Ferritin-Polyamine Conjugates for Targeted and Enhanced Delivery of siRNA

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Background: Small interfering RNA (siRNA) represents a powerful therapeutic modality for targeted gene silencing. However, clinical translation remains constrained by biological barriers, including targeted delivery, serum stability, and inefficient endosomal escape. To overcome these challenges, an engineered "humanized" chimeric Archaeal ferritin (HumAfFt) protein nanocage was developed as a biocompatible delivery platform. Functionalizing the inner cavity of HumAfFt with polycationic linkers offers an electrostatic mechanism designed to enhance siRNA encapsulation and facilitate intracellular release1 . Method: A novel synthetic library of linear polyamine linkers was designed to systematically establish a structure–interaction relationship (SIR) with negatively charged RNA duplexes. The linkers were synthesized via iterative amidation reactions coupling N-protected amino acid building blocks (methylamine, β-alanine, and glycine in varying ratios). A pentafluorobenzenesulfonamide moiety was incorporated to achieve chemoselective thiol-coupling to cysteine residues strategically engineered inside the HumAfFt cavity. Results: The iterative amidation route successfully yielded a modular library of positively charged, aliphatic polyamine linkers in high yield and purity, as confirmed by NMR spectroscopy and mass spectrometry analyses. By systematically varying the building block composition and sequence, linkers were produced with precisely controlled amine spacing, overall charge density, and conformational flexibility. Furthermore, the terminal pentafluorobenzenesulfonamide group was cleanly integrated without compromising linker solubility, yielding a suite of reactive polycationic linkers custom-designed for future site-specific bioconjugation within the ferritin nanocage. Conclusion: This work establishes a versatile synthetic platform for generating customized, electrophilically activated polyamine linkers tailored for internal protein functionalization. By enabling precise tuning of chemical composition, length, and charge density, this linker library provides the necessary chemical foundation for upcoming internal HumAfFt functionalization and subsequent siRNA complexation studies.