Supporting Information

Protease-Catalyzed L-Aspartate Oligomerization: Substrate Selectivity and Computational Modeling

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Table S1: A selection of relevant Rosetta scores **of papain and α-chymotrypsin acyl-intermediates with** the corresponding nucleophilic diester that illustrate the experimental trends.

	Nucleophile	Hydrolyzed Ester	Total		Catalytic Residues		Acyl Ester	
			Score	Constraints	Score	Constraints	Score	Constraints
α-chymotrypsin	Et_2-L-Asp	α	-448.4±1.2	21±2.9	0.3±0.4	3.2±0.1	-0.5±0.4	7.2±0.6
		β	-451.7±0.6	22.7±3.9	-0.5±0.4	3.8±0.6	-0.2±0.4	8.8±2.3
	Et_2-L-Glu	α	-442.7±5.7	25.2±7.3	1±0	6.7±4.5	1.4±1.8	10.7±4.3
		γ	-447.4±1.3	19.7±2.2	0.2±0.2	2.9±0.3	-0.8±0.1	8±1.7
papain	Et_2-L-Asp	α	-247.4±1.3	40.9±0.4	0.7±0.1	17.6±0.2	0.8±0.1	18.6±0.2
		β	-244±0.6	50.7±1.1	0.4±0	22.1±0.2	1.5±0.2	24.1±0.2
	Et_2-L-Glu	α	-245.7±0.4	38.1±2.1	1.2±0.1	16.3±0.2	1.2±0	17.5±0.4
		γ	-246.7±1.7	35.2±0.9	0.6±0.2	15.1±0.5	0.2±0.1	16.7±0.6

Table S2: The full atom intramolecular repulsive score of the acyl-ester scaled by 1000x demonstrating the preference of α polymerization in aspartic acid diethyl ester.

	nucleophile	hydrolyzed ester intermediate	Scaled fa_intra_rep of acylenzyme moiety
α-chymotrypsin	Et_2-L-Asp	α	7.6±0
		β	12.4±0.01
	Et_2-L-Glu	α	10.7±0.1
		γ	11.6±0.1
papain	Et_2-L-Glu	α	10±0
		γ	34±0

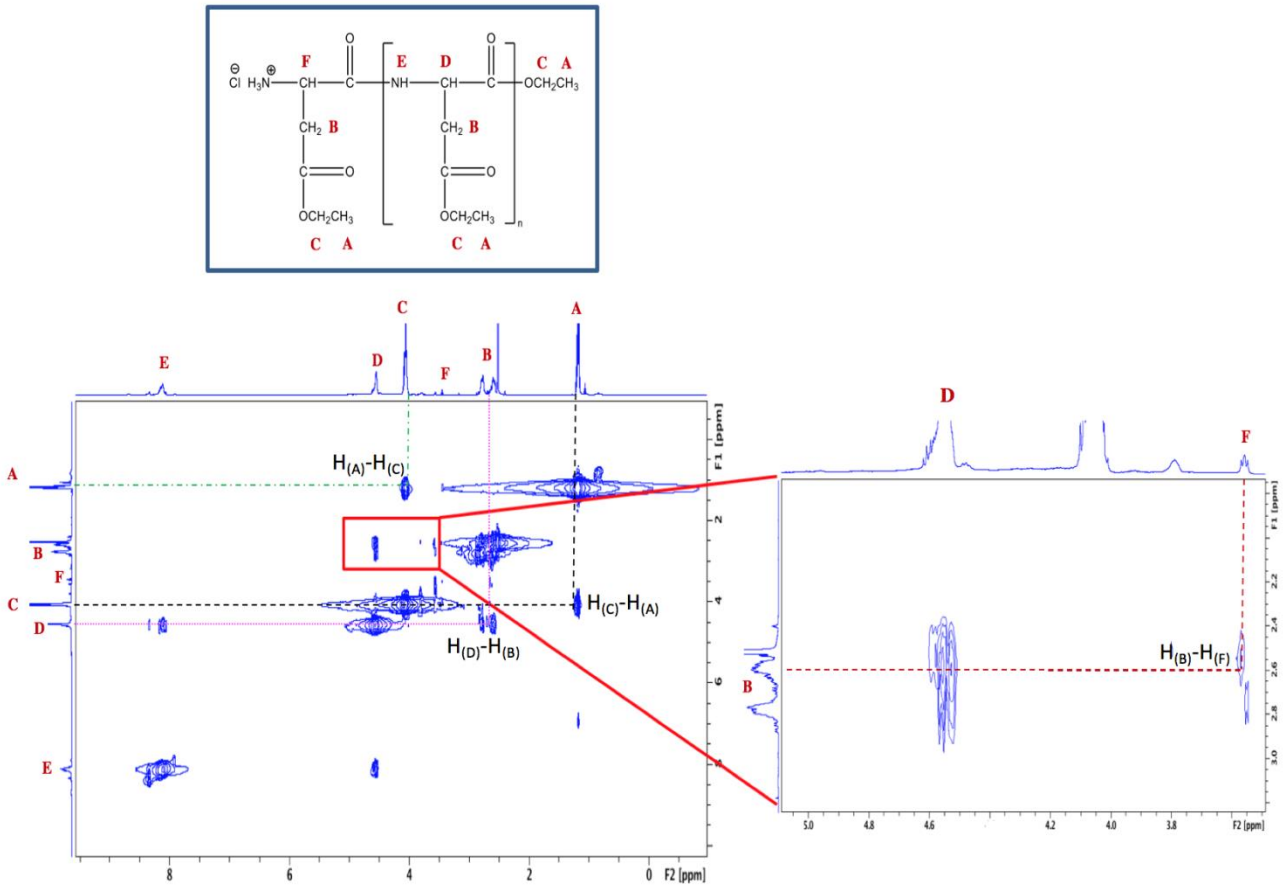

Figure S1: 2D ^{1}H-^{1}H COSY NMR (600MHz, DMSO-d_6) spectrum of oligo(L-aspartate) synthesized using 0.3 M L-aspartic acid diethyl ester hydrochloride, 2mg/mL α-chymotrypsin, 0.6 M phosphate buffer, at 40 °C, for 15 min, at pH 8.5.

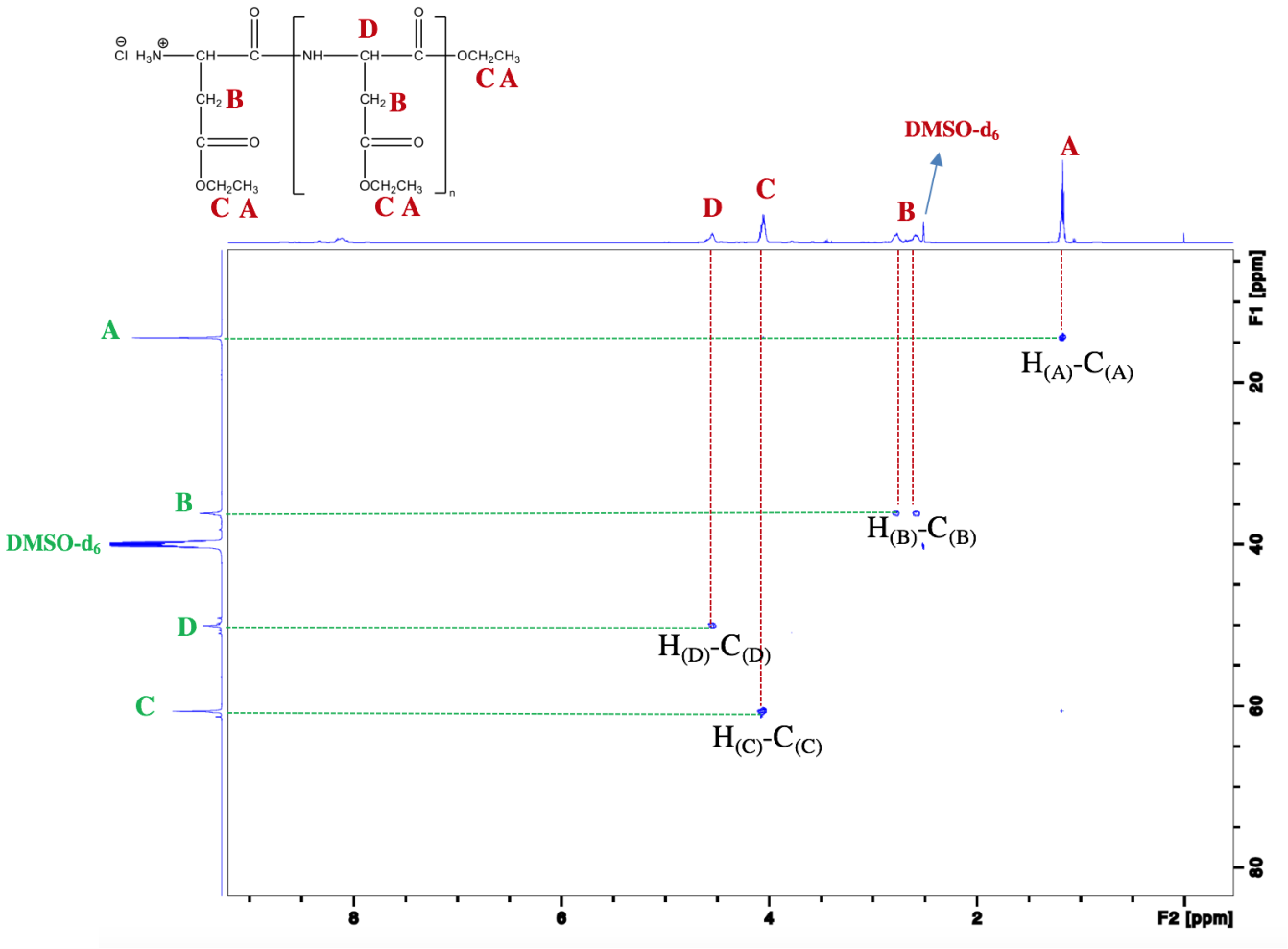

Figure S2: 2D HSQC NMR (600MHz, DMSO-d_6) spectrum of oligo(L-aspartate) synthesized using 0.3 M L-aspartic acid diethyl ester hydrochloride, 2mg/mL α-chymotrypsin, 0.6 M phosphate buffer, at 40 °C, for 15 min, at pH 8.5.

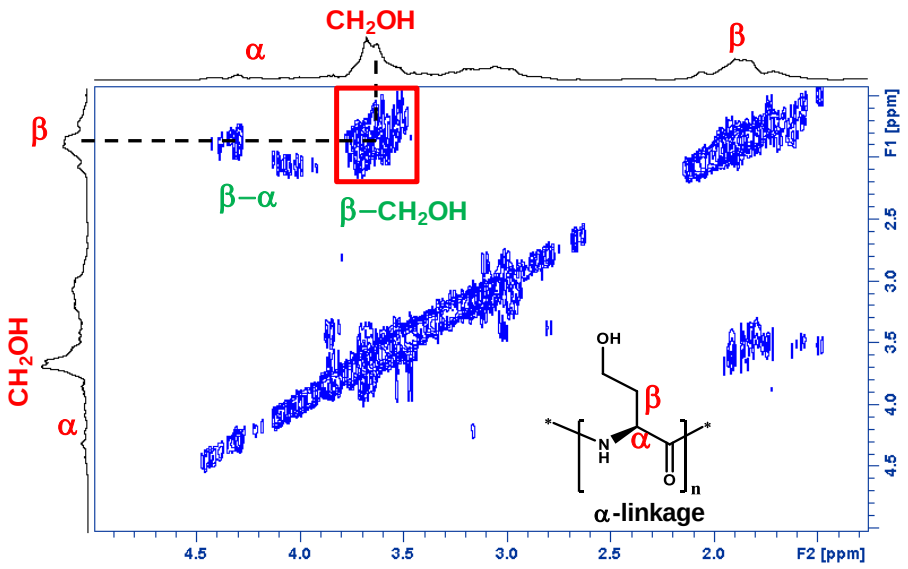

Figure S3. 2D $^{1}H,^{1}H$ COSY spectrum in D_2O of reduced oligoAsp. The cross-peak between β-CH_2s and CH_2OHs in the red box shows that the peptide is α-linked.

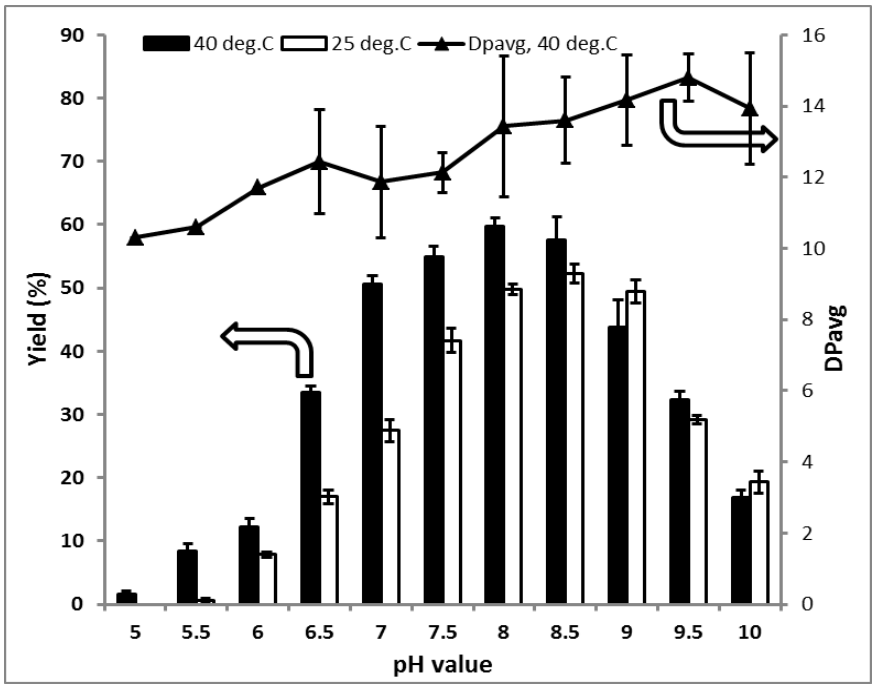

Figure S4. Relationship between reaction pH and oligo(Et-Asp) yield and DP_{avg}. Reactions were conducted using 0.5 M L-aspartic acid diethyl ester hydrochloride, 2mg/mL α-chymotrypsin, 0.6 M phosphate buffer at 40 °C for 5 min. Values are the mean from triplicate experiments. Error bars define the standard deviation.

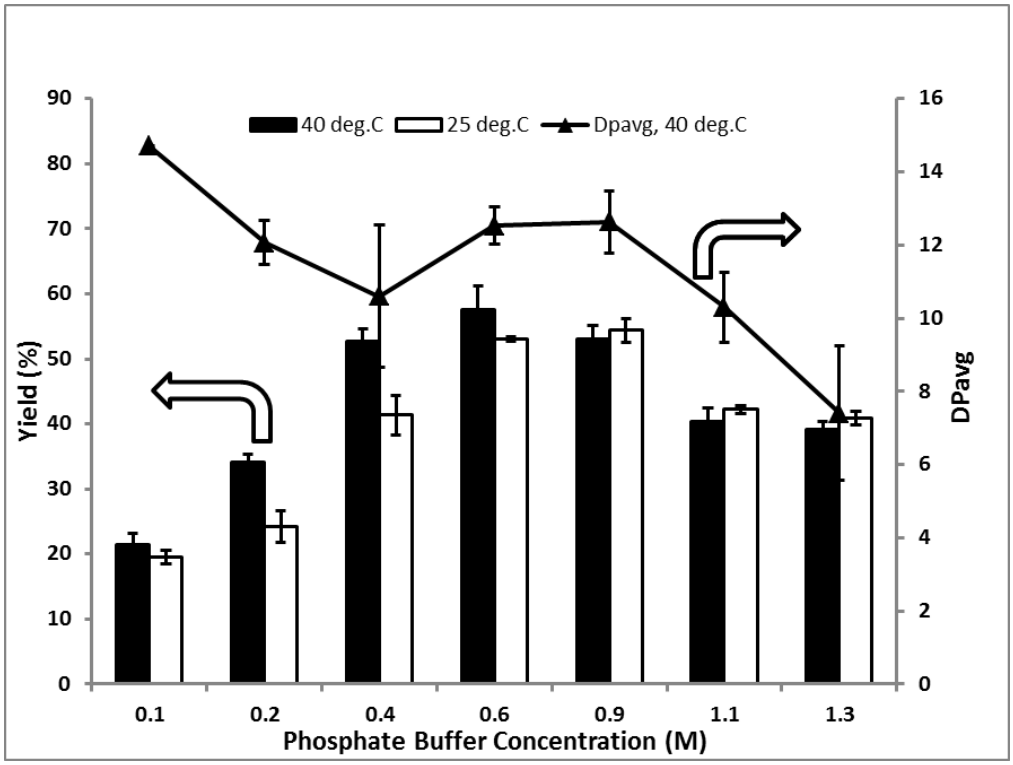

Figure S5. Effect of buffer concentration on oligo(Et-Asp) yield and DP_{avg}. Reactions were conducted using 0.5 M Et_2-L-Asp, 2mg/mL α-chymotrypsin, at 40 °C, for 5 min at pH 8. Values are the mean from triplicate experiments. Error bars define the standard deviation.