Supporting Information

Defining the Catechol-Cation Synergy for Enhanced Wet Adhesion to Mineral Surfaces

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1. Materials and Methods

Materials: 2,3-dihydroxybenzoic acid (2,3-DHBA), dicyclohexylcarbodiimide (DCC), and tris(2-aminoethyl)amine (TREN) were purchased from Aldrich. Ethanol, THF, trimethylamine (Et_3N), potassium hydroxide, trifluoroacetic acid (TFA) and DCM were purchased from Fisher. DMSO and acetic acid were purchased from EMD. Benzyl bromide and palladium on carbon were purchased from Alfa Aesar. N-hydroxysuccinimide (NHS) was purchased from Fluka. H-Lys(Z)-OH and Boc-Arg$(Z)_2$-OSu were purchased from Bachem. Unless otherwise stated, all chemicals were used as received without further purification or modification.

Siderophore Analog Synthesis: Tren-cam (TC) and Tren-Lys-Bam (TLB) were synthesized according to previously published methods.[1-3] Tren-Lys-Lys-Cam (TLLC) and Tren-Arg-Cam (TAC) were synthesized using previously published procedures with minor variation.[3] See Figures S1 and S5 for details of the synthesis procedure.

Surface Forces Apparatus (SFA): The full details of the SFA technique are elaborated elsewhere.[4] All measurements were performed with a SFA 2000, manufactured by SurForce LLC in Santa Barbara, California. Briefly, for each experiment, two mica surfaces are prepared by gluing a piece of freshly-cleaved, back-silvered mica (~1 cm^2), of equal mica and silver thicknesses, onto cylindrical glass disks (radius ~2 cm), with the pristine mica surface facing upward. The two mica surfaces are installed into the SFA, with the pristine mica surfaces facing each other. The surfaces are brought close together and small droplets of aqueous buffer are injected between the surfaces (~50 μL total volume). Normal force-distance measurements are then performed between the two surfaces in aqueous solution. The contact area between the mica surfaces is verified as free from asperities or contaminants based on the interferometric profile of the contact zone and the measured forces, which are well documented for mica interacting in aqueous solution.[5-7] Following, a small amount (~10 μL) of siderophore analog in aqueous buffer is injected into the gap solution between the surfaces, and the system is allowed to equilibrate for 20 minutes as the siderophore analogs adsorb to the mica surfaces. While remaining at the same contact position, force-distance measurements are then performed between the mica surfaces in the siderophore analog solutions. The aqueous solution used in all SFA experiments was a 50 mM acetate + 150 mM KNO_3 buffer solution at pH 3.3. The force-distance data shown are representative of measurements performed over at least 4 separate experiments for each molecule and solution condition. The adhesion values, F_{ad}, and compressed film thicknesses, D_T, are reported as the sample mean and standard deviation.

2. Synthesis and Characterization of Siderophore Analogs

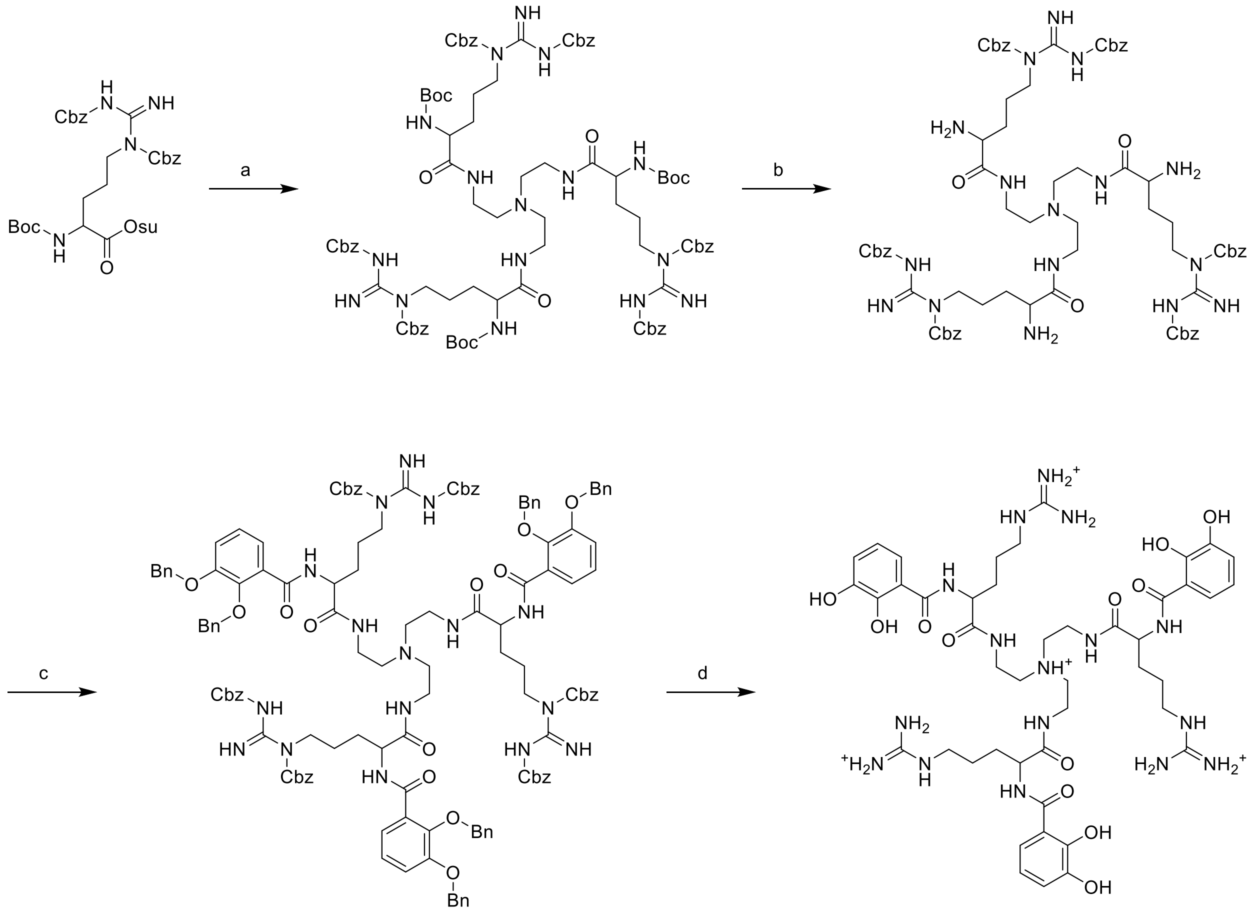
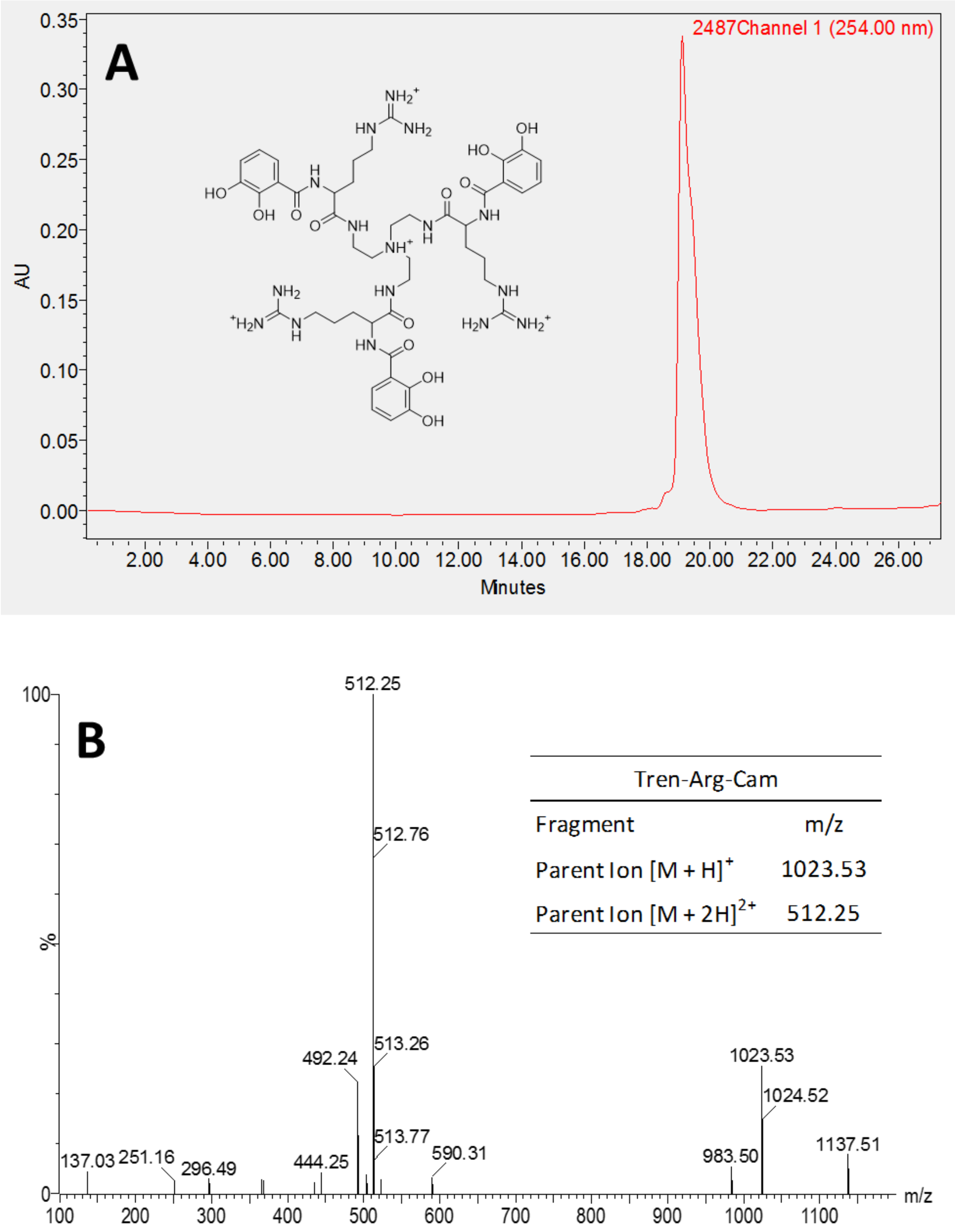


Figure S1. Synthesis Scheme for Tren-Arg-Cam. Reaction Conditions: (a) anhydrous THF under N_2, tris(2-aminoethyl)amine, Et_3N, stirred overnight. (b) 50% TFA in DCM added dropwise and stirred for 2 hours. (c) anhydrous THF under N_2, Et_3N, Bn-2,3-DHBA-OSu. Bn-2,3-DHBA-OSu was synthesized as described in Fig. S5 (d) 25% EtOAc in EtOH, 5% HOAc, Pd/C, stirred overnight under hydrogen atmosphere.



Tren-Arg-Cam	
Fragment	m/z
Parent Ion $[M + H]^+$	1023.53
Parent Ion $[M + 2H]^{2+}$	512.25

Figure S2. (A) Reverse phase HPLC purification of Tren-Arg-Cam (TAC) with TAC structure. RP-HPLC was performed on a preparative C_4 column (22-mm internal diameter, ID x 250-mm length, Vydac) with a gradient from H_2O (doubly deionized with 0.05% trifluoroacetic acid (TFA)) to 50% MeOH (with 0.05% TFA) in H_2O over 15 minutes. The column was exchanged back to 100% H_2O with 0.05% TFA over the next 10 minutes. (B) ESI Mass Spectrometry of TAC with several prominent fragments identified.

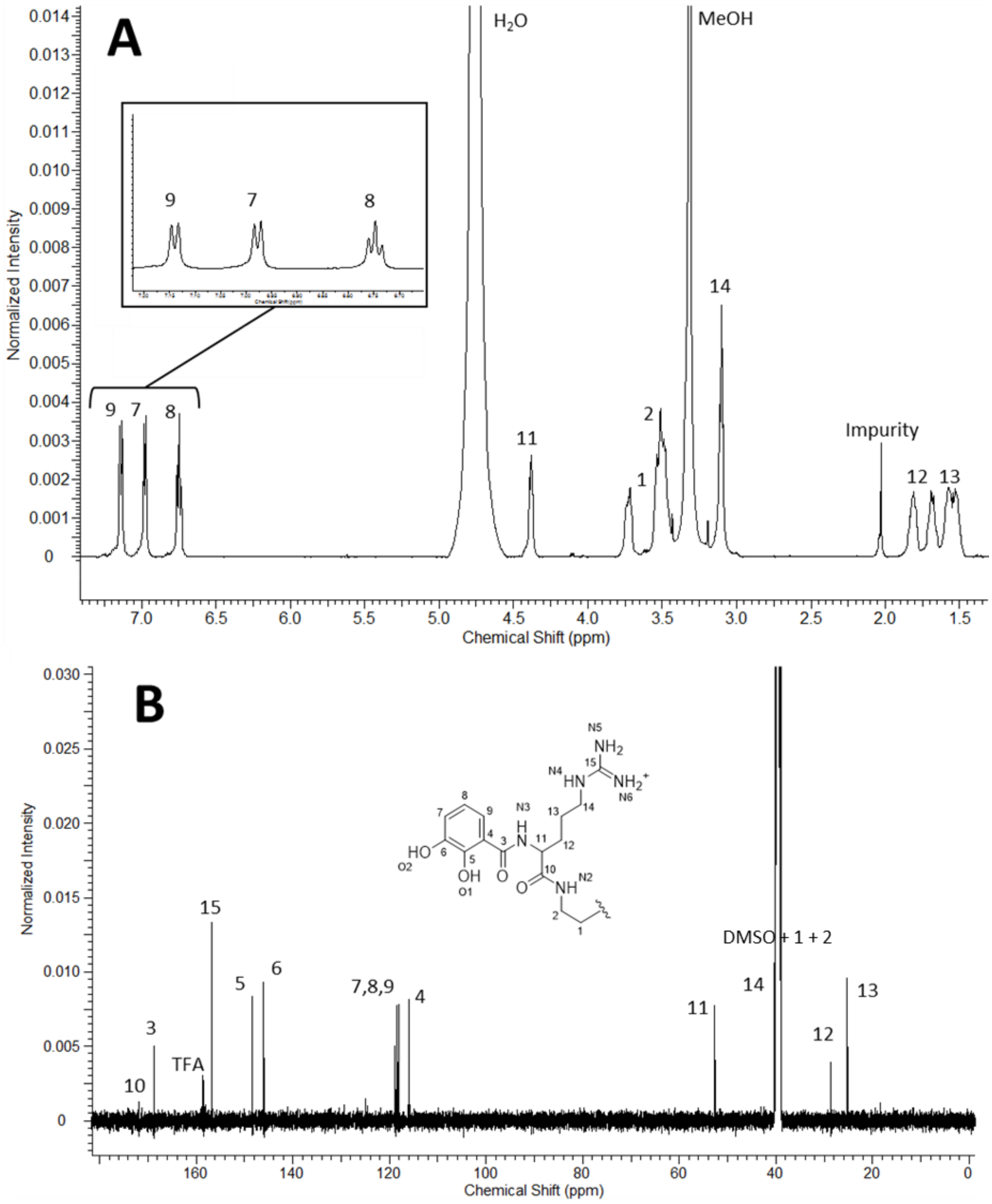


Figure S3. (A) ^{1}H NMR of TAC in D_2O. Data was collected using a Varian Unity Inova 600 MHz spectrometer. (B) ^{13}C NMR of TAC in DMSO. Data was collected using a Varian Unity Inova 500 MHz spectrometer.

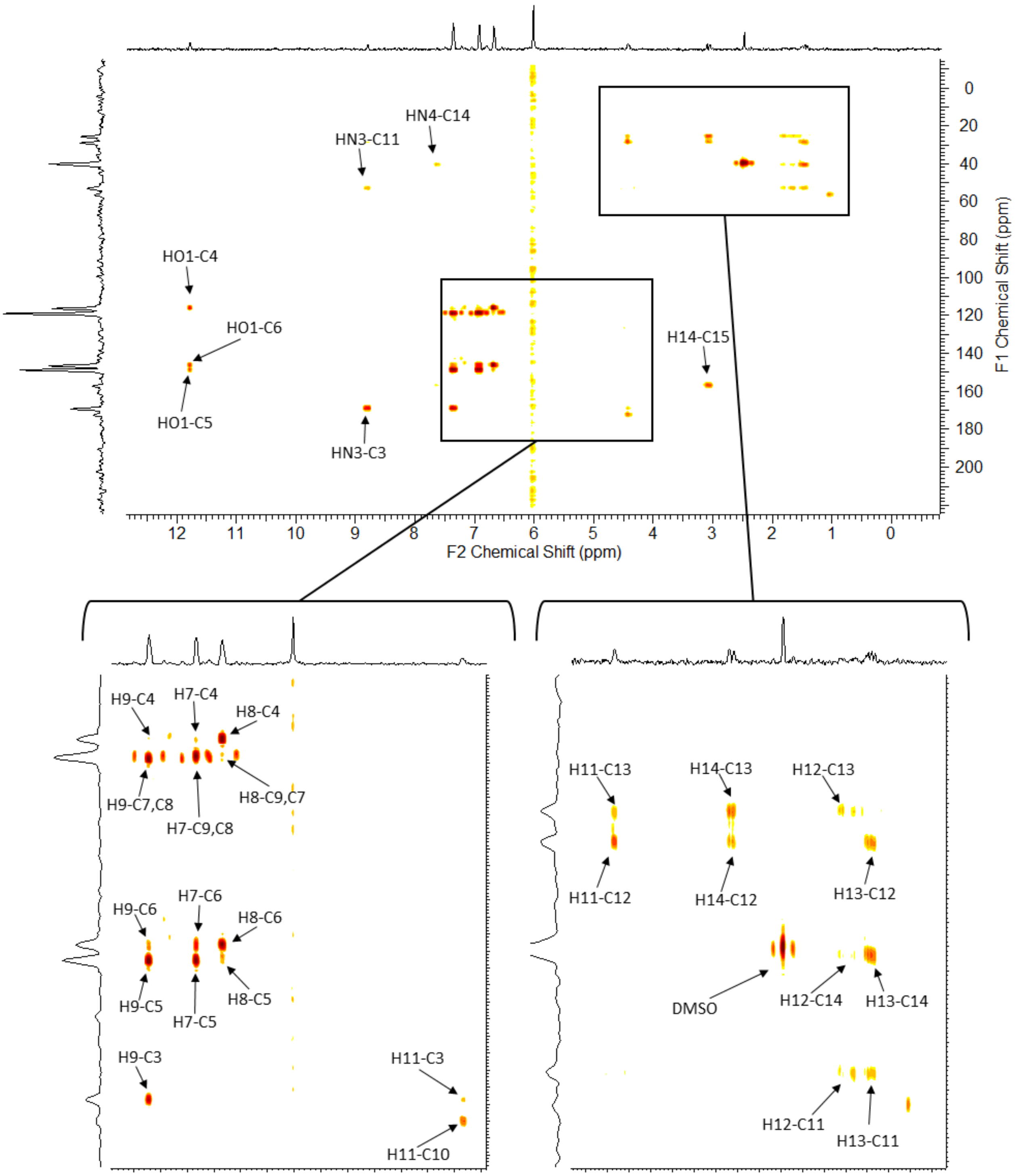


Figure S4. ^{1}H-^{13}C HMBC NMR for TAC. NMR (600 MHz) in DMSO. Enlarged regions of the ^{1}H-^{13}C HMBC NMR for TAC are in bottom panel. The spectrum is annotated with the correlations between specific carbons and hydrogens. See figure S3B for numbered positions.

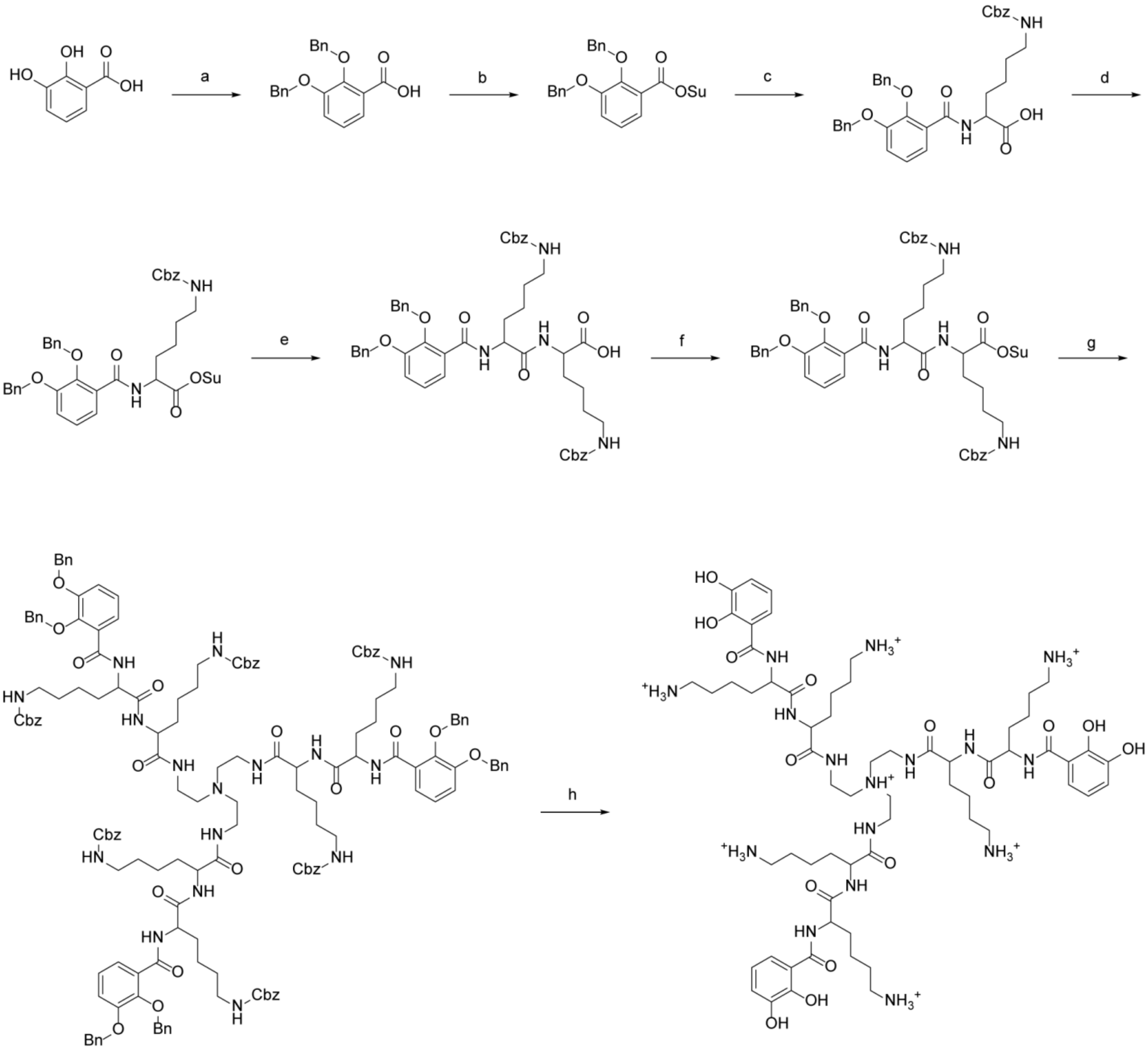
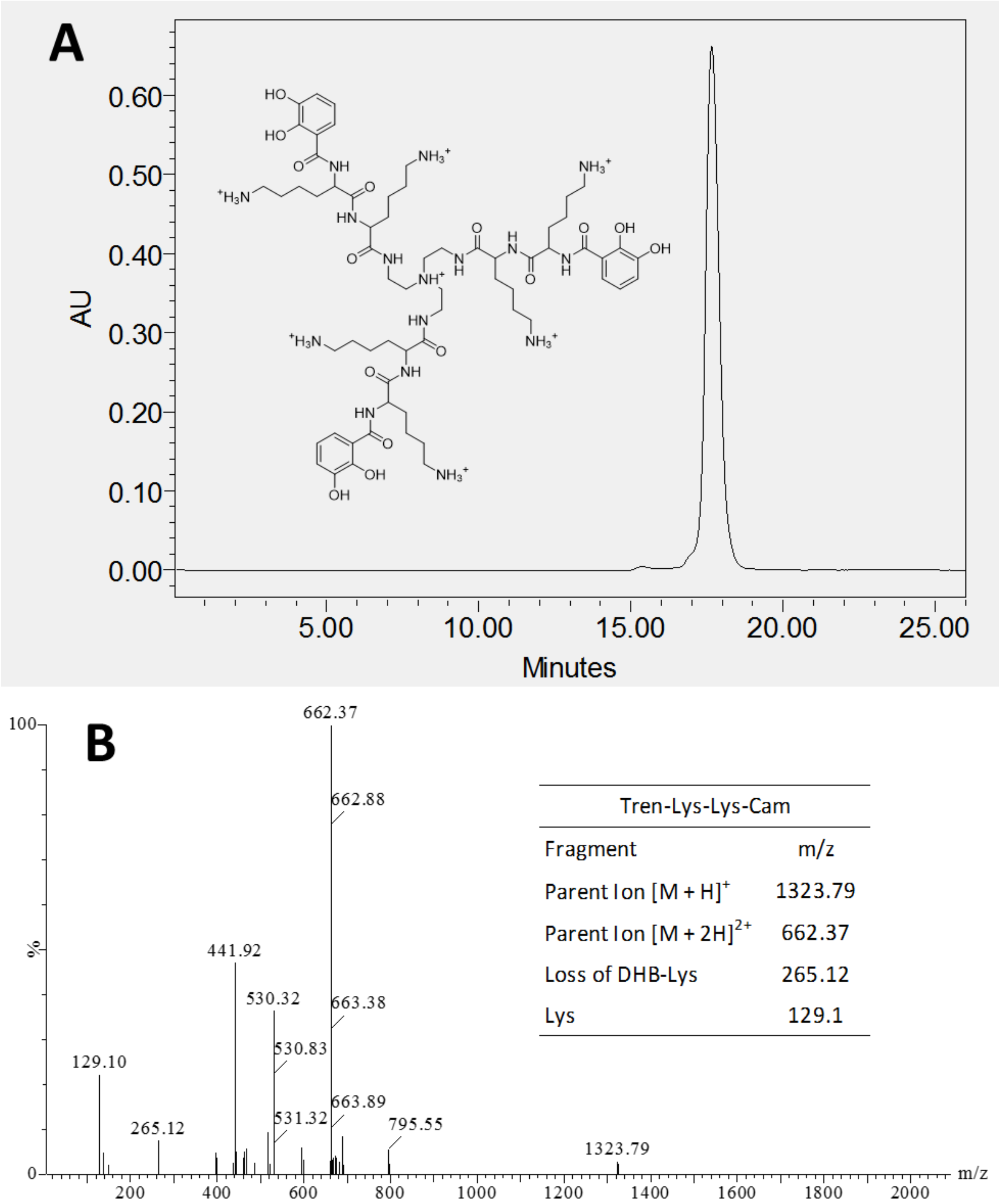


Figure S5. Synthesis Scheme for Tren-Lys-Lys-Cam. Reaction Conditions: (a) KOH, DMSO, benzyl bromide, stirred for 4 hours. (b) NHS, DCC, anhydrous THF under N_2, stirred overnight. (c) THF, H_2O, Et_3N, H-Lys(Z)-OH, stirred overnight. (d) NHS, DCC, anhydrous THF under N_2, stirred overnight. (e) THF, H_2O, Et_3N, H-Lys(Z)-OH, stirred overnight. (f) NHS, DCC, anhydrous THF under N_2, stirred overnight. (g) Et_3N, tris(2-aminoethyl)amine, anhydrous DCM under N_2, stirred overnight. (f) EtOH, 5% HOAc, Pd/C, stirred overnight under hydrogen atmosphere.



Tren-Lys-Lys-Cam	
Fragment	m/z
Parent Ion $[M+H]^+$	1323.79
Parent Ion $[M+2H]^{2+}$	662.37
Loss of DHB-Lys	265.12
Lys	129.1

Figure S6. (A) Reverse phase HPLC purification of Tren-Lys-Lys-Cam (TLLC) with TLLC structure. RP-HPLC was performed on a preparative C_4 column (22-mm internal diameter, ID x 250-mm length, Vydac) with a gradient from H_2O (doubly deionized with 0.05% trifluoroacetic acid (TFA)) to 50% MeOH (with 0.05% TFA) in H_2O over 15 minutes. The column was exchanged back to 100% H_2O with 0.05% TFA over the next 10 minutes. (B) ESI Mass Spectrometry of TLLC with several prominent fragments identified.

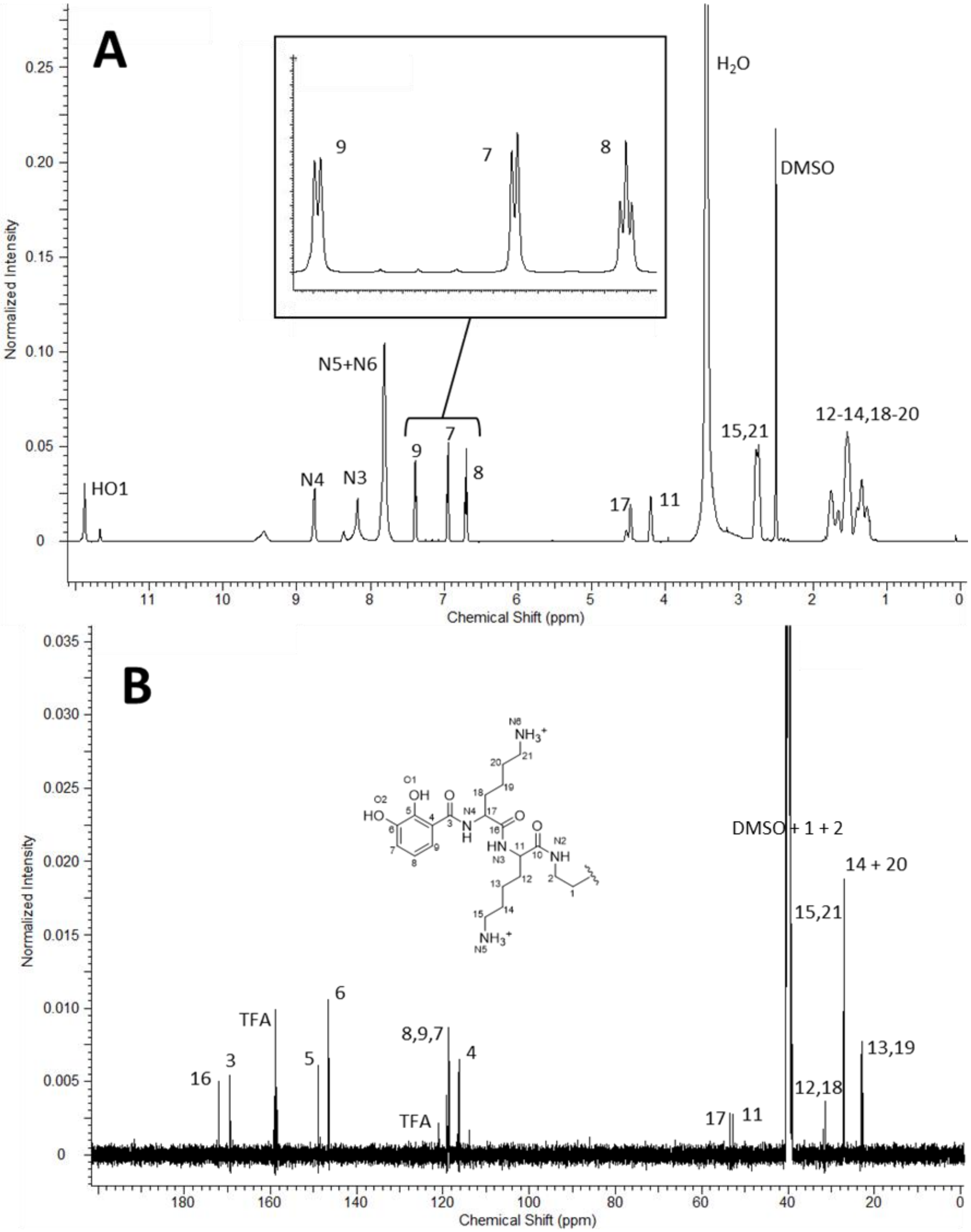


Figure S7. (A) ^{1}H NMR of TLLC in DMSO. Data was collected using a Varian Unity Inova 600 MHz spectrometer. (B) ^{13}C NMR of TLLC in DMSO. Data was collected using a Varian Unity Inova 500 MHz spectrometer.

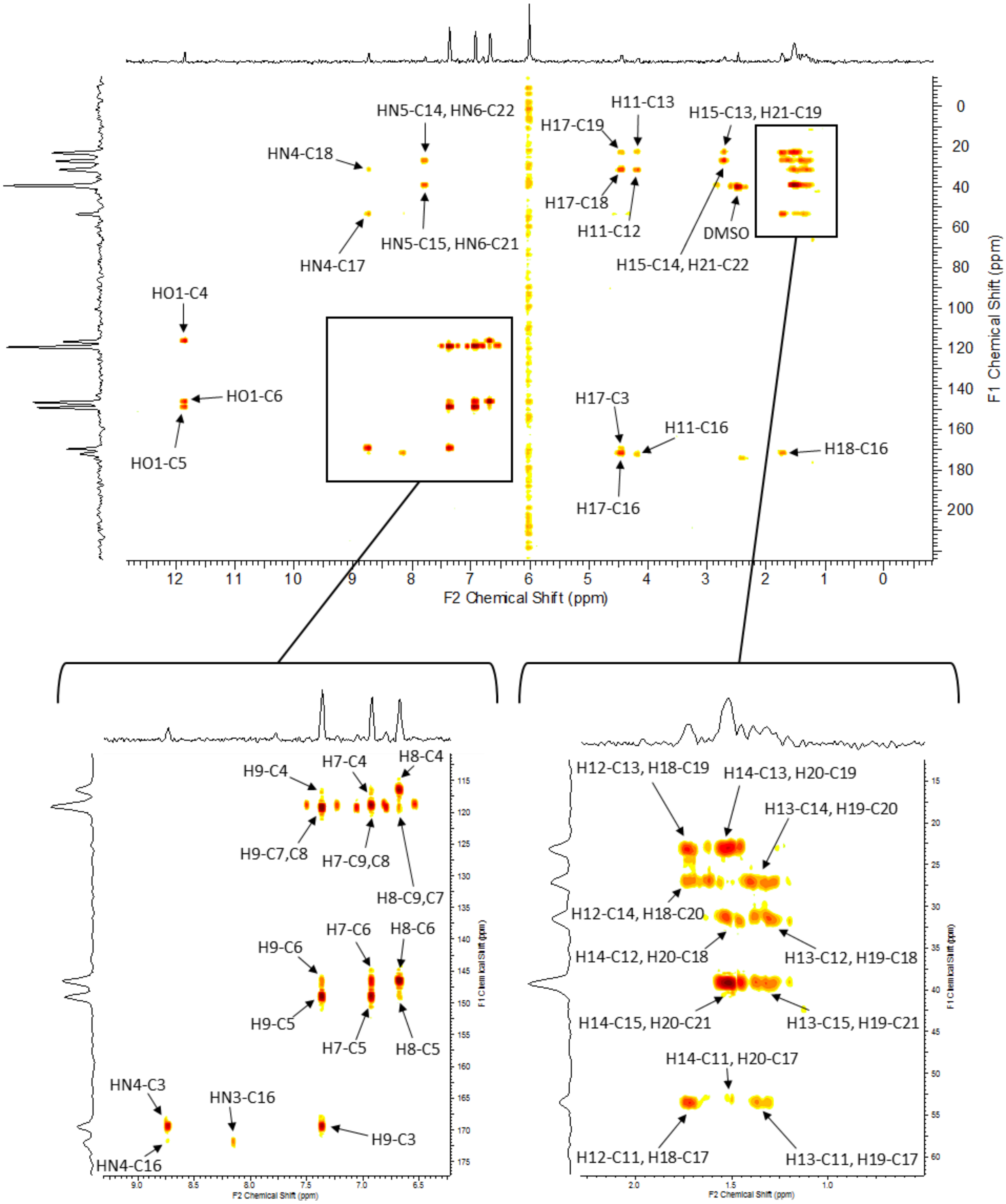


Figure S8. ^{1}H-^{13}C HMBC NMR for TLLC. NMR (600 MHz) in DMSO. Enlarged regions of the ^{1}H-^{13}C HMBC NMR for TLLC are in bottom panel. The spectrum is annotated with the correlations between specific carbons and hydrogens. See figure S9B for numbered positions.

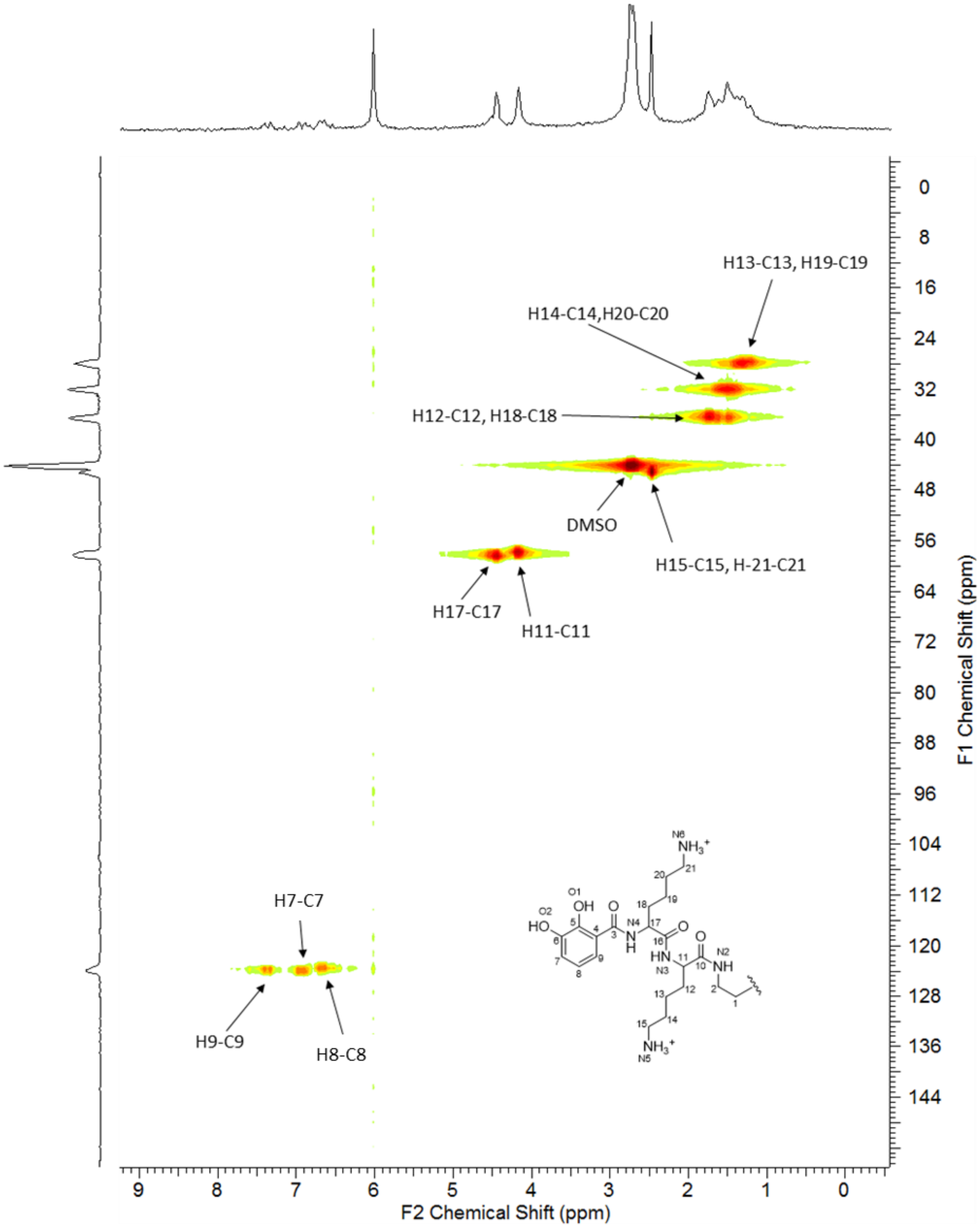


Figure S9. ^1H-^{13}C HSQC NMR for TLLC. NMR (600 MHz) in DMSO. The spectrum is annotated with the correlations between specific carbons and hydrogens.

Table S1. NMR Data for Tren-Arg-Cam and Tren-Lys-Lys-Cam. NMR (^{1}H on a Varian Unity Inova 600 MHz spectrometer and ^{13}C on a Varian Unity Inova 500 MHz spectrometer) was taken in D2O or DMSO. See Figs. S3 and S7 for NMR spectra.

	Tren-Arg-Cam (TAC)		Tren-Lys-Lys-Cam (TLLC)	
Position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
TREN				
1	Obscured by DMSO	3.61, m	Obscured by DMSO	Obscured by Water
2	Obscured by DMSO	3.51, m	Obscured by DMSO	Obscured by Water
DHBA				
3	168.76, C	-	168.69, C	-
4	115.93, C	-	114.83, C	-
5	148.38, C	-	148.50, C	-
6	146.04, C	-	146.10, C	-
7	118.18, CH	6.98, d (7.8)	118.20, CH	6.70, t (7.8)
8	118.81, CH	6.75, t (7.8)	118.81, CH	6.95, d (7.8)
9	118.44, CH	7.14, d (8.1)	118.37, CH	7.39, d (7.8)
Lys/Arg				
10	171.81, C	-	-	-
11	52.71, CH	4.38, m	52.48, CH	4.20, m
12	28.63, CH2	1.75, m	31.28, CH2	1.70, m
13	25.31, CH2	1.55, m	22.67, CH2	1.34, m
14	40.39, CH2	3.10, t (6.9)	26.63, CH2	1.53, m
15	156.71, C	-	38.76, CH$_2$	2.75, m
16	-	-	171.54, C	-
17	-	-	53.04, CH	4.48, m
18	-	-	30.94, CH2	1.70, m
19	-	-	22.33, CH2	1.34, m
20	-	-	26.63, CH2	1.53, m
21	-	-	38.68, CH2	2.75, m
Heteroatoms				
O1	-	-	-	11.88, s
O2	-	-	-	-
N1	-	-	-	-
N2	-	-	-	-
N3	-	-	-	8.17, m
N4	-	-	-	8.76, m
N5	-	-	-	7.81, m
N6	-	-	-	7.81, m

3. Supporting SFA Adhesion Measurements

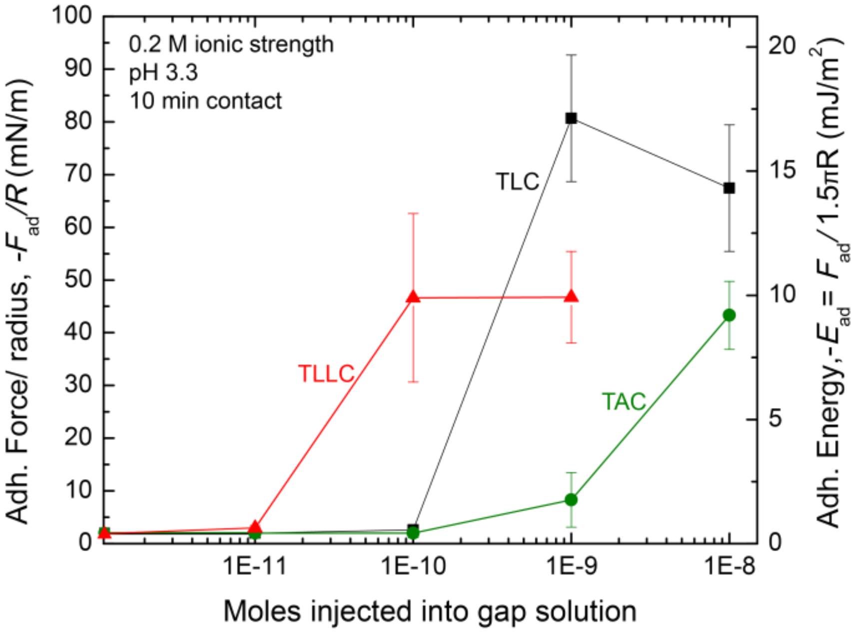


Figure S10: TLC-, TAC-, and TLLC-mediated adhesion force (and energy) required to separate two mica surfaces in aqueous solution, as a function of the number of moles of the siderophore analogs injected into the intervening gap solution between the mica surfaces. The total volume of intervening solution between the two mica surfaces was approximately 50 μL. Error bars represent one standard deviation.

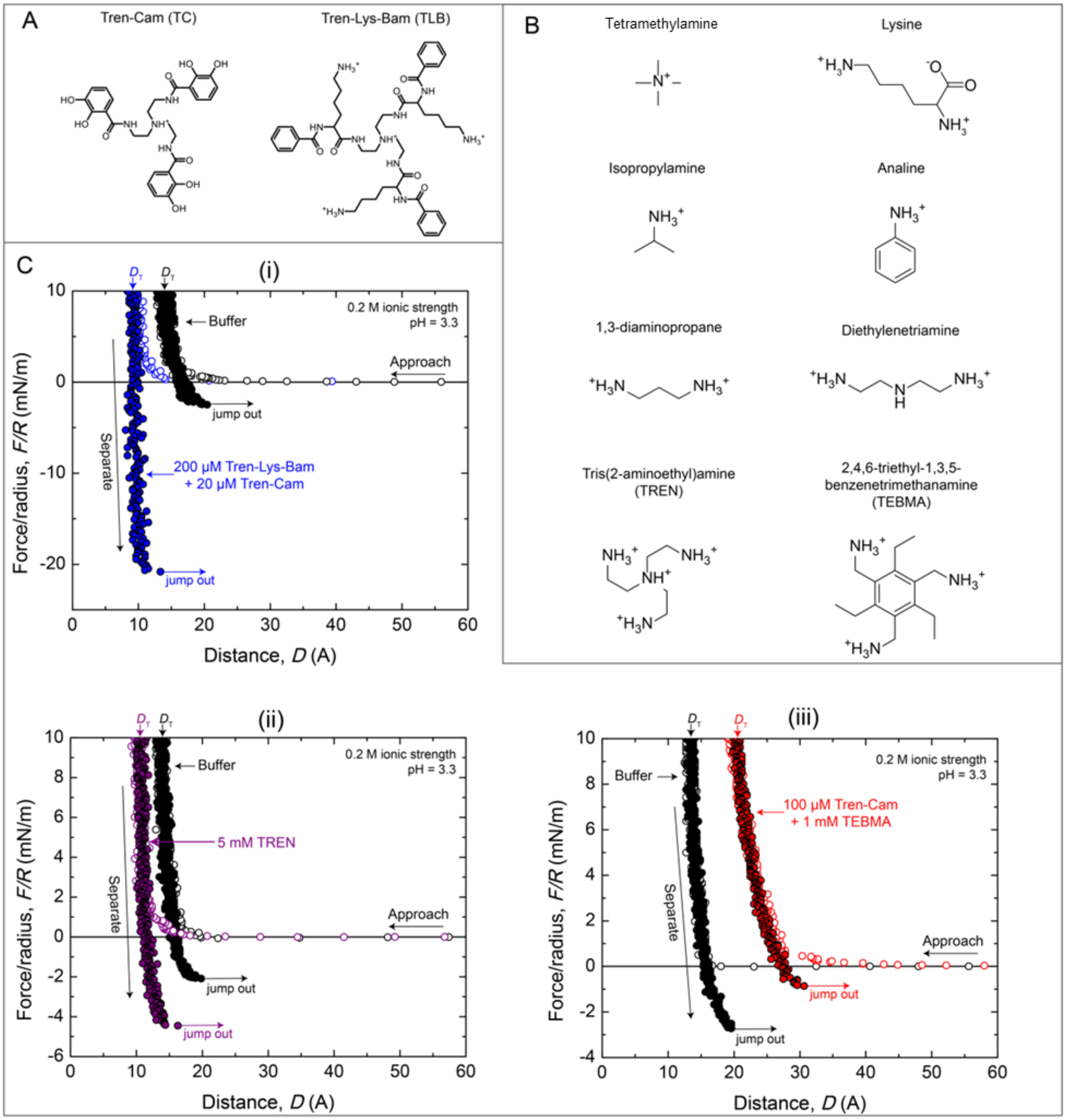


Figure S11: (A) Structures of siderophore analogs TC and TLB. (B) Structures of amine-containing compounds. Mixtures of varying ratios of the amine-containing compounds (0.1-5 mM) and Tren-Cam (0.02-1 mM) were prepared in buffer solution (150 mM KNO_3 + 50 mM acetate, pH 3.3) and injected into the gap solution between two mica surfaces in SFA measurements. (C) Representative SFA force-distance interaction for two mica surfaces in aqueous buffer (black circles) and various amine/catechol solutions (colored circles). Open circles represent measurements during the approach of the two surfaces, while closed circles represent measurements during separation. Mixtures of amine-containing compounds and Tren-Cam (contains no cationic functionalities) were unable to recreate the strong adhesive synergy of the siderophore analogs that contain both catechol and cationic moieties (TLC, TAC, TLLC). (i) Mixture of the siderophore anaologs TC and TLB (contains no catechol groups) produce force-distance interactions that are identical to the interactions in pure TLB solution. Thus, TLB outcompetes TC for adsorption sites and TLB mediates all adhesion between mica surfaces in mixtures with TC. (ii) 5mM solutions of TREN in buffer were able to displace some of the hydrated salt layer at the mica surfaces, and promote a minute increase in adhesion. Additions of TC into the TREN solution yielded no change to the interactions measured. (iii) In mixtures of 1 mM TEBMA and 0.1 mM TC, TEBMA adsorbs to form a thicker layer on the mica surfaces. No adhesive synergy is measured.

4. References

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