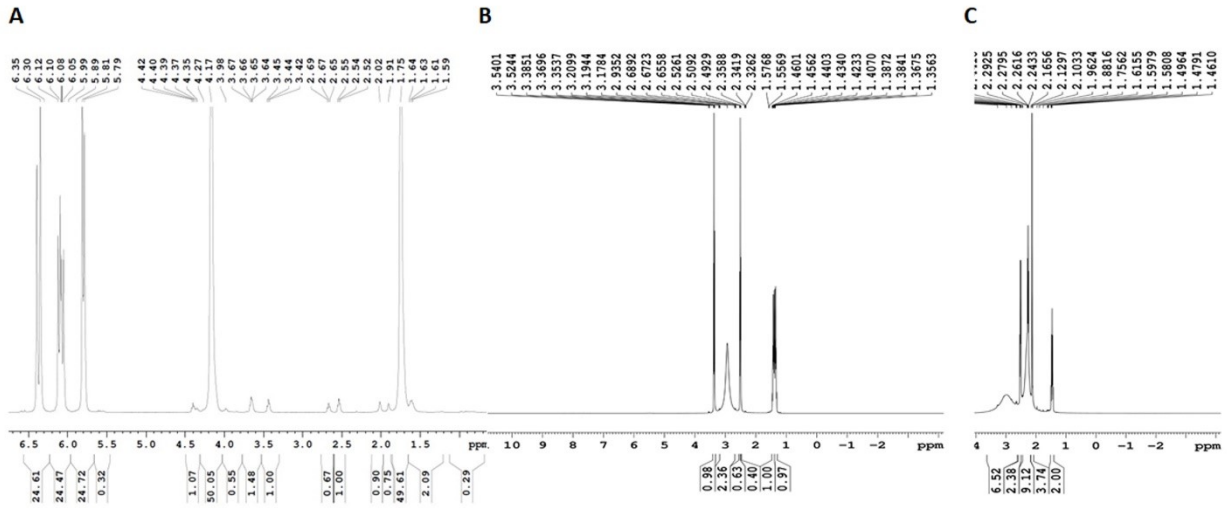

Figure S1: H-NMR spectra of key reagents used in the synthesis of poly(β-amino ester) (PBAE) polymers. A, H-NMR spectrum of 1,4-butanediol diacrylate, showcasing characteristic peaks that confirm the presence of acrylate and butanediol functional groups, essential for polymer backbone formation. B, H-NMR spectrum of 4-amino-1-butanol, highlighting the unique peaks corresponding to the amino and hydroxyl groups, which are critical for the cross-linking and flexibility of the polymer. C, H-NMR spectrum of 1-(3-Aminopropyl)-4-methylpiperazine, illustrating the distinct peaks associated with the piperazine ring and amino group, contributing to the polymer's structure and functionality.

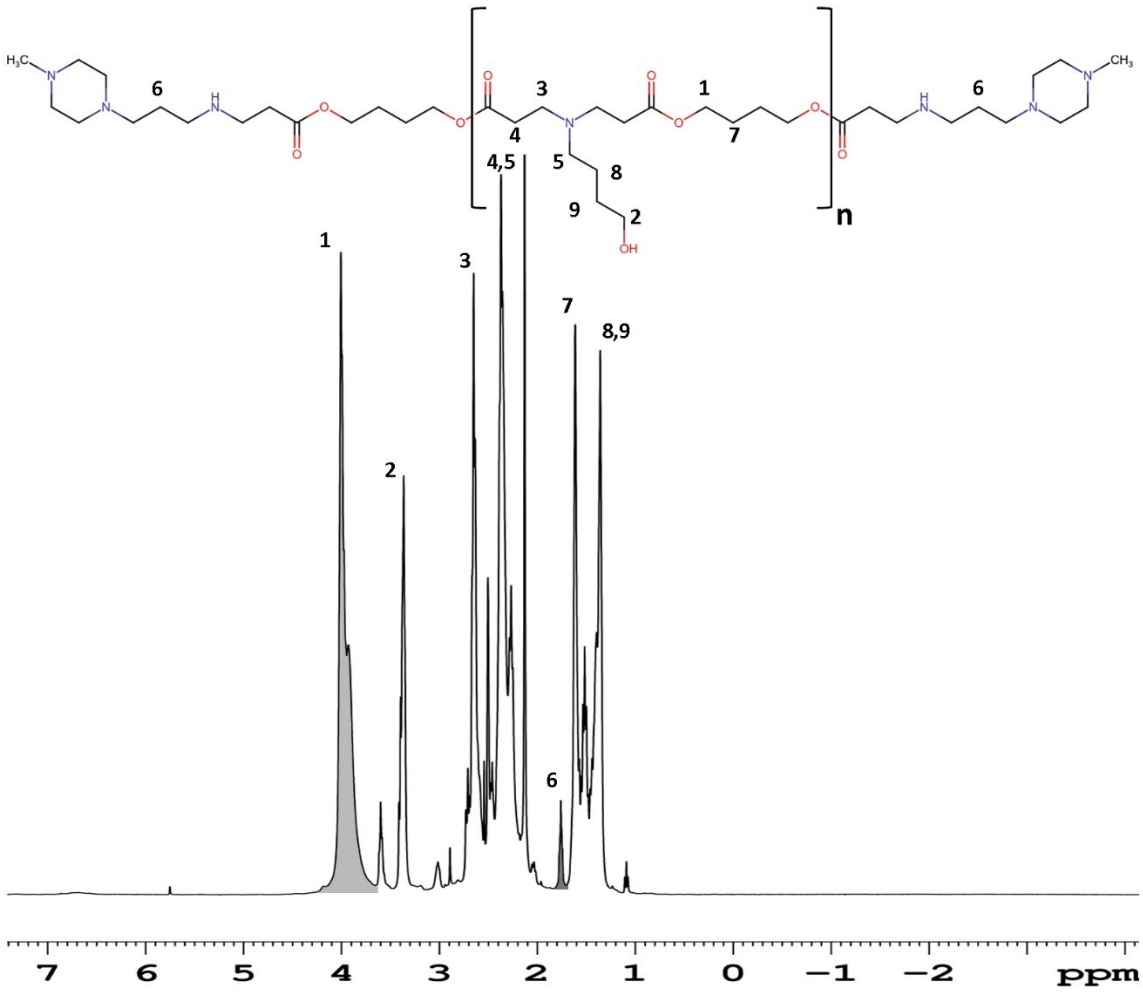

Figure S2: H NMR spectrum of a low molecular weight poly(β-amino ester) (PBAE) recorded in deuterated solvent. The spectrum reveals the characteristic proton signals associated with the polymer's structural units. Key peaks are labeled according to their corresponding protons in the chemical structure. The singlet at ~4.1 ppm corresponds to the methylene protons adjacent to the ester group (labeled as 1). Peaks at ~3.3-3.5 ppm are attributed to the methylene protons (2) adjacent to the hydroxyl group and amine groups. Signals around ~2.7-2.9 ppm are assigned to methylene protons (6) in the β-amino backbone. The peak clusters at ~1.2-1.8 ppm represent the aliphatic methylene groups (4, 5, 7, 8, 9) in the polymer chain. The characteristic peak at ~3.7 ppm is indicative of methylene groups (3) near the ester and amine functionalities.

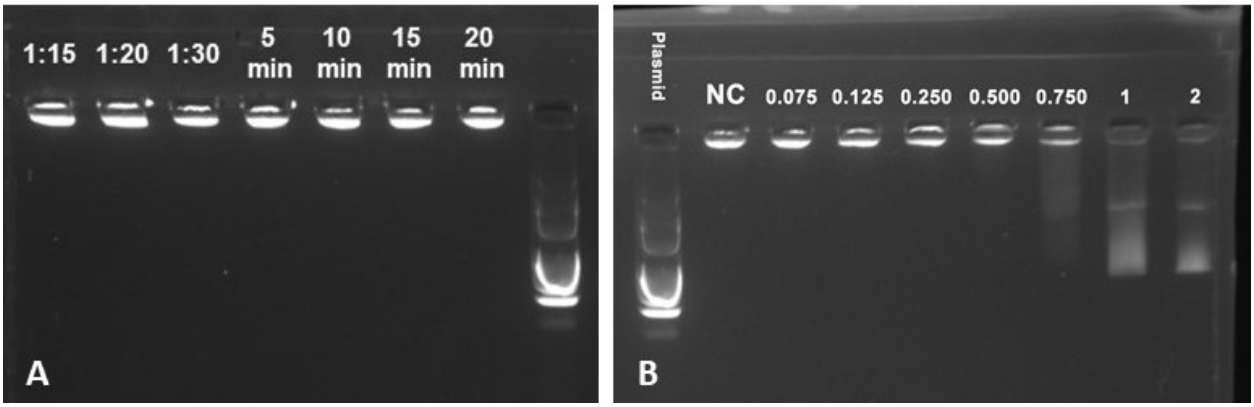

Figure S3: A, Gel retardation assay of nanocarrier-DNA complexes at three different DNA/polymer ratios (1:15, 1:20, and 1:30). Additionally, the assay includes varying incubation times for the 1:20 ratio (5, 10, 15, and 20 minutes), demonstrating the efficiency and stability of complexation over time. B, Heparin stability assay showing the effect of different heparin concentrations (75 ng to 2 µg) on the stability of the nanocarrier-DNA complexes. This assay highlights the robustness of the complexes in the presence of heparin, an important factor for in vivo applications. Panels A and B are from a single gel, with the overlapping control plasmid appearing in both panels.