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SUPPLEMENTARY INFORMATION

PROPERTIES OF POLYPLEXES FORMED BETWEEN A CATIONIC POLYMER DERIVED FROM L-ARABINITOL AND NUCLEIC ACIDS

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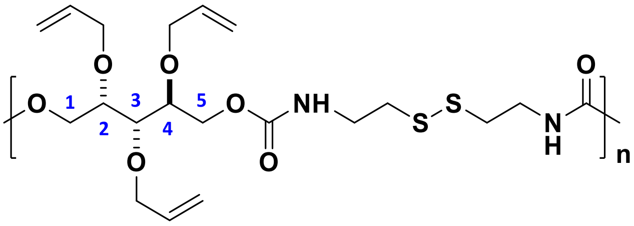
Synthesis of Polyurethanes

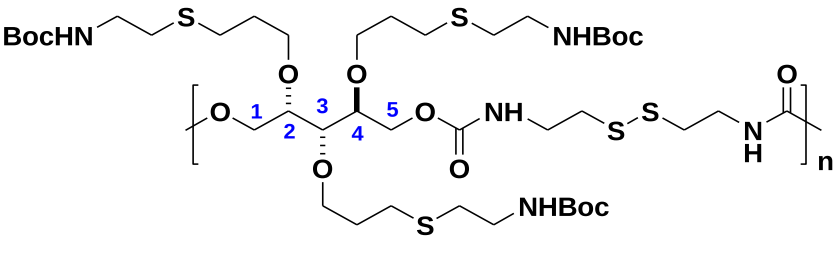
[(AAl)DTDI]



A round-bottom flask was loaded with 2,3,4-di-*O*-allyl-L-arabinitol (AAl) (1.09 g, 4 mmol) and the system was treated with three cycles of vacuum-argon before the addition of dry THF (2.5 mL). The mixture was stirred to get a solution and then dithiodiethyldiisocyanate (DTDI) (0.82 g, 4 mmol) was added under inert atmosphere, followed by one drop of the catalyst [dibutyltin (II) dilaurate]. The stirring was maintained during 24 h at 40 °C. Finally, the reaction mixture was treated with *tert*-butyl alcohol (1.2 ml) for 30 minutes and added dropwise into cold diethyl ether (200 ml). The precipitated polymer was filtered, washed with diethyl ether and dried under vacuum at 40 °C for 24 h, to afford the title compound (1.6 g, 85%).

IR: δ_{max} (cm^{-1}) 3329 (N-H), 1697 (C=O), 1522 (N-H), 1249 (N-CO-O).
^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 2.72-2.85 (m, 4H, CH_2S), 3.22-3.34 (m, 4H, CH_2NH), 3.51-3.60 (m, 1H, H-3), 3.61-3.78 (m, 2H, H-2, H-4), 3.89-4.21 (m, 8H, H-1$_a$, H-5$_a$, OCH_2-CH=CH$_2$), 4.30-4.38 (m, 2H, H-1$_b$, H-5$_b$), 5.07-5.30 (m, 6H, CH=CH_2), 5.79-5.99 (m, 3H, CH=CH$_2$), 7.37 (s, 2H, NH).
^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 37.7 (CH_2S), 39.9 (CH_2NH), 62.8, 63.5 (C-1, C-5), 70.3, 71.5, 72.8 (OCH_2-CH=CH$_2$), 76.7, 77.8 (C-2, C-3, C-4), 116.3, 116.6 (CH=CH_2), 135.4 (CH=CH$_2$), 155.8 (CO).

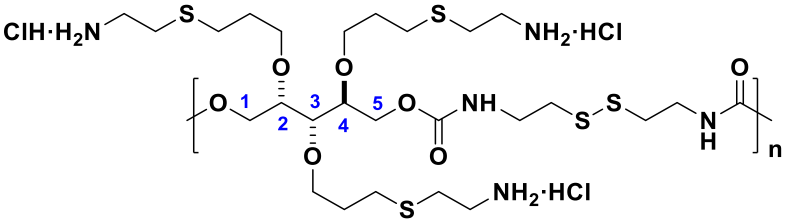
[(ABocCis)DTDI]



Polyurethane [(AAl)DTDI] (0.17 g, 0.37 mmol) was dissolved in DMF (1.2 mL) and the resulting solution was deoxygenated under a stream of argon during 30 minutes. Then, BocCis (0.78 g, 5.1 mmol) and 2,2'-azobisisobutyronitrile (AIBN) as initiator (0.15 g, 0.88 mmol) were added under an inert atmosphere and the mixture was stirred at 80 °C for 24 h. After this time, the obtained solution was added dropwise into cold diethyl ether (300 mL). The precipitated polymer was filtered and subsequently purified by dissolving in dichloromethane and reprecipitation in diethyl ether. The formed solid was filtered, washed with diethyl ether and dried under vacuum at 40 °C for24 h (1.0 g, 96%).

IR: δ_{max} (cm^{-1}) 3354 (N-H), 1671 (C=O), 1516 (N-H), 1250 (N-CO-O).
^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 1.40 [bs, 27H, C(CH_3)$_3$], 1.68-1.78 (m, 6H, OCH$_2$CH$_2$CH_2S), 2.41-2.60 (m, 12H, OCH$_2$CH_2CH$_2$S, SCH_2CH$_2$NHBoc), 2.70-2.77 (m, 4H, CH$_2$S-S), 3.02-3.10 (m, 6H, CH_2NHBoc), 3.14-3.26 (m, 4H, S-SCH$_2$CH_2NH), 3.35-3.74 (m, 9H, H-2, H-3, H-4, OCH_2CH$_2$CH$_2$S), 3.83-4.44 (m, 4H, H-1, H-5), 6.87, 6.96, (bs, 3H, NHBoc), 7.98 (bs, 2H, NH).
^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm) 28.3 (CH$_3$), 30.9 (OCH$_2$CH$_2$CH$_2$S), 35.5 (CH$_2$S, SCH$_2$), 37.6 (CH$_2$S-S), 39.0 (CH$_2$NH), 43.4 (CH$_2$NHBoc), 62.9, 64.5 (C-1, C-5), 70.1 (OCH$_2$), 74.0, 74.5, 78.1 (C-2, C-3, C-4), 77.9 [C(CH$_3$)$_3$], 155.6 (CO), 162.4 (CO Boc).

PUArab



The *N*-Boc protected polyurethane [(ABocCis)DTDI] (230 mg, 0.23 mmol) was treated with 4N HCl solution in dry dioxane (17.2 mL) under an argon atmosphere, at room temperature for 4 h. After this time, the mixture was filtered and the obtained solid was dried under vacuum at 40 °C for 24 h to afford the title compound (79 mg, 50%).

IR: δ_{max} (cm^{-1}) 3359 (N-H), 2899 (ammonium), 1696 (C=O), 1500 (N-H), 1250 (N-CO-O).
^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 1.68-1.80 (m, 6H, OCH$_2$CH_2CH$_2$S), 2.54-2.79 (m, 12H, CH$_2$S), 2.90-3.21 (m, 14H, CH$_2$S-S, CH_2NH$_2$, CH_2NH), 3.34-3.72 (m, 9H, OCH_2CH$_2$CH$_2$S, H-2, H-3, H-4), 3.89-4.40 (several m, 4H, H-1$_{a/b}$, H-5$_{a/b}$), 7.46 (bs, 2H, NH), 8.34 (bs, 6H, NH$_2$).

^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm) 27.4, 27.7 ($\mathrm{OCH_2CH_2}\mathit{C}\mathrm{H_2S}$), 29.6 ($\mathrm{S}\mathit{C}\mathrm{H_2CH_2NH_2}$), 33.9 ($\mathrm{OCH_2}\mathit{C}\mathrm{H_2CH_2S}$), 37.8 ($\mathrm{CH_2NH_2}$), 38.1, 38.5 ($\mathrm{CH_2S\text{-}S}$), 41.9 ($\mathrm{C}\mathit{H}_2\mathrm{NH}$), 63.1 (C-1/C-5), 66.3 ($\mathrm{OC}\mathit{H}_2\mathrm{CH_2CH_2S}$), 77.3 (C-2/C-3/C-4), 156.0 (C=O chain).

Table S1. Yields and molecular weights of the cationic polyurethane PUArab and their precursors.

		GPC		
Polyurethane	**Yield (%)**	**M_w[a]**	**M_n[a]**	**M_w/M_n[a]**
[(AAl)DTDI]	85	39,500	32,100	1.2
[(ABocCis)DTDI]	96	30,400	29,000	1.0
PUArab	50	-	4,950[b]	-

[a] Weight and number average molecular weight in g mol^{-1} and polydispersity measured by GPC analysis in DMF/LiBr as mobile phase against polystyrene standards.
[b] Estimated by ^{1}H RMN.

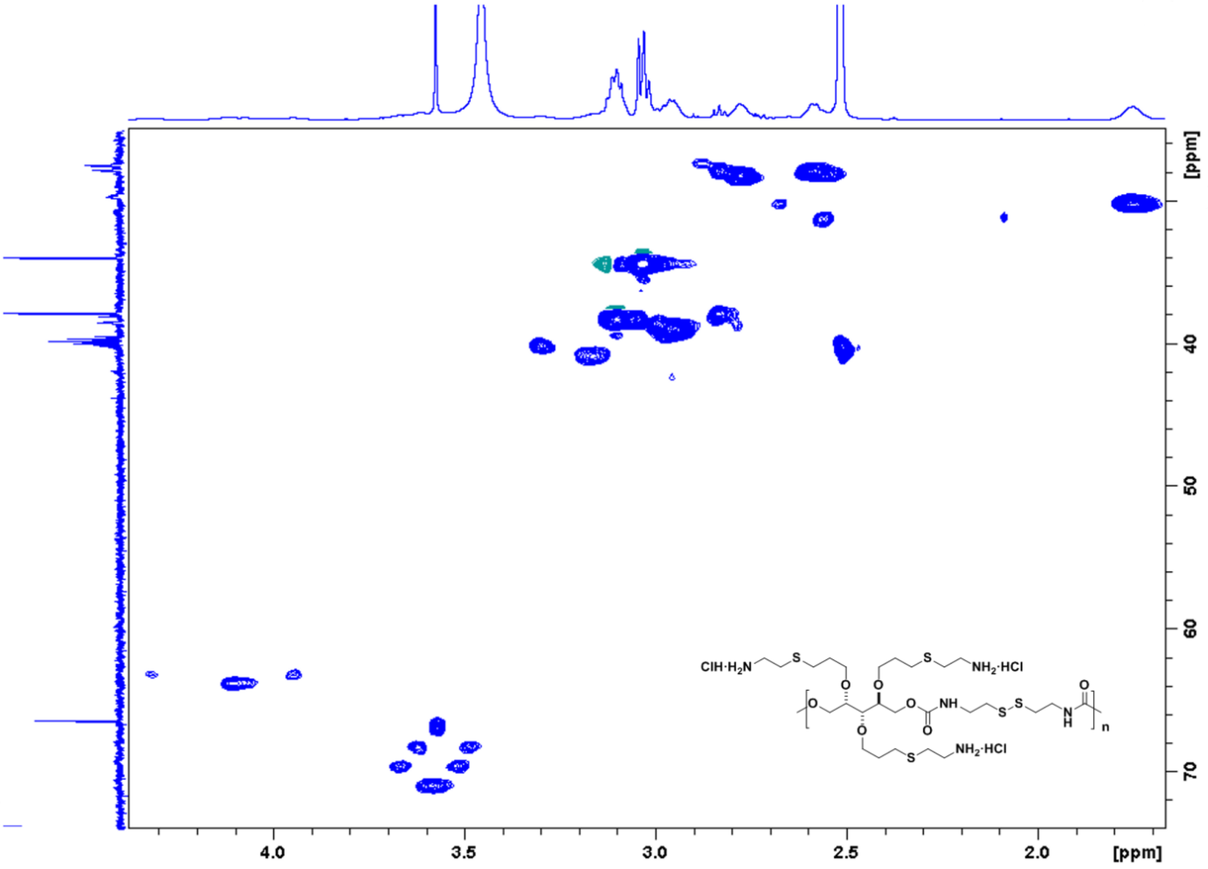


Figure S1. ^{1}H-^{13}C HETCOR NMR spectra for PUArab.

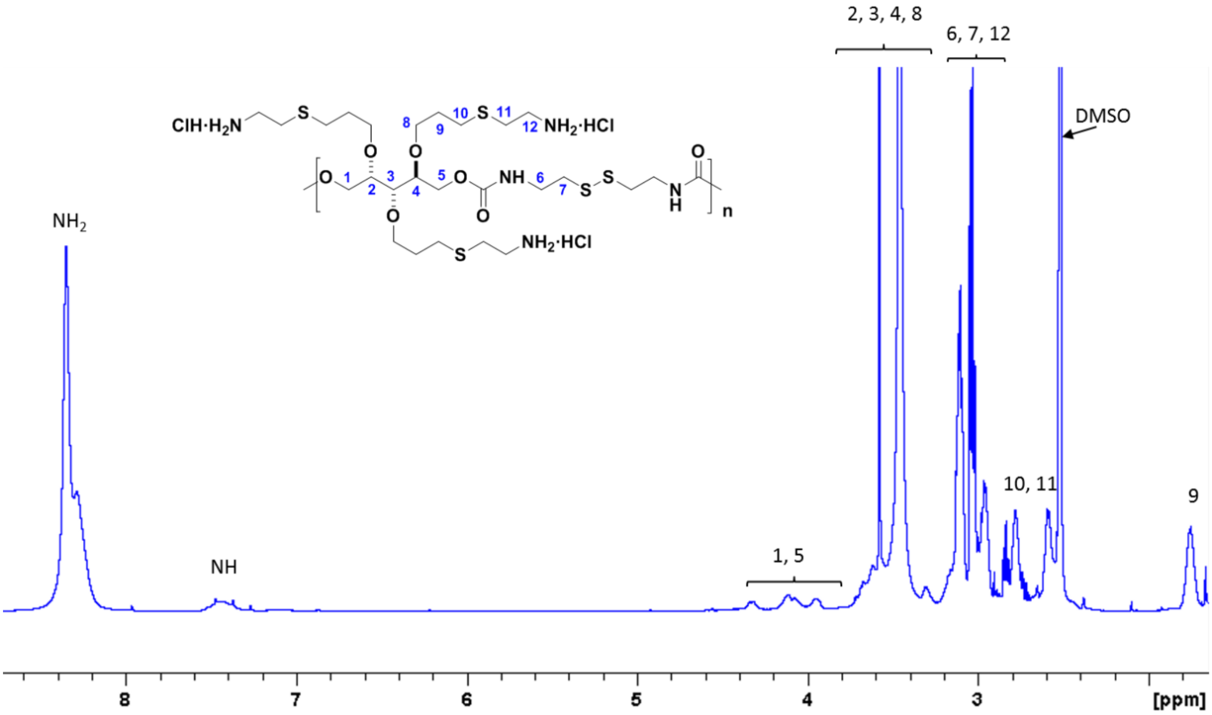


Figure S2. ^{1}H NMR spectra for PUArab.

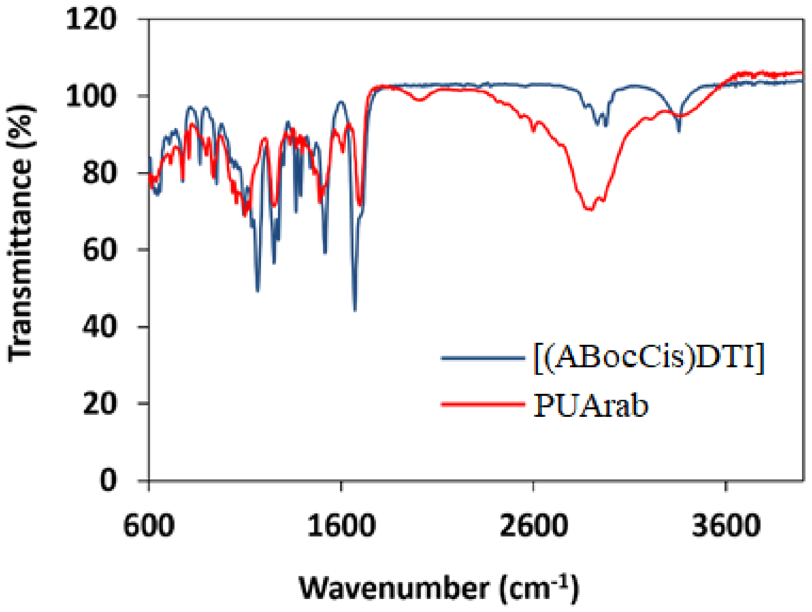


Figure S3. Infrared spectrum of PURarab and its precursor [(ABocCis)DTDI].

Thermal properties

The results of thermogravimetric analysis, TGA, and differential scanning calorimetry, DSC, are collected in Table S2. TGA studies of the *O*-allyl polymer showed thermal stability up to 222 °C for the L-arabinitol derivative. The rest of the polyurethanes as well as the cationic PUArab, were stable up to 240 °C. In all cases, decomposition processes took place in two stages: the first one at temperatures between 256 and 259 °C, and the second one in the range 324-333 °C. The decomposition profiles obtained for the cationic PUArab is shown in Figure S2.

The DSC study of polyurethanes showed low T_g values, in the range 2-18 °C, corresponding the lowest value, 2 °C, to the cationic PUArab. Endothermic peaks associated to melting process were only observed for the *N*-Boc protected polyurethane [(ABocCis)DTDI] around 75°C. The rest of the polyurethanes manifested an amorphous behavior.

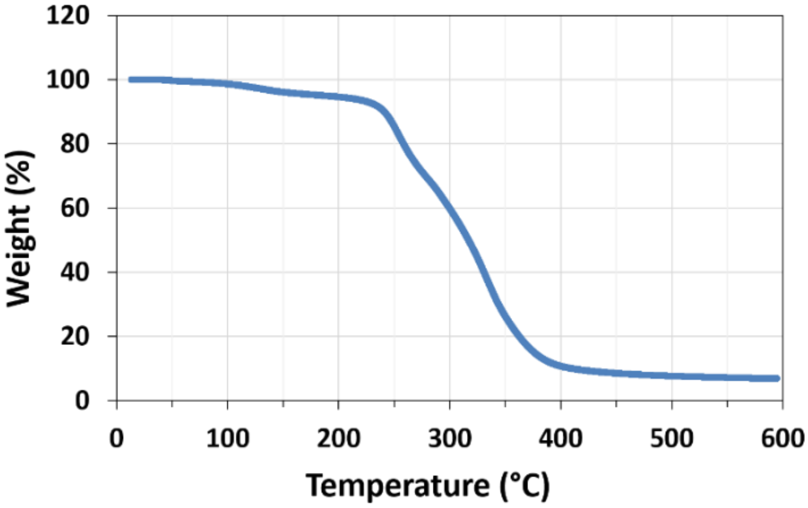


Figure S4. Curve of thermal degradation under inert atmosphere of PUArab (weight (%) versus temperature).

Table S2. Thermal properties of the cationic PUArab and thei precursors.

	TGA			DSC		
Polyurethane	$T_d{}^a$ (°C)	$T_{ds}{}^b$ (°C)	ΔW^c (%)	$T_g{}^d$ (°C)	$T_m{}^e$ (°C)	$\Delta H_m{}^e$ (J g^{-1})
[(AAl)DTDI]	222	256/324	42/41	18		
[(ABocCis)DTDI]	240	257/331	38/50	10	77/114	59/86
PUArab	241	255/333	25/62	4		

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[a] Temperature at which 10% weight loss was observed in the TGA traces °C · min^{-1}.

[b] Temperature for maximum degradation rate.

[c] Remaining weight at 600 °C.

[d] Glass transition temperature taken as the inflection point of the heating DSC traces of melt-quenched samples recorded at 20 °C · min^{-1}.

[e] Melting temperature (T_m) and respective enthalpy (ΔH_m) measured by DSC at a heating rate of 10 °C · min^{-1}.

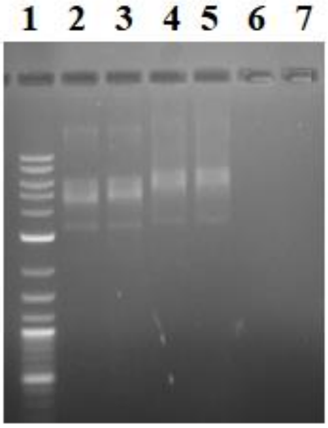

Figure S5.- Electrophoretic mobility shift assay on an agarose gel (1%) for PUArab/pDNA polyplexes. Experiments were run in TAE buffer (40 mM tris-acetate, 1 mM EDTA) and stained with Red Safe. Electrophoresis was performed at 90 V until completion. 1) K_b DNA ladder; 2) Pure pDNA; 3) N/P=1.8; 4) N/P=5; 5) N/P=7; 6) N/P=22; and 7) N/P=50.

Table S3.- PUArab concentrations for the different N/P ratios investigated in the transfection assays.

N/P	8.5	14	17	25	42
[PUArab]/μg mL^{-1}	3.7	6.1	7.5	9.8	18.2