Supplementary Information

Optimized Gadolinium-DO3A loading in RAFT-Polymerized Copolymers for Superior MR Imaging of aging blood-brain barrier

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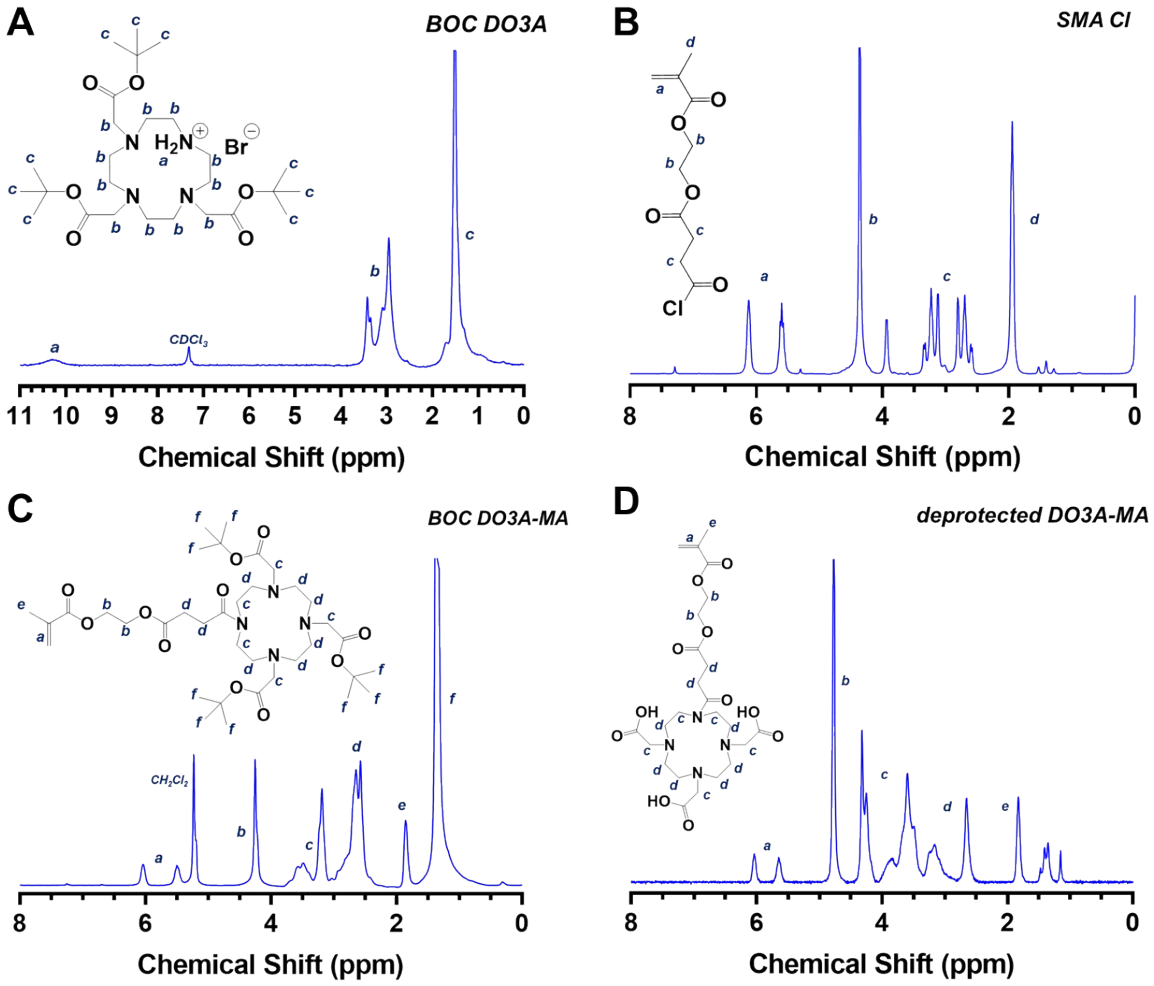


ESI Figure 1. ^{1}H NMR spectra for each step of the DO3A-MA monomer synthesis process. (A) ^{1}H NMR of BOC DO3A precursor powder synthesized *via* trialkylation of cyclen by *tert*-butyl bromoacetate. (B) ^{1}H NMR of SMA acid chloride monomer synthesis *via* reaction between SMA and oxalyl chloride. (C) BOC DO3A-MA monomer synthesis *via* reaction of SMA Cl (B) and "tri-boc" precursor (A). (D) Deprotection step of BOC DO3A-MA monomer via reaction with TFA (trifluoroacetic acid) for removal of boc groups to make DO3A-MA monomer.

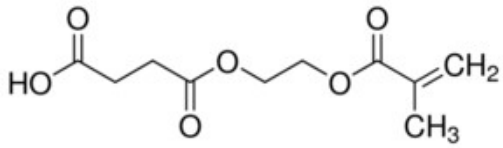
Peptides were synthesized automatically by using standard Fmoc chemistry as described previously by:

Zwanziger, D., Hackel, D., Staat, C., Böcker, A., Brack, A., Beyermann, M., Rittner, H., & Blasig, I. E. (2012). A peptidomimetic tight junction modulator to improve regional analgesia. Molecular Pharmaceutics, 9(6), 1785-1794. https://doi.org/10.1021/mp3000937

The N-terminus was then functionalized with the carboxylic acid-functional monomer SMA as shown below.

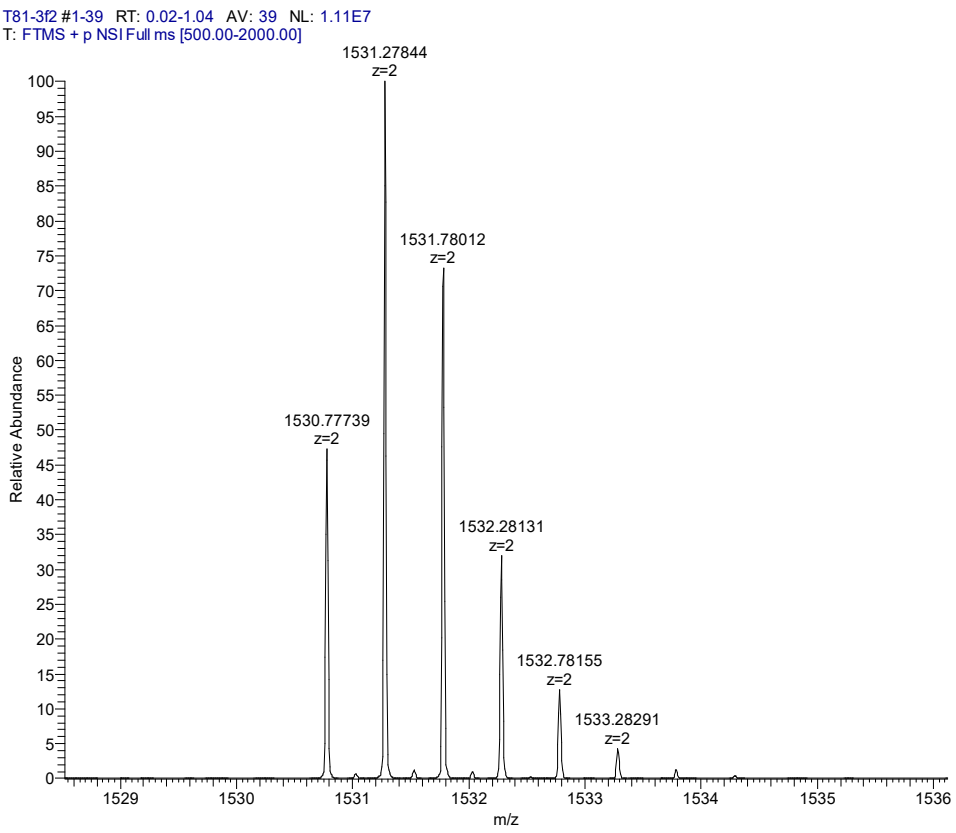
"Monomer"- SSVSQSTGQIQSKVDSLLNLNSTQATR-conh2

Where Monomer is the acid below connected to the N-terminal of the peptide by amide bond



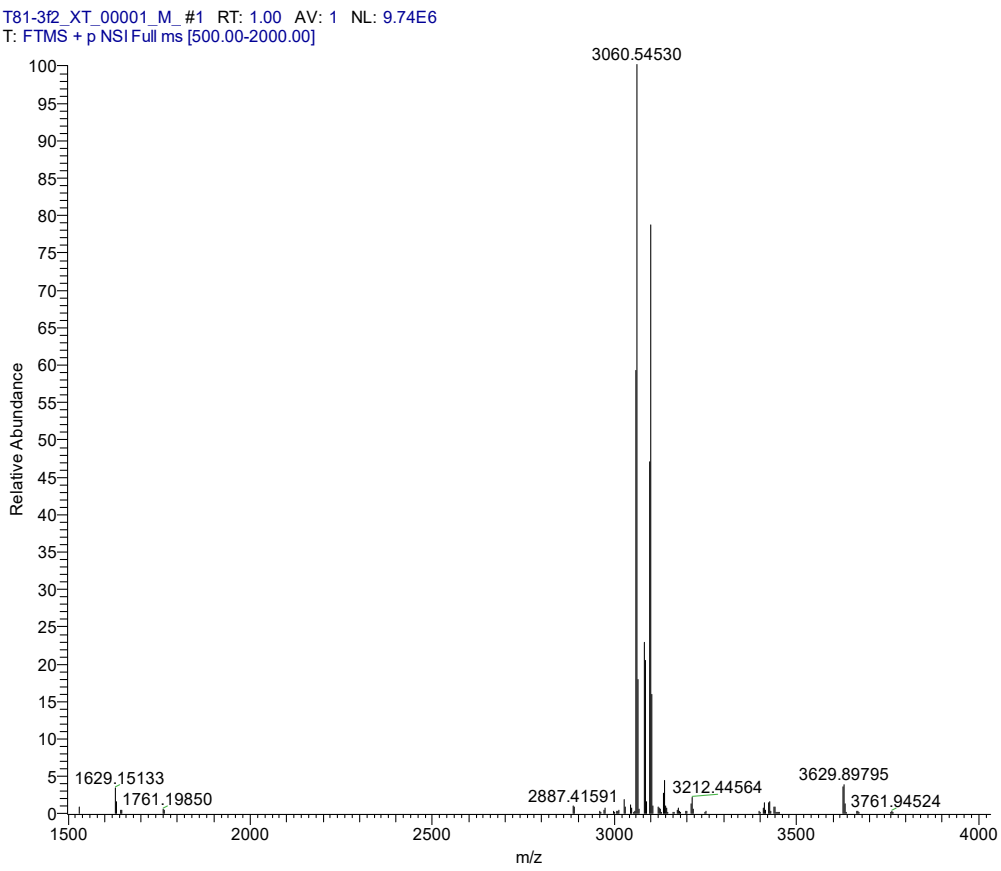
Monomer

Sample processing and Analytical Methods: Sample was resuspended at 1mg/mL in 60% acetonitrile and diluted 10x with 70% acetonitrile,0.1% formic acid. A static nanospray emitter (Econo12) was filled with 10uL of sample and data acquired by FTMS positive ion on an LTQ Orbitrap XL. The sample was loaded into a static nanospray ECONO 12 tip (Proxeon) and analyzed by nano-electrospray ionization in positive-ion mode on a ThermoScientific LTQ Orbitrap XL mass spectrometer. Typical flow rates from these tips are estimated to be 50nL/min. FTMS data were collected in the Orbitrap (100,000 resolving power, 500-2000 m/z, 1 microscan, maximum inject time of 100ms, AGC= 5e5) over 1 min of infusion. FTMS spectra were internally calibrated using a lock mass of 531.40777 m/z (didodecyl 3,3'-thiodipropionate oxidized to sulfoxide), a common background ion.

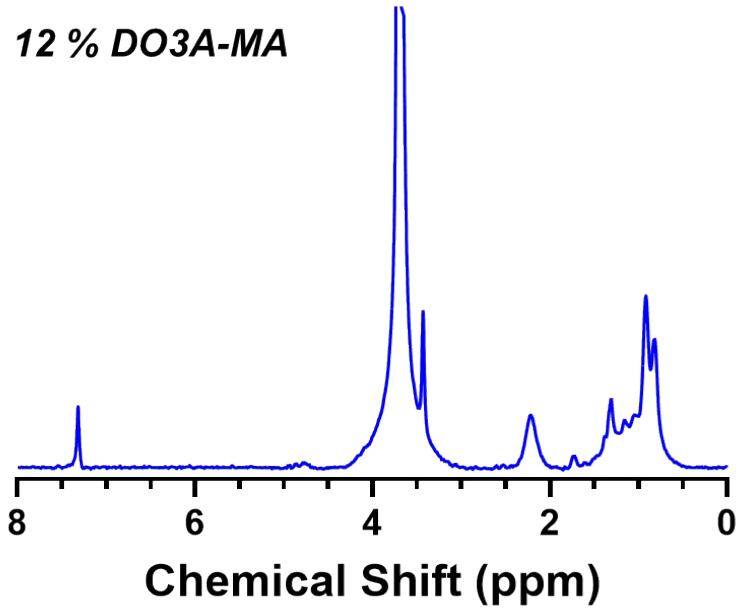


Theoretical m/z $[M+2H]^+$ = 1530.77583
Observed m/z (monoisotopic peak) = 1530.77739
Mass error = 1.02 ppm

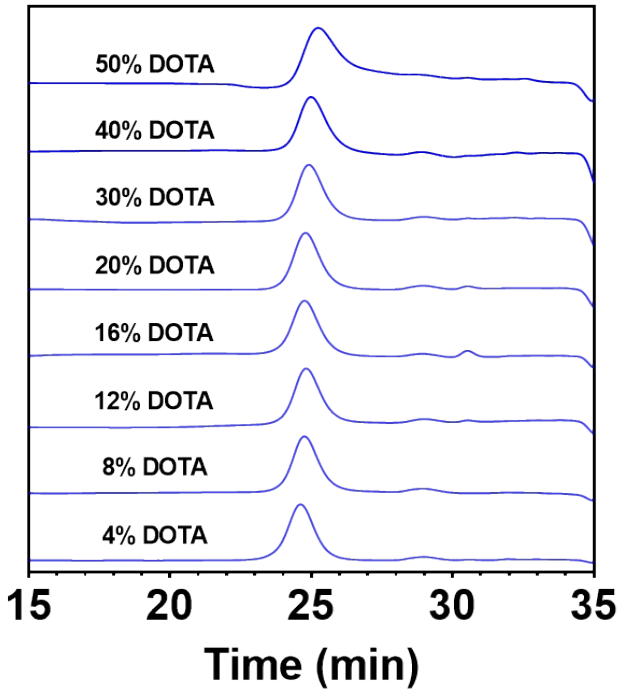
ESI Figure 2. Mass Spectroscopy (MS) of C1C2 peptide monomer.



ESI Figure 3. Mass Spectroscopy (MS) of C1C2 peptide monomer



ESI Figure 4. Representative 1H NMR spectrum of a (DO3A-MA)-co-O950 series polymer using the 12% DO3A-MA polymer.



ESI Figure 5. Gel Permeation Chromatography (GPC) traces for (DO3A-MA)-co-O950 series polymers for MRI contrast testing. The shift in retention time is subtle from 4% DOTA to 50% DOTA due to the similar targeted DPs and the log scale of molecular weight associated with GPC measurements. As shown, all molar mass distributions were unimodal and narrow (50% being the one exception).