Supplementary Material (ESI) for Chemical Communications

Supporting Information for

Poly(β-aminosulfonamides) as Gene Delivery Vectors: Synthesis and in Vitro Screening

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I. Monomer synthesis

All amine monomers 1-24 were purchased from commercial suppliers and used without further purification.

For the synthesis of divinyl sulfonamide monomers A – E: To a 50 mL solution of 42 mmol β-chloroethanesulfonylchloride in dry dichloromethane, a 50mL solution of 20mmol of 2-methylpiperazine and 85mmol of triethylamine in dry dichloromethane was added dropwise at 0 ^{o}C under nitrogen flow. The reaction mixture was stirred for 2 hours at 0 ^{o}C and then overnight at room temperature. The solid formed was filtered, washed with water, brine and then water. The organic layer was dried over dry sodium sulfate and concentrated. A pure product, pale yellow solid or liquid, was obtained through column chromatography using a mixture of dichloromethane and ethyl acetate as eluent. Solid monomers were further purified by recrystallization from a mixture of diethyl ether and dichloromethane (Yield: 30 – 60 %). ^{1}H NMR spectra were recorded at 400 or 500 MHz (^{13}C spectra at 100 or 125 MHz) on Bruker DRX-400 or DRX-500 spectrometers, respectively.

A: ^{1}H NMR (500 MHz, CDCl3): δ 6.38 – 6.44 (two dd, overlapping, 2H), 6.27 (d, J= 16.5 Hz, 1H), 6.24 (d, J= 16.5 Hz, 1H),6.07 (d, J= 10.0 Hz, 1H), 6.00 (d, J= 10.0 Hz, 1H), 4.18 (m, 1H), 3.68 (d, J = 11.7 Hz, 1H), 3.59 (d, J = 11.7 Hz, 1H), 3.50 (d, J = 11.7 Hz, 1H), 3.25 (m, 1H), 2.89 (m, 1H), 2.71 (m, 1H), 1.35 (d, J=6.8 Hz, 3H). ^{13}C NMR (100 or 125 MHz, CDCl3): δ 135.6, 132.2, 129.5, 127.4, 122.3, 50.89, 50.86, 48.55, 48.50, 45.7, 44.7, 39.5, 14.9]. MS m/z 281.0.

B: ^{1}H NMR (400 MHz, CDCl3): δ 6.42 (dd, 2H), 6.20 (d, J= 16.7 Hz, 2H), 6.02 (d, J= 9.9 Hz, 2H), 3.70 (d, J= 12.0 Hz, 4H), 2.55 (t, J = 11.5 Hz, 4H), 1.75 (d, J=9.8 Hz, 4H), 1.21-1.27 (m, 12H). ^{13}C NMR (100 or 125 MHz, $CDCl_3$): δ 132.8, 128.2, 46.1, 36.3, 35.4, 31.8, 23.7. MS m/z 391.1.

C: ^{1}H NMR (400 MHz, CDCl3): δ 6.42 (dd, 2H), 6.15 (d, J= 16.5 Hz, 2H), 5.95 (d, J= 9.8 Hz, 2H), 3.22 (s, 4H), 2.77 (s, 6H). ^{13}C NMR (100 or 125 MHz, CDCl3): δ 132.9, 127.9, 48.1, 34.9. MS m/z 269.0.

D: ^{1}H NMR (500 MHz, CDCl3): δ 6.46 – 6.55 (two dd, overlapping, 2H), 6.26 (d, J= 16.4 Hz, 1H), 6.25 (d, J= 16.4 Hz, 1H),5.98 (d, J= 10.1 Hz, 1H), 5.92 (d, J= 10.1 Hz, 1H), 4.99 (m, 1H), 4.30 (m, 1H),

4.21 (q, J = 7.4 Hz, 2H), 3.88 (m, 1H), 3.00 (q, J = 6.6 Hz, 2H),1.85 (m, 1H),1.43-1.71 (m, 5H), 1.29 (t, J=7.4 Hz, 3H). MS m/z 355.0.

E: ^{1}H NMR (400 MHz, CDCl3): δ 6.42 (dd, 2H), 6.22 (d, J= 16.7 Hz, 2H), 5.98 (d, J= 9.9 Hz, 2H), 3.07 (t, J=3.5 Hz, 4H), 2.77 (s, 6H), 1.58 (m, 6H), 1.38 (m, 4H). ^{13}C NMR (100 or 125 MHz, CDCl3): δ 132.8, 128.2, 46.1, 36.3, 35.4, 31.8, 23.7. MS m/z 325.0.

F: ^{1}H NMR (400 MHz, CDCl3): δ 6.58 (dd, 2H), 6.23 (d, J= 16.7 Hz, 2H), 5.96 (d, J= 9.9 Hz, 2H), 5.30 (s, 2H), 3.63 (m, 8H, overlapping), 3.19 (t, J= 6 Hz, 4H). MS m/z 663.4.

G: ^{1}H NMR (400 MHz, CDCl3): δ 6.43 (dd, 2H), 6.26 (d, J= 16.7 Hz, 2H), 6.11 (d, J= 9.9 Hz, 2H), 3.28 (s, 8H). ^{13}C NMR (100 or 125 MHz, CDCl3): δ 132.1, 129.7, 45.4, 45.3. MS m/z 267.0.

II. Polymer synthesis

Several model reactions were carried out to optimize the reactions conditions. Steric hindrance of both amine monomers and divinylsulfonamides plays an important role in the Michael reaction. Chloroform was chosen as the solvent because of its good solubility for both monomers and resulting polymers. Isopropanol accelerated the reaction by helping with proton transfer. No adduct product of isopropanol with divinylsulfonamides could be detected.

Typical polymerization conditions were carried out for both bis(secondary amines) and mono(primary amine) monomers: 2 mmol of both amine monomer and divinylsulfonamide were weighed into vials equipped with Teflon coated stir bars. Next, 4.0 mmol of $LiClO_4$, then 1 mL anhydrous chloroform and 1 mL IPA were added. After capping with Teflon lined screw caps, the vials were kept at 75 ^{o}C in the dark for one week.

For bis(primary amines), gelation is observed due to crosslinking, affording insoluble solids under the above conditions. Thus, the reaction was carried out at room temperature for 3 days. No gelation was observed even after storage for two months.

When polymerization was complete, the polymers were precipitated in diethyl ether. The precipitates were purified by repeatedly dissolving in DMSO, DMF, or chloroform and precipitating in diethyl ether or THF. Finally, the polymers were dried under vacuum at 60^{o}C for 2 days.

III. Polymer characterization

1. ^{1}H and ^{13}C NMR spectra

^{1}H NMR spectra were recorded at 400 or 500 MHz on a Bruker DRX-400 spectrometer. ^{13}C spectra were measured at 100 or 125 MHz on a DRX-500 spectrometer. Spectra for polymer E24 are illustrated in Figures S1 and S2.

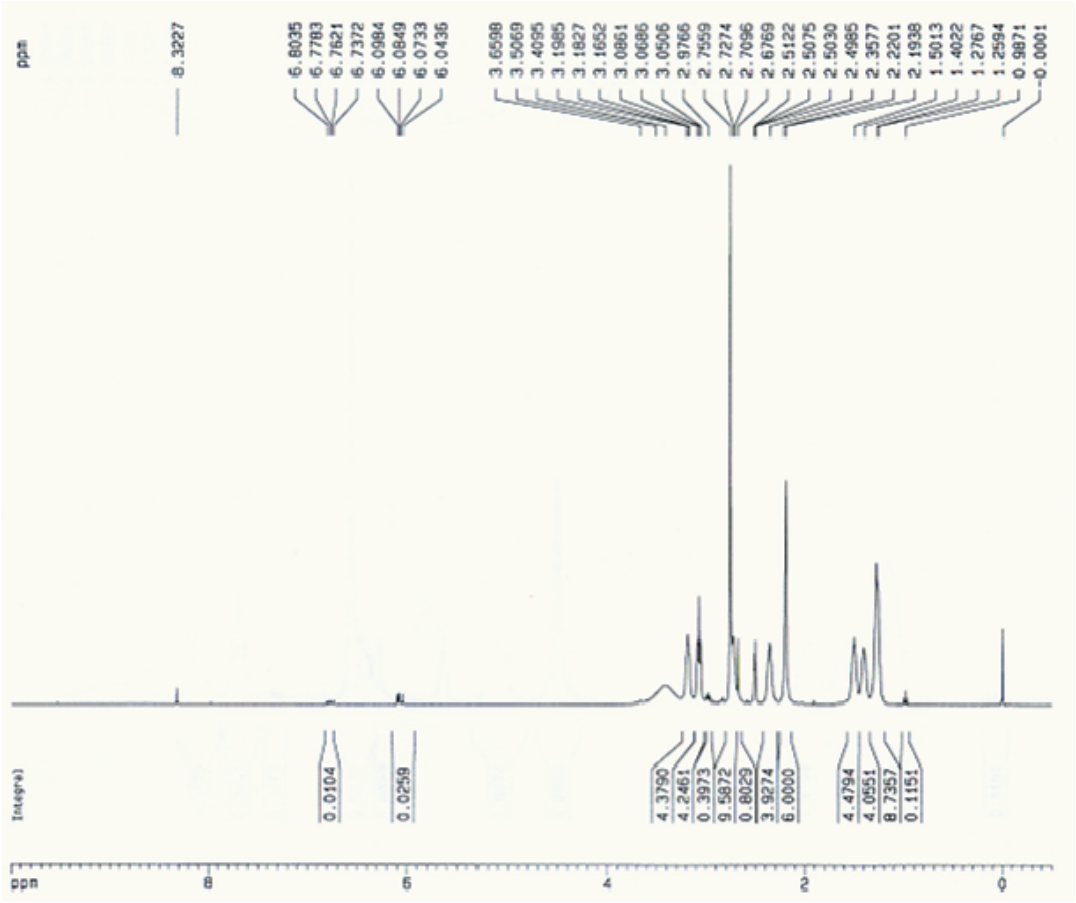

Figure S1 ^{1}H NMR spectrum of representative polymer E24

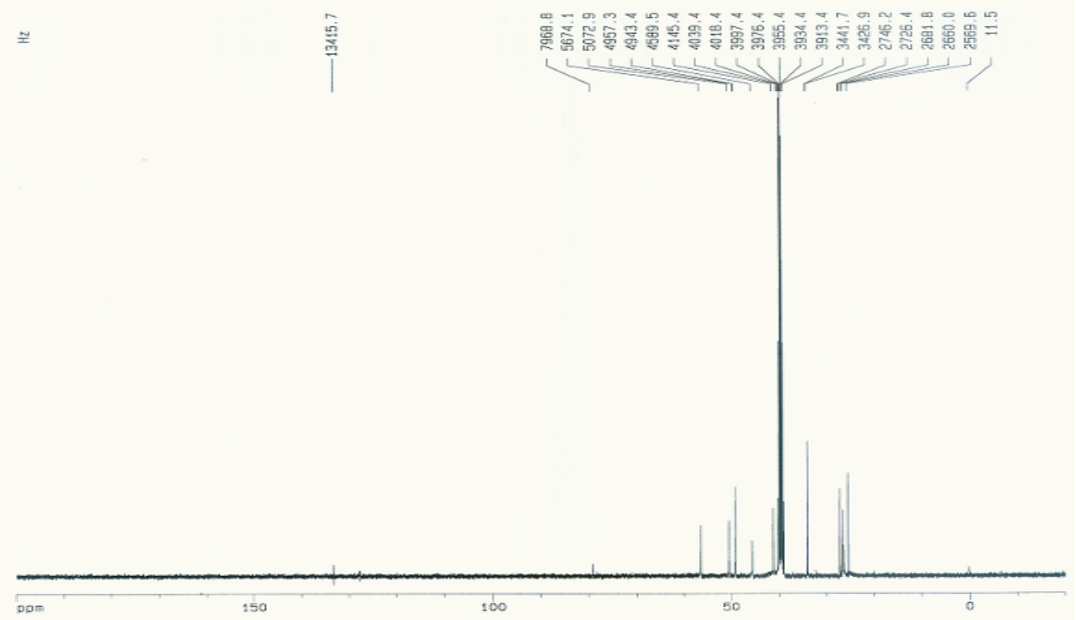

Figure S2 ^{13}C NMR spectrum of representative polymer E24

2. Molecular weights determination by MALDI-MS.

MALDI-TOF MS was used to determine the molecular weights and polydispersity of the resulting polymers [1]. Shown in Figures S3 and S4 are a typical MALDI spectrum and an expansion of the apex of its polydispersity curve. From the expansion, the end groups could be determined. In this case, vinyl-vinyl, vinyl-amine or amine-amine end groups could be detected.

To prepare the samples, dried droplet method was used. Polymers were dissolved in DMSO to a final concentration of 5 mg/mL. Sinapic acid, dithranol, α-cyano-hydroxy-cinnamic acid, indole acrylic acid, and 2,5-DHB were tested as matrices; 2,5-DHB gave the best spectra and was used at 15 mg/mL in methanol. 0.5 microliters of a 1:1 mixture of analyte and matrix was pipetted onto a roughened 2.5 mm diameter sample position on a stainless steel plate and dried under vacuum.

Mass spectral data were collected in a linear mode on Applied Biosystems Voyager System 6187. The ions were generated using the 337 nm laser beam from a nitrogen laser, having a pulse width of 3 ns. No correction of 1/ (dm/dt) [1] was applied to the mass spectra during the conversion of the time domain to the mass domain.

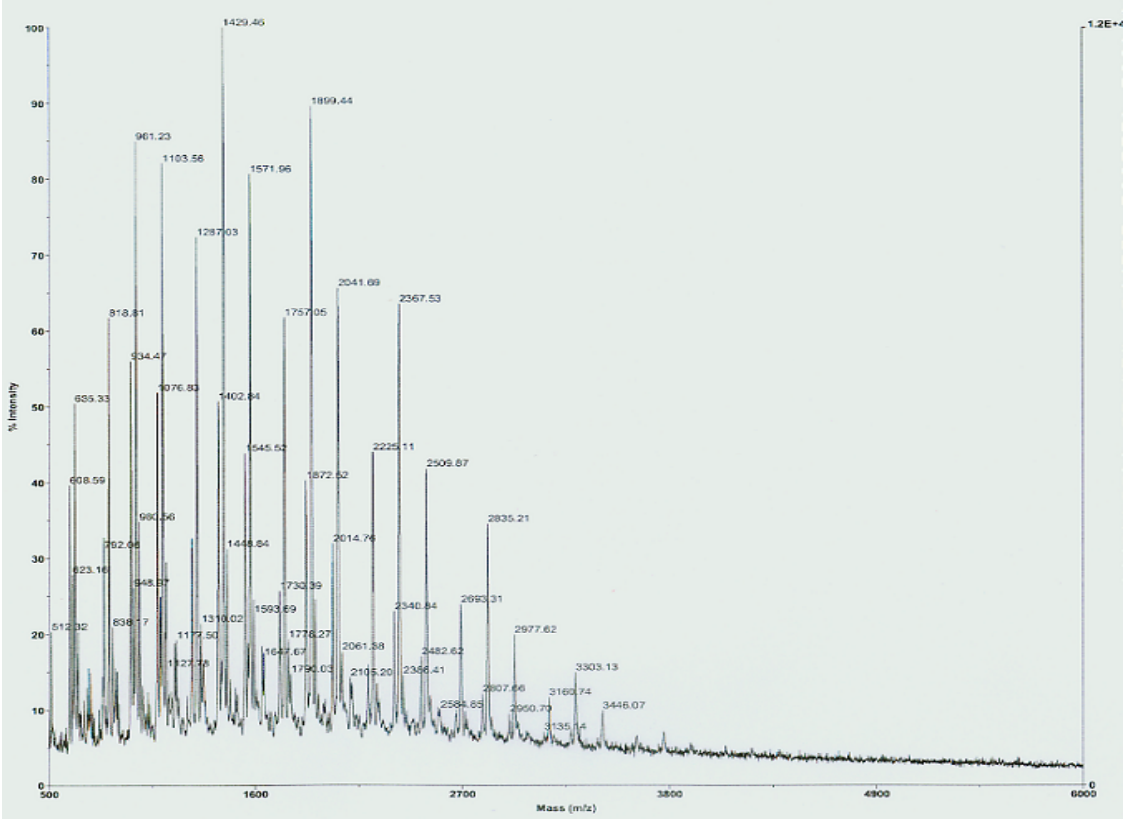

Figure S3 MALDI MS spectrum of representative polymer E24

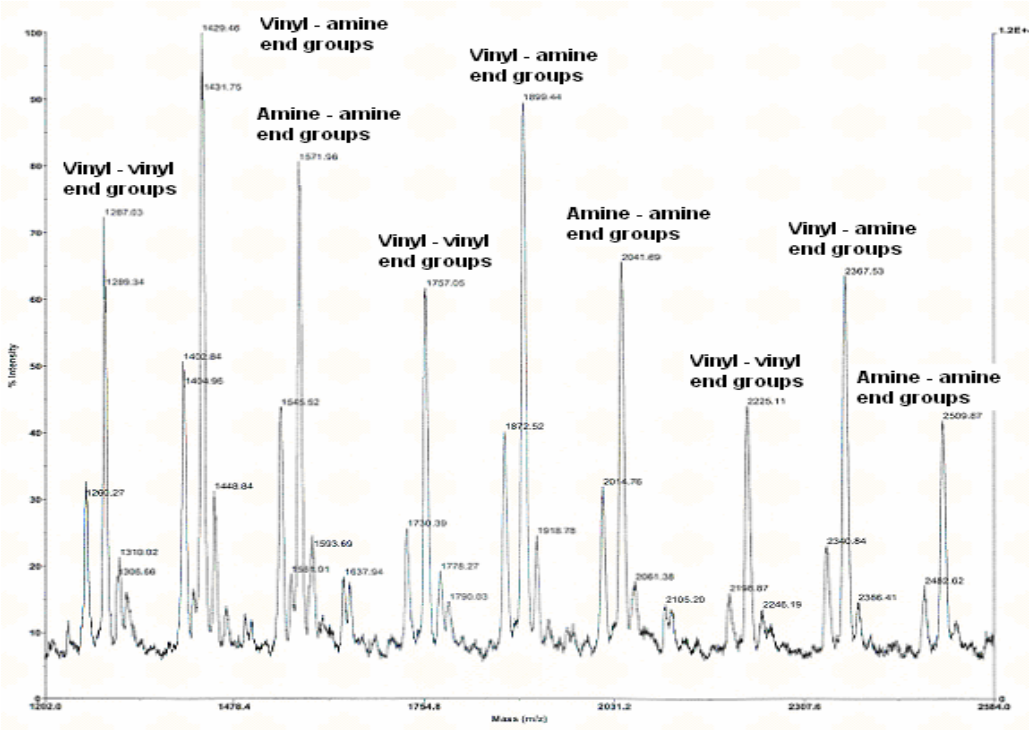

Figure S4 MALDI MS spectrum (expansion) of representative polymer E24

3. Solubility

Polymer solubility was tested in pH 7 double processed cell culture water (Sigma), 25mM pH5 sodium acetate buffer, and DMSO (Hybri-Max, Sigma). There was no difference between the solubility profiles of water and buffer. Water was used for suspension when possible; DMSO was used when necessary (see Table S1).

4. Acid-Base Titration

Polymers and NaCl were dissolved in water and adjusted to pH 2 with 0.1 N HCl solution followed by titration with 0.1 N NaOH to pH 12. The pH value was monitored with Accumet Basic AB15/15+. The results are summarized in Table S1. Insouble compounds are represented by "—". Compounds soluble only in DMSO were not tested.

Polymer	MW	pH buffering range
A6	3900	4.2-7.7
A7	3100	4.9-6.9
A10	5100	--
A11	4900	--
A12	4900	(DMSO)
B6	2000	4.7-7.2
B8	3700	--
B9	5700	(DMSO)
B10	5000	5.3-6.8
B11	4100	--
B12	2000	5.4-6.9
B17	2100	(DMSO)
B18	5200	(DMSO)
B19	5500	(DMSO)
B21	3300	6.7-7.5
B22	4000	(DMSO)
C2	6800	5.0-5.7
C3	3900	5.3-6.0
C4	7500	5.9-6.7
C6	**1600**	**4.5-7.2**
C7	3900	5.0-6.7
C8	4500	5.4-6.7
C9	4000	4.7-6.8
C10	3400	5.0-6.9
C11	3100	5.1-6.5
C15	1200	5.0-6.7
C16	5500	--
C17	4700	(DMSO)
C18	**5200**	**7.0-8.1**
C19	5500	6.9-7.8
C21	2900	6.8-7.7
C22	3000	7.1-8.2
D2	3600	(DMSO)
D3	5700	5.2-5.8
D11	4800	5.2-6.7
D12	5000	(DMSO)
D16	5300	(DMSO)
E1	7100	--
E2	8300	5.1-5.9
E3	4500	5.1-5.5
E6	2200	4.1-7.4
E8	2400	5.0-7.1
E9	4600	5.2-7.2
E13	4000	6.0-6.7
E14	7000	--
E17	1900	6.9-7.9
E18	4550	6.6-8.0
E19	3300	6.8-7.9
E20	4100	(DMSO)
E21	**1700**	**6.8-8.1**
E23	3300	(DMSO)
E24	**1600**	**5.0-5.6**
F2	--	--
G4	3000	6.0-6.7
G13	1000	6.2-6.8

Table S1 Molecular weights and pH buffer range of PBASs

IV. Characterization of the polyplexes

1. Gel retardation Assay

Stock solutions of polymers were solubilized at 6.3mM in water or DMSO. Stock solutions were diluted to 1.3mM with double processed cell culture water. The two ratios of pDNA:polymer were created by adding 3 or 30uL of the polymer solution to 100uL of 0.02uL pEFX-AP and adjusting the final volume to 60uL. Plasmid pEFX-AP is described elsewhere [2]. 5uL of colorless loading buffer (30% glycerol in 100mM Tris-HCl pH 8, 100mM EDTA) was added before running 20ul on a 1% agarose gel for 30 minutes at 90V. A composite of gel retardation experiments for all soluble PBAS polymers is shown in Figure S5.

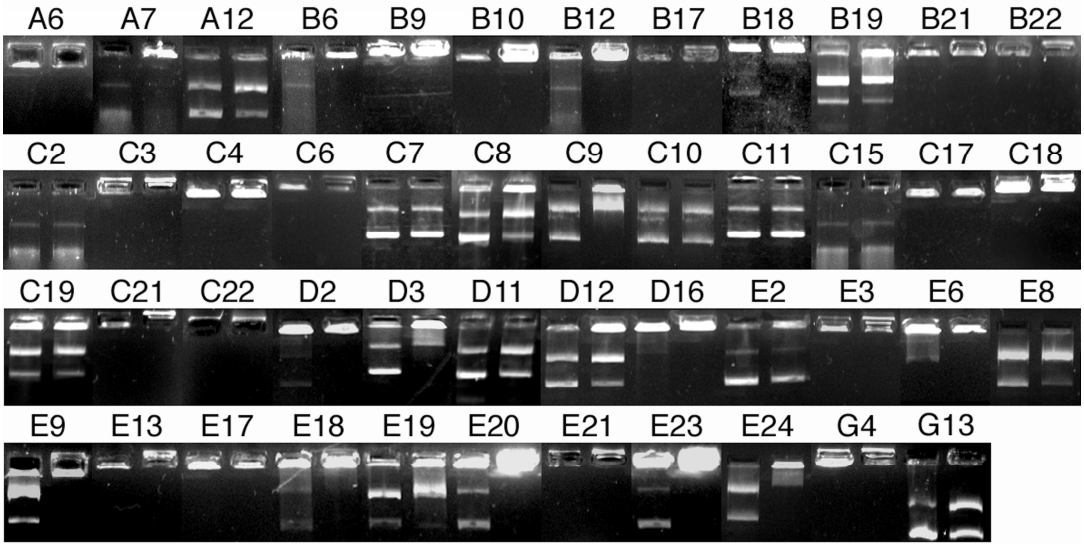

Figure S5 Composite of gel retardation assays for all soluble PBAS

2. Atomic Force Microscopy

For the E24 polyplex, equal volumes of 100ug/mL pEFX-AP pDNA and 1mg/ml E24 were combined. The pDNA control was prepared at 50ug/mL; the E24 control, at 0.5mg/mL. 5uL of each solution was dropped on freshly cleaved mica and dried with pressurized air. Images were acquired with a Digital Instruments Nanoscope IIIa AFM in tapping mode at a scan rate of 2Hz with a standard silicon probe. Height images were flattened.

V. In vitro assays

1. Transfection assay

COS-7 cells were seeded at 12,000 to 16,000 cells/well in 96-well plates in growth media (90% phenol red-free DMEM/F12 (Gibco, Invitrogen), 10%FBS (Gemini Biologicals), 100units/ml penicillin/streptomycin(Gemini Biologicals)) one day before testing. For transfections, the medium was replaced with 100ul Opti-MEM (Gibco, Invitrogen). Polymer solutions were made by diluting the 6.3mM stock solutions to 1.6mM with double processed tissue culture water. Polyplexes were prepared by adding 5ul, 25ul, or 100ul of polymer solution to 100ul of 12ng/ul pRSV-Luc (plasmid generously provided by Dr. M. Domowicz, Univ. Chicago) and adjusting the final volume to 200ul. After a 30-minute room temperature incubation, 50ul of polyplex was added to each well. Experiments were performed in triplicate. After a 4-hour incubation at 37°C 5%CO_2, transfection media was exchanged for 200ul growth media and the cells were incubated for 72 hours. Luciferase activity was detected with the Tropix luciferase assay kit (Applied Biosystems) and quantified with a Victor 3 multilabel counter (Perkin-Elmer) bioluminescence plate reader.

We determined the optimal pDNA:polymer ratio for each polymer in scout transfection experiments (5ul for A7, C22, E3; 25ul for B12, C2, C21, E6, E8, E9, E21; 100ul for A6, B6, C3, C6, C15, C18, E24, G4, G13). Polymers were then assayed in parallel to determine the best transfectants and the data on the leading compounds from this experiment are presented in Figure 3.

2. Cell toxicity assay

The COS-7 cells were prepared as described above. Transfection media was exchanged for 100ul growth media. 10ul 5mg/ml MTT (Sigma) was added to each well. After a 2-hour incubation, we changed the media to 100ul of Opti-MEM (Gibco, Invitrogen) and added 100ul of the polyplex solution. After an incubation of 4 hours at 37°C, 100ul of 10%SDS 0.01N HCl was added and the plates were left overnight at 37°C 5% CO_2. Colorometric data was obtained at 495nm with a 10 second reading. Data reported as a percent based on the untreated control cells having 100% viability. Findings on the 47 soluble PBAS are illustrated in Figure S6. E23 is blank because this polymer interacts with the MTT.

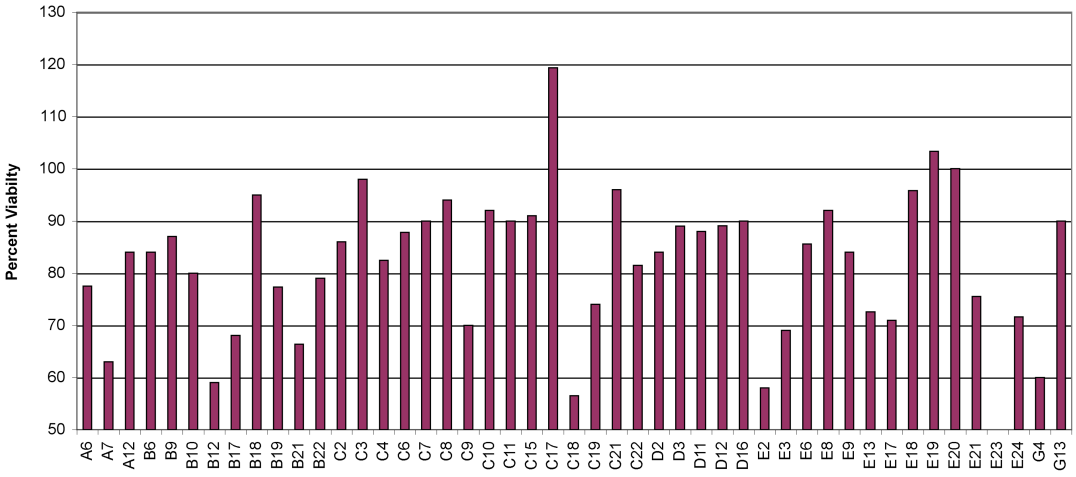

Figure S6 Viability after PBAS transfection

3. Fluorescence reporter transfections

COS-7 cells were seeded at 60,000 cells/well in 24 well plates. Transfection was carried out as described above with 2ug pEFX-3xVenus per well and 167ul of 1.6mM E24. The MP OmniPORTER control transfection followed the protocol specified in the product literature.

VI. References

[1] Hanton, S. D., *Chem. Rev*. **2001**, 101, 527-569.

[2] Agarwala, S., Sanders, T.A. and Ragsdale, C.W. (2001) *Science*, **291**, 2147-2150.