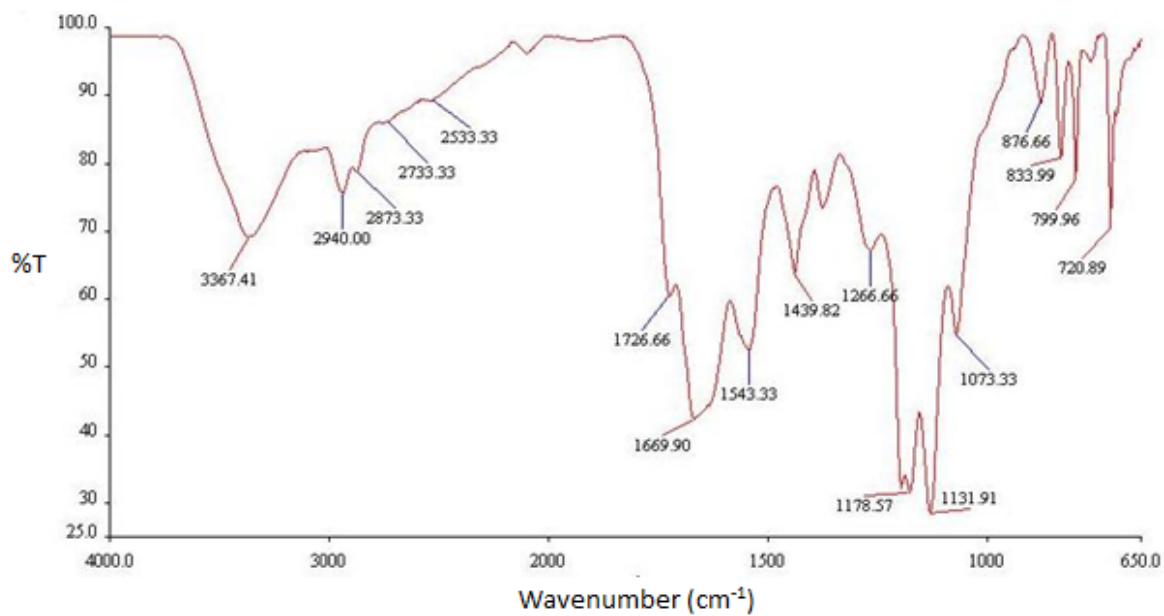


Figure S.21: ATR-FTIR spectra of P5.

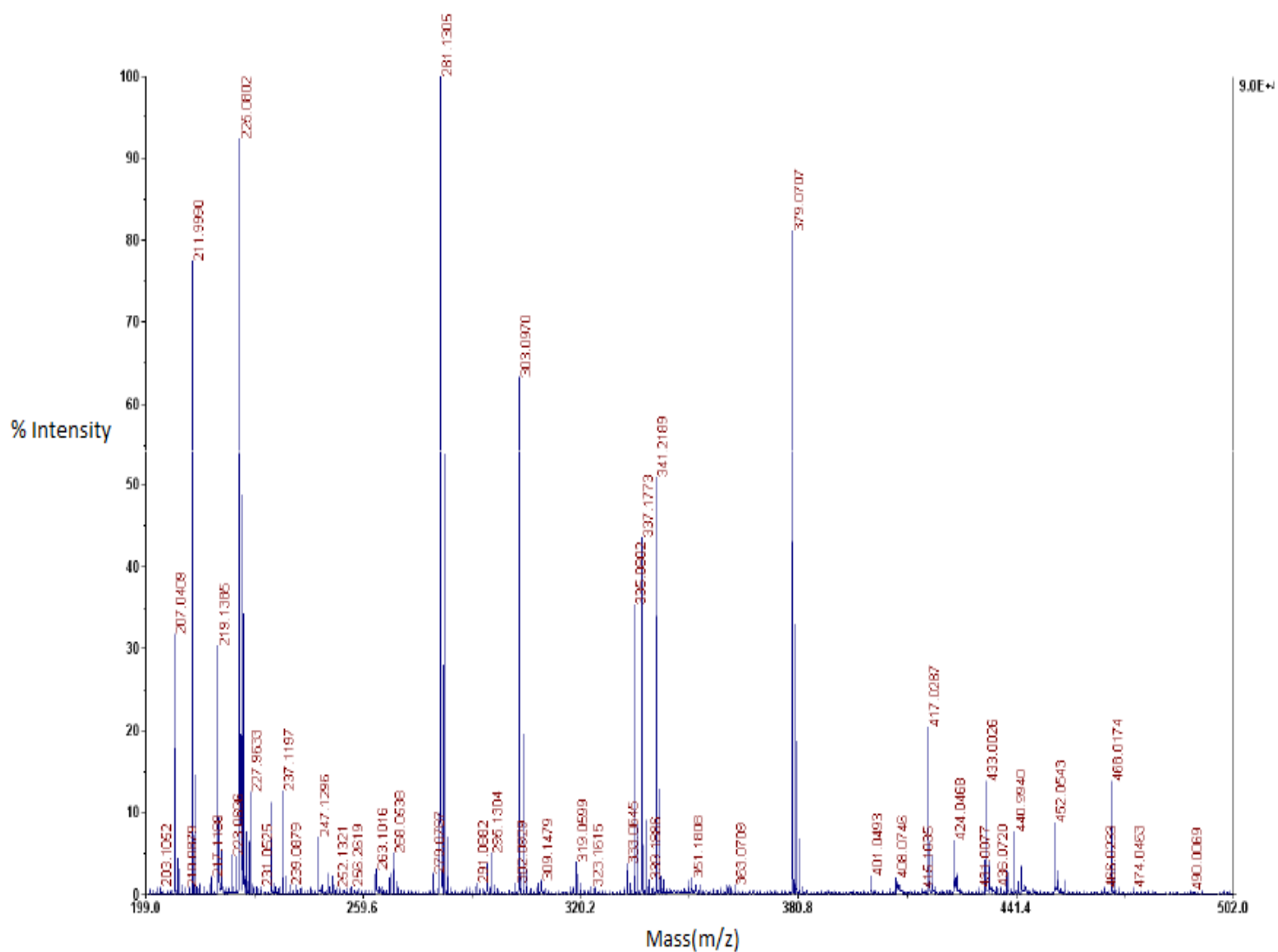


Figure S.22 : Mass spectra of 3-(tert-butoxycarbonylamino)propyl isonicotinate.

Mass spectra calcd for $C_{14}H_{21}N_2O_4$ (H^+), $m/z = 281.1501$, obtained $m/z = 281.1305$

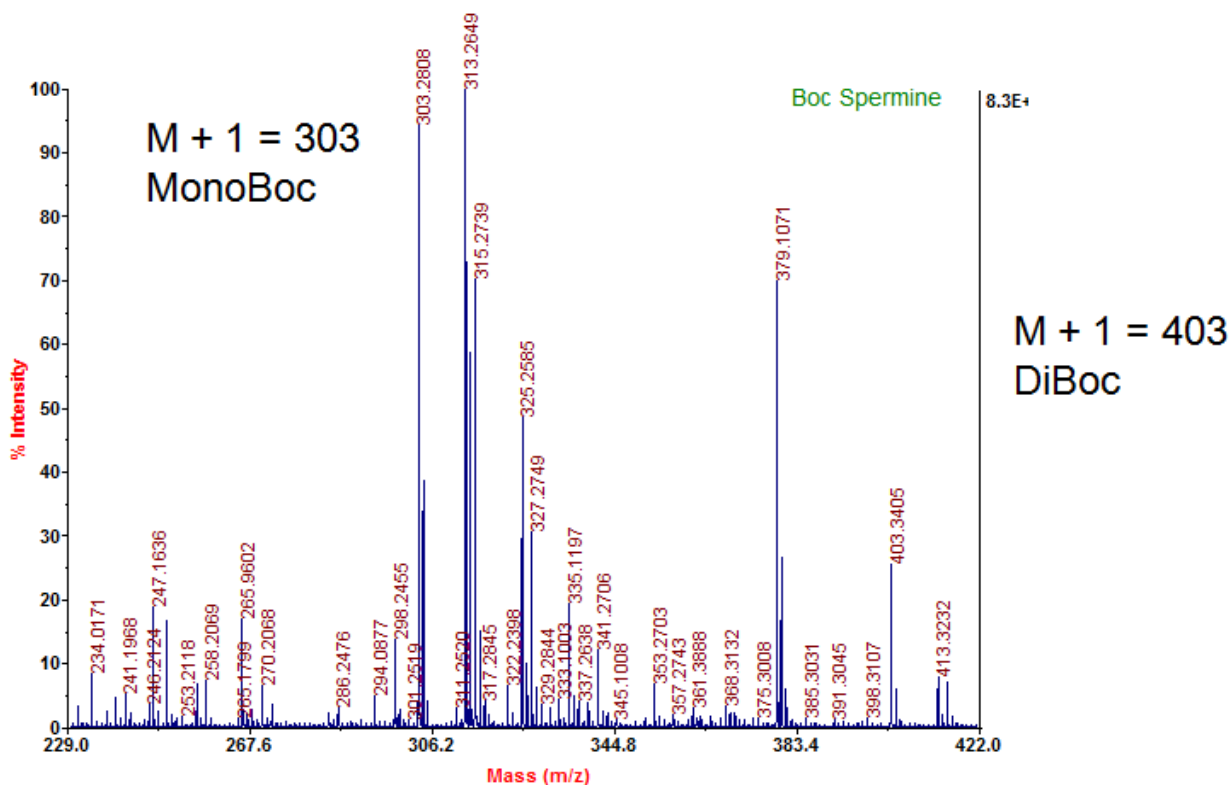
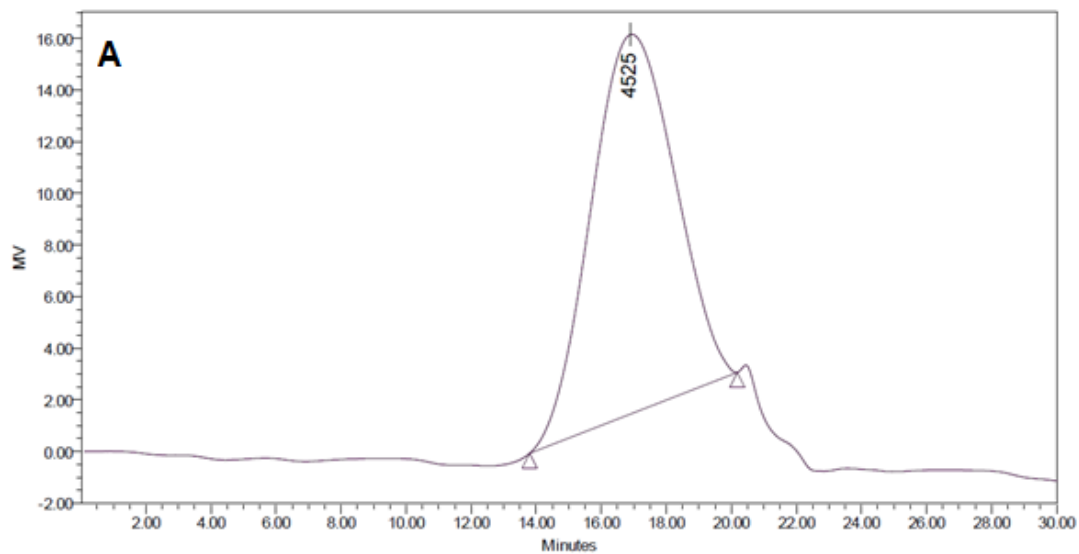


Figure S.23 : Mass spectra of Boc- protected spermine.

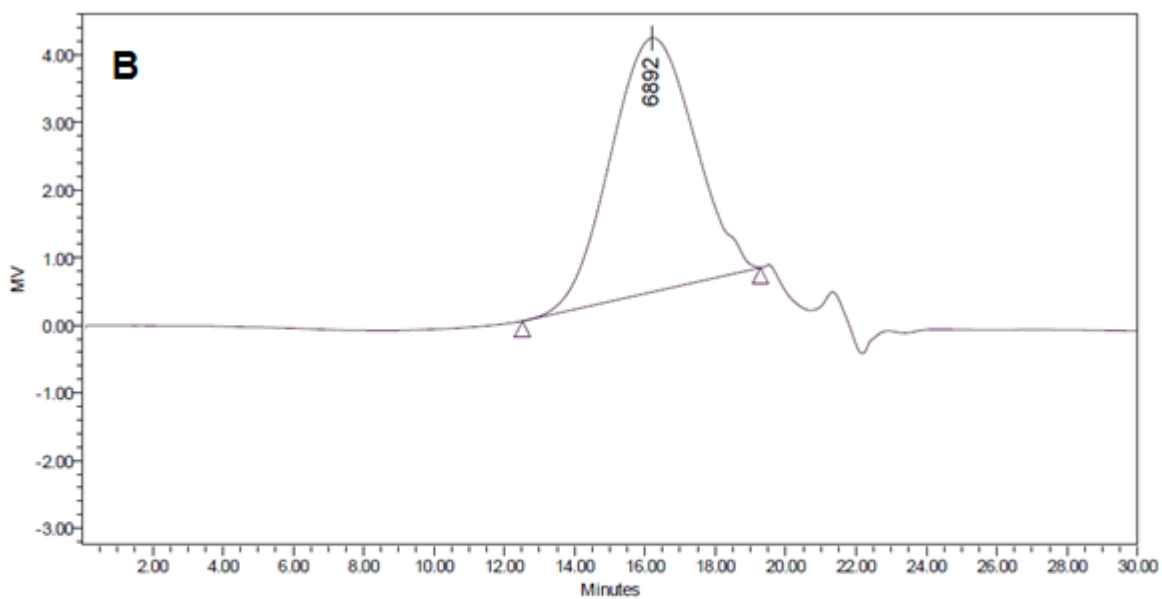
S.4 Determination of molecular weights and cationic amine content of polymeric derivatives (P1-P5)

Average molecular weights of the parent polymer (unmodified PSMA) and the polymeric derivatives were determined by gel permeation chromatography (Waters, USA, Styragel HR column) using Waters 2414 RI detector. Among the various polymers employed in the study, **P4** (L-arginine grafted PSMA) and **P5** (spermine grafted PSMA) were insoluble in THF and CHCl₃ and formed semi gel in DMF. Due to this practical difficulty, immediate precursors of **P4** (Methyltosyl and Boc protected arginine – PSMA conjugate) and **P5** (Mono Boc spermine – PSMA conjugate) were characterized using GPC. The parent polymer (PSMA) and all the quaternized derivatives (**P1-P3**) were used as such for GPC characterization.



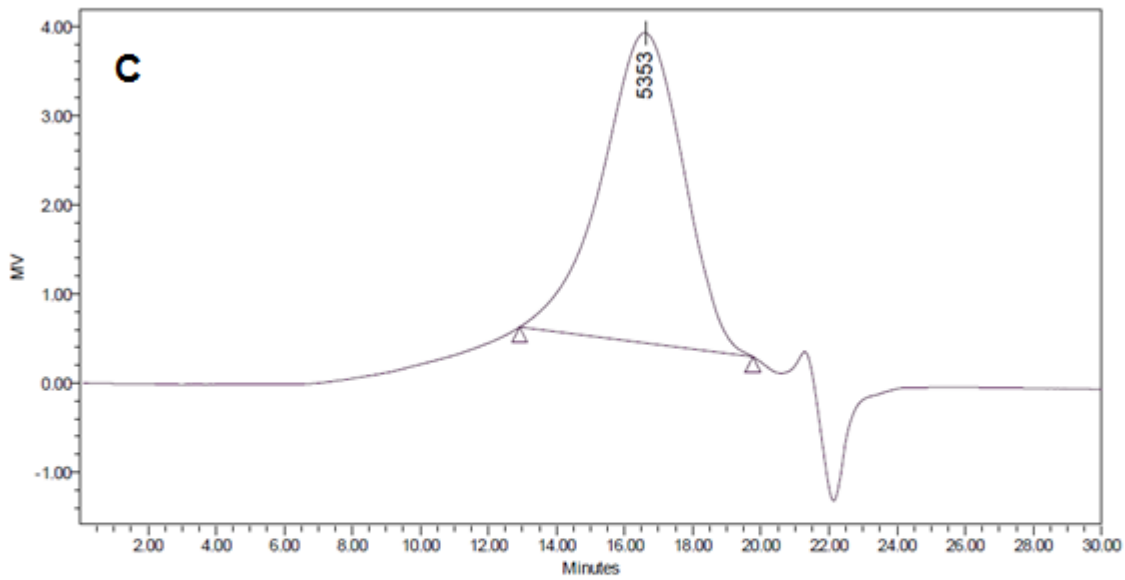
Broad Unknown Relative Peak Table

Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1	2896	5132	4525	7706	10222	1.771912	1.501625	1.992014



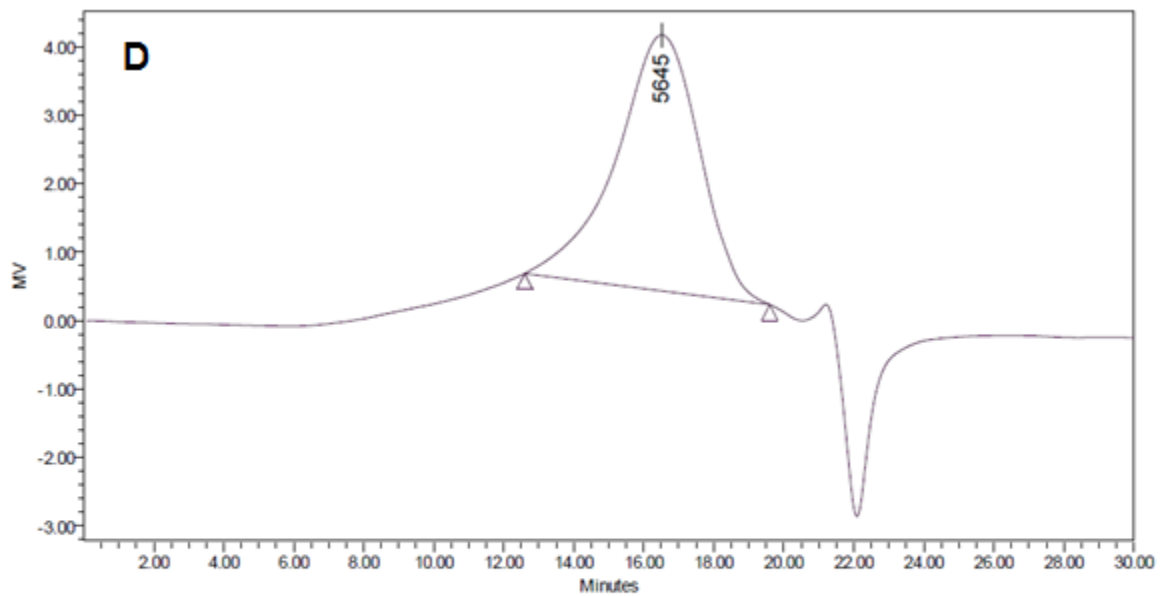
Broad Unknown Relative Peak Table

Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1	5242	8005	6892	11354	15101	1.527219	1.418283	1.886397



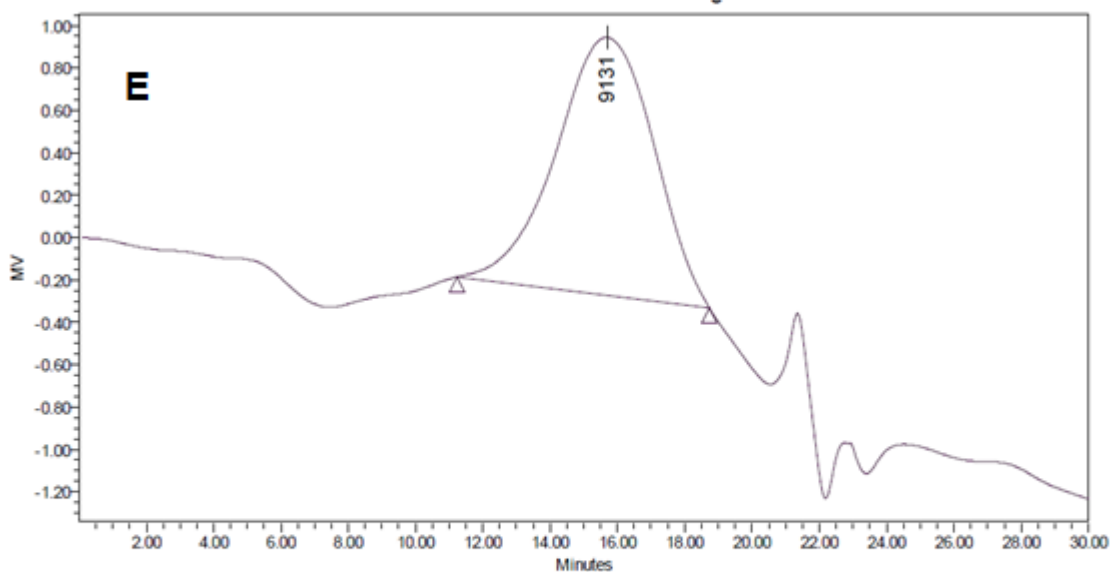
Broad Unknown Relative Peak Table

Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1	4368	7195	5353	11432	16758	1.647266	1.588862	2.329054



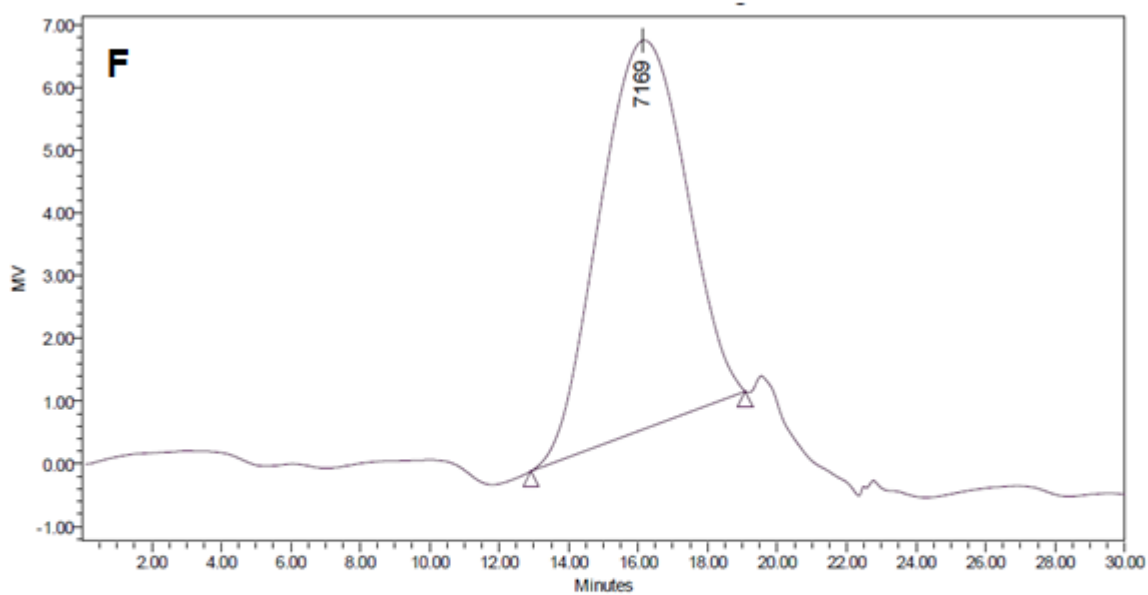
Broad Unknown Relative Peak Table

Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1	4846	8049	5645	13390	20504	1.661019	1.663607	2.547477



Broad Unknown Relative Peak Table

Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1	7313	13162	9131	24529	42546	1.799878	1.863607	3.232529



Broad Unknown Relative Peak Table

Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1	5553	8478	7169	12000	15680	1.526882	1.415453	1.849480

Figure S.24: GPC spectra of (A) unmodified PSMA (B) P1 (C) P2 (D) P3 (E) Methyl tosyl and Boc protected L-arginine grafted PSMA (F) Mono Boc spermine grafted PSMA.

Cationic amine content of various polymeric derivatives was determined as follows.

The parent polymer (unmodified PSMA) is composed of styrene and maleic anhydride in the ratio 2:1. Hence one monomer unit of parent polymer could be considered to have 2 styrene units and one maleic anhydride unit with a molar mass of 307 Da.

ie. Average no. of monomer units per parent polymer chain = M_n of parent polymer /307

$$= 2896/307$$

$$= 9.4 (\sim 9)$$

Now, \sim no. of grafting moieties per polymer chain for each polymeric graft could be determined from their ^1H NMR spectra. For this purpose, integration value of one distinguishable proton signal of grafting molecule was compared with the integration value of the aromatic protons (constant for all polymeric grafts) of styrene units.

Example below shows how the \sim no. of isonicotinic acid molecules per monomer unit was determined from the ^1H NMR of isonicotinic acid grafted PSMA.

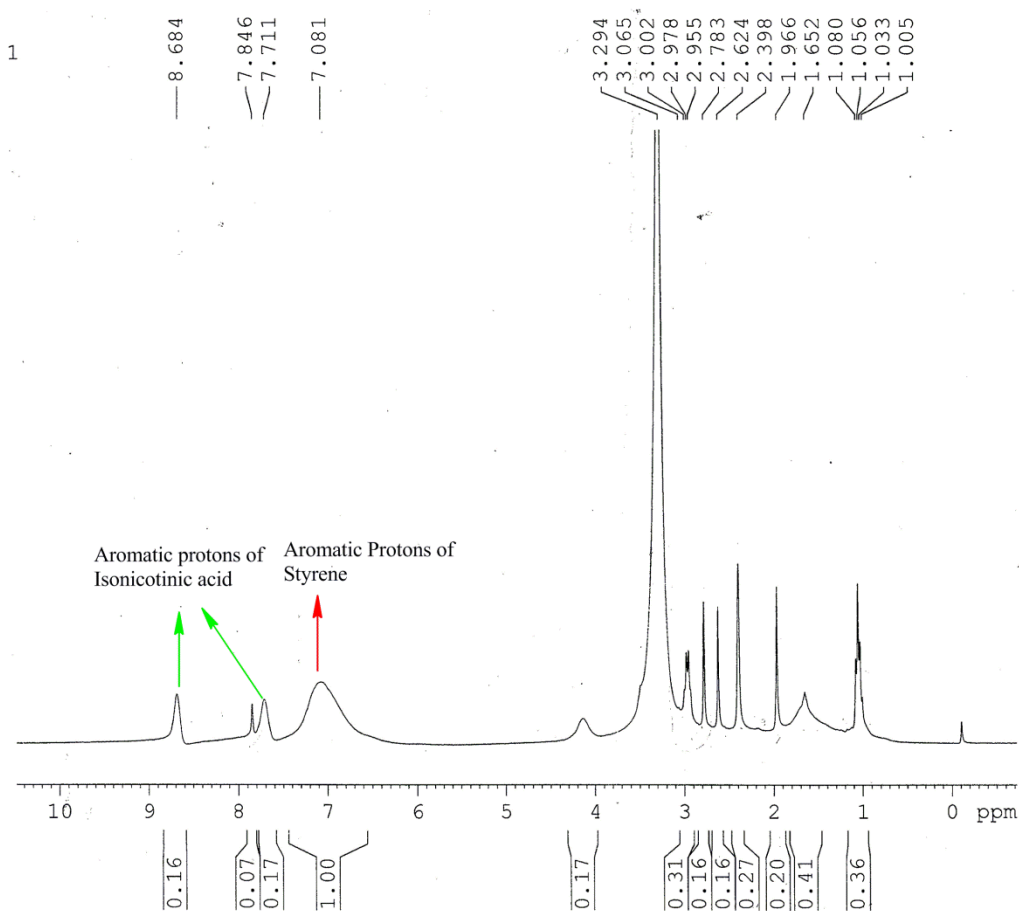


Figure. S.25: ^1H NMR spectra (300 MHz, DMSO- d_6) of isonicotinic acid grafted PSMA

As shown in figure S.25, the signals marked at δ 8.6 and δ 7.7 corresponds to symmetric aromatic hydrogens of grafted isonicotinic acid moiety.

Here integration of 1 at δ 7.2 corresponds to 10 protons of two styrene units. Hence no. of protons corresponding to the signal of isonicotinic acid (at δ 8.6) is $0.16 \times 10 = 1.6$.

If 100% grafting has occurred, the integration value of the aromatic proton signal of isonicotinic acid at δ 8.6 would have been 2.

From the integration values of NMR spectra, number of grafting moieties per monomer unit is $1.6/2 = 0.8$

Since one grafting moiety (isonicotinic acid) contains one cationic amine, ~ no. of cationic amines per polymer chain = ~ no. of monomer units of parent polymer chain \times ~ no. of grafting moieties per monomer unit

$$= 9 \times 0.8$$

$$= 7.2 (\sim 7)$$

Similarly cationic amine content/polymer chain was calculated for all polymeric grafts (Table S.1).

Table S.1: Cationic amine content of different polymeric grafts

Polymer	Average molecular weight , Mn, from GPC (Daltons)	~ No. of grafting moieties per monomer unit	~ No. cationic amines per polymer chain
Unmodified PSMA	2896	-	-
P1	5242	0.8	7
P2	4368	0.84	15
P3	4846	0.88	8
Immediate precursor of P4	7313	0.9	16
Immediate precursor of P5	5553	0.87	24

In case of quaternized grafting moieties, each nitrogen was considered as a cation. For L-arginine grafted PSMA, no. of cationic amines in one L – arginine molecule was taken as 2. This is due to the fact that, 3 nitrogens forming the guanidino group corresponds to one cation and the secondary amine of L-arginine constitutes second one. In case of spermine grafted PSMA, no. of cationic amines per spermine molecule was considered to be 3, due to the utilization of one primary amine in amide bond formation.

S.5 Transfection studies of the polyplexes of P1-P3

Transfection studies of the polyplexes of **P1-P3** were performed as explained in experimental part of main article (in vitro transfection studies). DsRed-Express-N1 plasmid vector (4.7 Kb) was purified using HiPurATM Plasmid DNA Midiprep Purification Spin Kit (MB509, HiMedia). The plasmid used was 2 ug per well of a 24-well plate.

Flow cytometry analysis of various polyplexes of **P1-P3** is quantitatively represented in figure S.26. Polyplexes of **P3** exhibited negligible mean fluorescence intensity at all weight ratios tested. In case of **P1** maximum fluorescence was observed at polymer/DNA weight ratio of 5 where as **P2** exhibited maximum efficiency at weight ratio of 10. Maximum mean fluorescence obtained with different polymeric derivatives w.r.t positive control (PEI, 25 KDa) is given in table S.2.

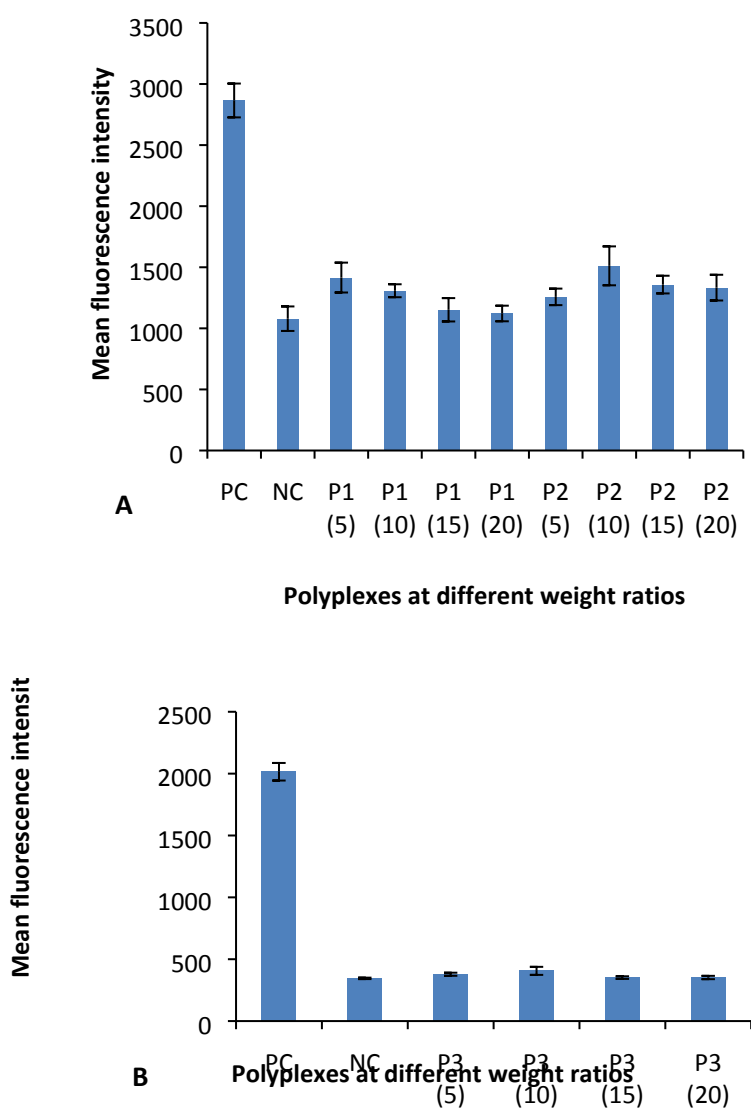


Figure S.26. Transfection efficiency of the polyplexes of (A) **P1, P2** and (B) **P3** at different weight ratios viz. 5,10,15 and 20 in MCF-7 cell line (Mean \pm SD, $n = 3$). NC – untransfected cells (negative control); PC – PEI, 25000 Da – DNA complex at optimal N/P ratio of 10 (positive control).

Table S.2 : Comparison of the transfection efficiency of various polyplexes

Polymer	Maximum transfection achieved w.r.t positive control (PEI, 25 KDa)	Polymer/DNA weight ratio
P1	0.49 fold	5
P2	0.53 fold	10
P3	0.2 fold	10
P4	0.96 fold	20
P5	1.45 fold	15