Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information

The pH Dependent Mechanisms of Non-enzymatic Peptide Bond Cleavage Reactions

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1. Confidence interval calculation.

The uncertainty in the parameter estimates are calculated based on statistical mesures.[1] The quantity N_m is the number of measured variables, which is equal to 4 here, since we have four measured variables: G, GG, cGG and GGG; N_d is the number of samples of each measured variable, which is equal to 7 in our case, since we have seven time points (not counting the initial point); N_p is the number of parameters, which is equal to 7 in our case, for k_1–k_4 and the three initial conditions for GG, cGG, and GGG.

The error covariance matrix (V_{ii}) is calculated is calculated following Equations (5)–(6).

$$V_{ii} = \frac{1}{N_d}\sum_{j=1}^{N_d} {e_{ij}}^2(k) \quad (5)$$

$$e_{ij} = y_{ij} - \tilde{y}_{ij} \quad (6)$$

where y_{ij} and $\tilde{y}_{ij}$represent the actual and model prediction of the i[th] measured variable and j[th] sampling time. Assuming the model can be represented by linear functions, the Jacobian matrix is obtained as

$$B_{ij} = \frac{\partial \tilde{y}_{ij}}{\partial k}\Big|_{k=k^*} \quad (7)$$

The 95% confidence interval for each parameter is obtained with $k - k^*$ as the boundary following Equations (8)–(10).

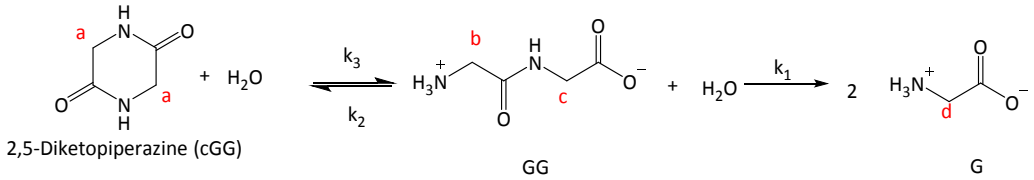
$$V_\theta^{-1} = \sum_{i=1}^{N_m}\sum_{j=1}^{N_d} B_{ij}^T V^{-1} B_{ij} \quad (8)$$

$$(k - k^*)^T V_\theta^{-1}(k - k^*) = \chi^2_{Np}(0.95) \quad (9)$$

$$(k - k^*) = \sqrt{\chi^2_{Np}(0.95)/V_\theta^{-1}} \quad (10)$$

II. Supporting materials

Figure S1. Stacked ^{1}H NMR spectra for GG decomposition at 95°C under pH 3. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.4ppm to 4.0ppm.



2,5-Diketopiperazine (cGG) GG G

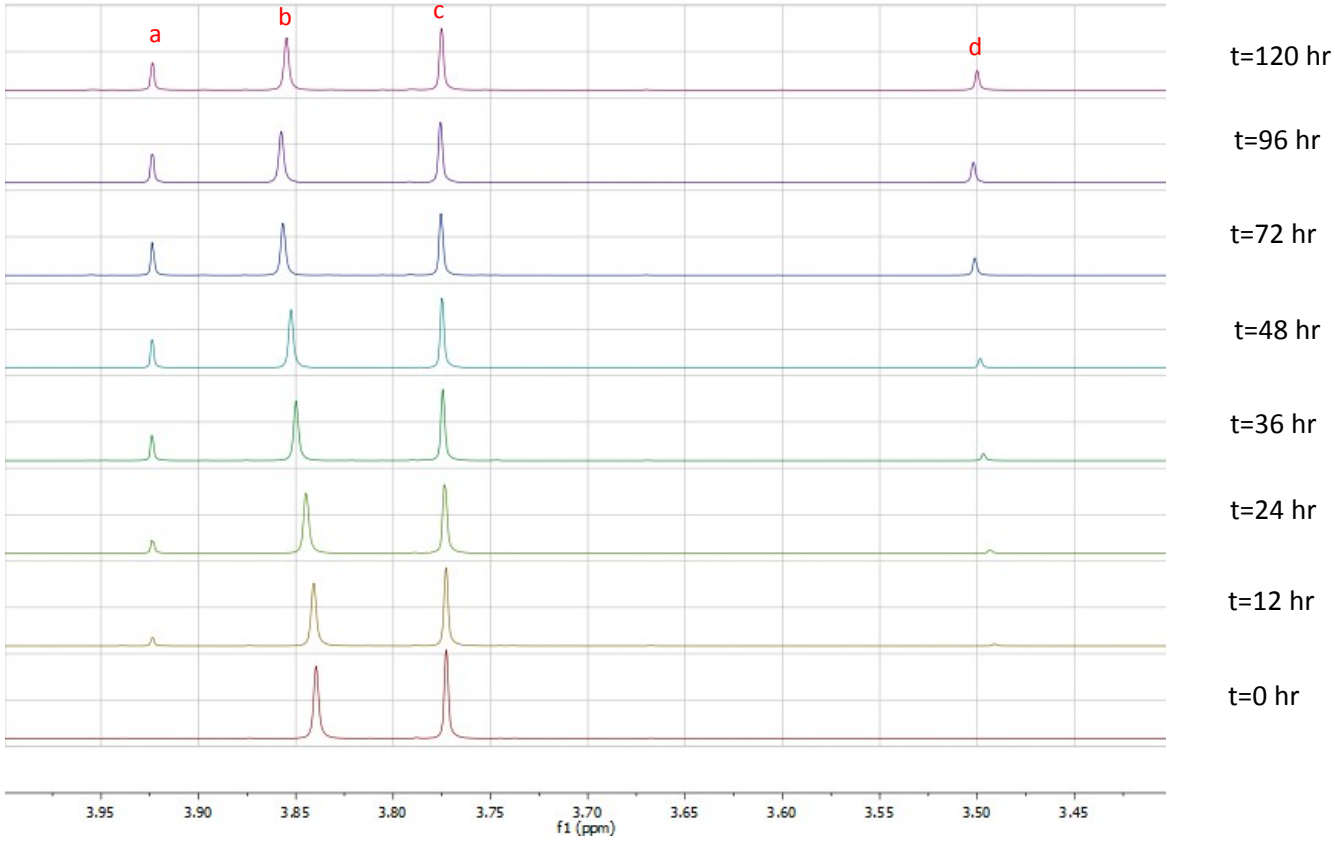
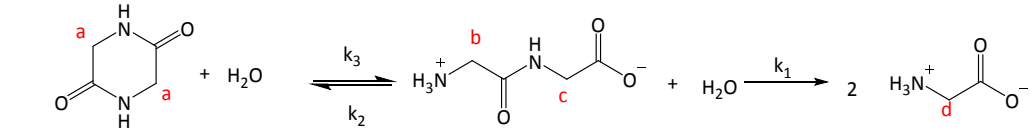


Table S1. GG degradation reactants and products amounts at varying reaction time under pH 3 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=3 GG analysis	NMR integrations				actual amount (μmol)		
time(h)	KHP	G	GG	cGG	G(μmol)	GG(μmol)	cGG (μmol)
0	1	0	2.1	0.01	0	31.5	0.15
0	1	0	2.1	0.01	0	31.5	0.15
0	1	0	2.12	0.01	0	31.8	0.15
12	1	0.02	1.9	0.08	0.6	28.5	1.2
12	1	0.03	1.93	0.1	0.9	28.95	1.5
12	1	0.02	1.92	0.09	0.6	28.8	1.35
24	1	0.04	1.85	0.14	1.2	27.75	2.1
24	1	0.05	1.84	0.16	1.5	27.6	2.4
24	1	0.04	1.85	0.15	1.2	27.75	2.25
36	1	0.08	1.68	0.25	2.4	25.2	3.75
36	1	0.09	1.65	0.25	2.7	24.75	3.75
36	1	0.08	1.76	0.24	2.4	26.4	3.6
48	1	0.11	1.63	0.27	3.3	24.45	4.05
48	1	0.11	1.63	0.28	3.3	24.45	4.2
48	1	0.11	1.65	0.28	3.3	24.75	4.2
72	1	0.2	1.47	0.31	6	22.05	4.65
72	1	0.17	1.52	0.3	5.1	22.8	4.5
72	1	0.2	1.52	0.31	6	22.8	4.65
96	1	0.25	1.49	0.3	7.5	22.35	4.5
96	1	0.25	1.47	0.3	7.5	22.05	4.5
96	1	0.23	1.49	0.29	6.9	22.35	4.35
120	1	0.23	1.49	0.27	6.9	22.35	4.05
120	1	0.26	1.46	0.27	7.8	21.9	4.05
120	1	0.24	1.48	0.28	7.2	22.2	4.2

Figure S2. Stacked ^{1}H NMR spectra for GG decomposition at 95°C under pH 5. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.4ppm to 4.05ppm.



2,5-Diketopiperazine (cGG) GG G

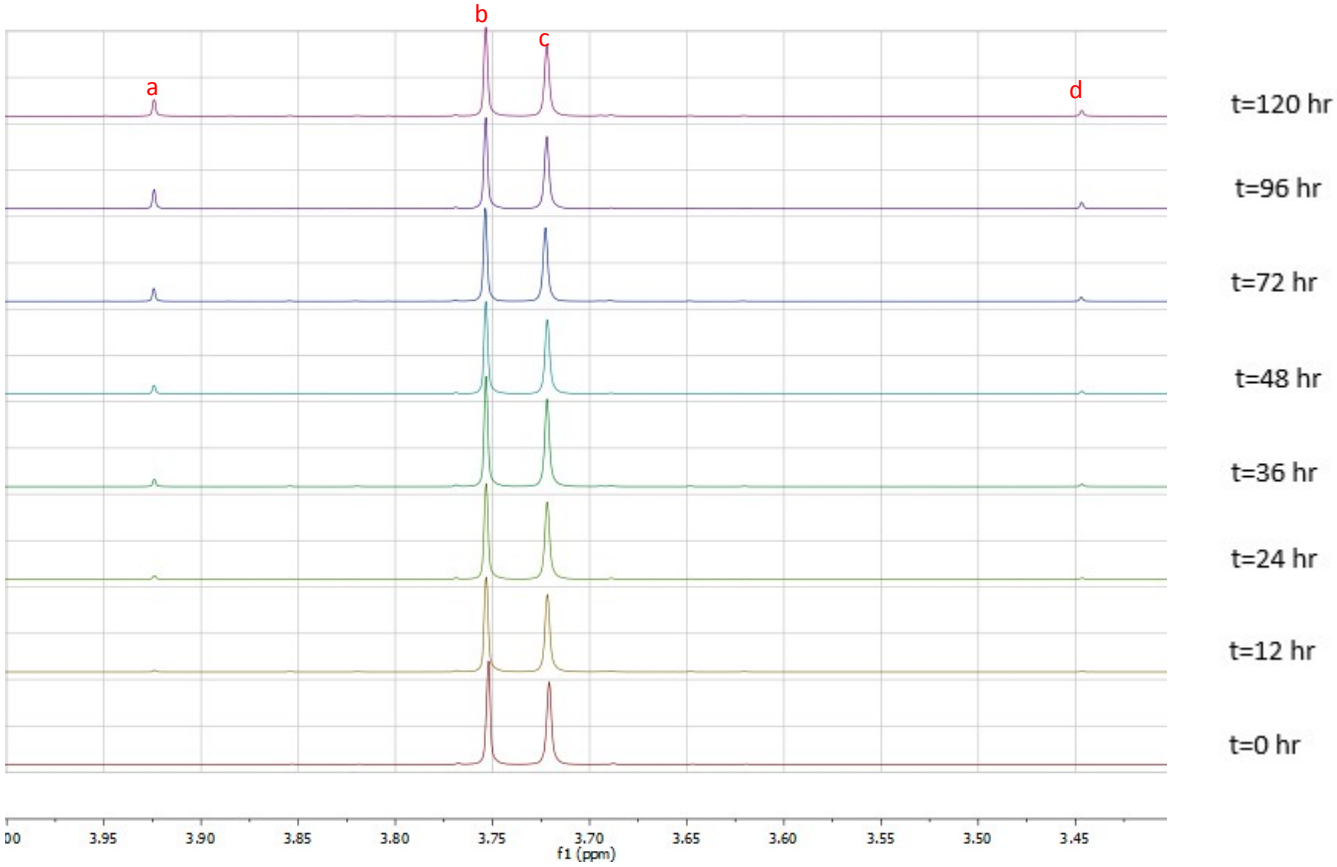
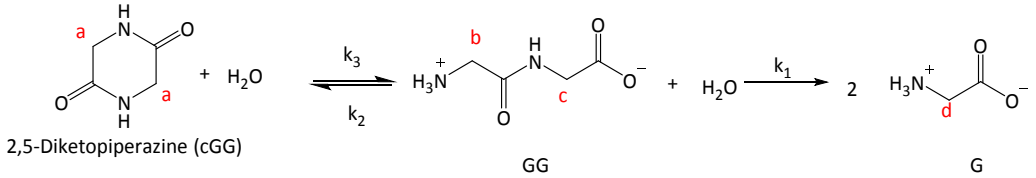


Table S2. GG degradation reactants and products amounts at varying reaction time under pH 5 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=5 GG analysis	NMR integrations				actual amount (µmol)		
time(h)	KHP	G	GG	cGG	G (µmol)	GG (µmol)	cGG (µmol)
0	1	0	2.62	0	0	39.3	0
0	1	0	2.62	0	0	39.3	0
0	1	0	2.64	0	0	39.6	0
12	1	0.01	2.5	0.02	0.3	37.5	0.3
12	1	0.01	2.47	0.03	0.3	37.05	0.45
12	1	0.01	2.47	0.02	0.3	37.05	0.3
24	1	0.01	2.46	0.04	0.3	36.9	0.6
24	1	0.01	2.45	0.03	0.3	36.75	0.45
24	1	0.01	2.48	0.03	0.3	37.2	0.45
36	1	0.02	2.43	0.07	0.6	36.45	1.05
36	1	0.02	2.42	0.06	0.6	36.3	0.9
36	1	0.03	2.43	0.08	0.9	36.45	1.2
48	1	0.03	2.36	0.09	0.9	35.4	1.35
48	1	0.03	2.36	0.07	0.9	35.4	1.05
48	1	0.04	2.37	0.1	1.2	35.55	1.5
72	1	0.04	2.25	0.13	1.2	33.75	1.95
72	1	0.06	2.27	0.17	1.8	34.05	2.55
72	1	0.05	2.31	0.15	1.5	34.65	2.25
96	1	0.07	2.2	0.19	2.1	33	2.85
96	1	0.07	2.21	0.21	2.1	33.15	3.15
96	1	0.06	2.27	0.17	1.8	34.05	2.55
120	1	0.06	2.25	0.18	1.8	33.75	2.7
120	1	0.07	2.18	0.19	2.1	32.7	2.85
120	1	0.08	2.16	0.23	2.4	32.4	3.45

Figure S3. Stacked ^{1}H NMR spectra for GG decomposition at 95°C under pH 7. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.3ppm to 4.2ppm.



2,5-Diketopiperazine (cGG) GG G

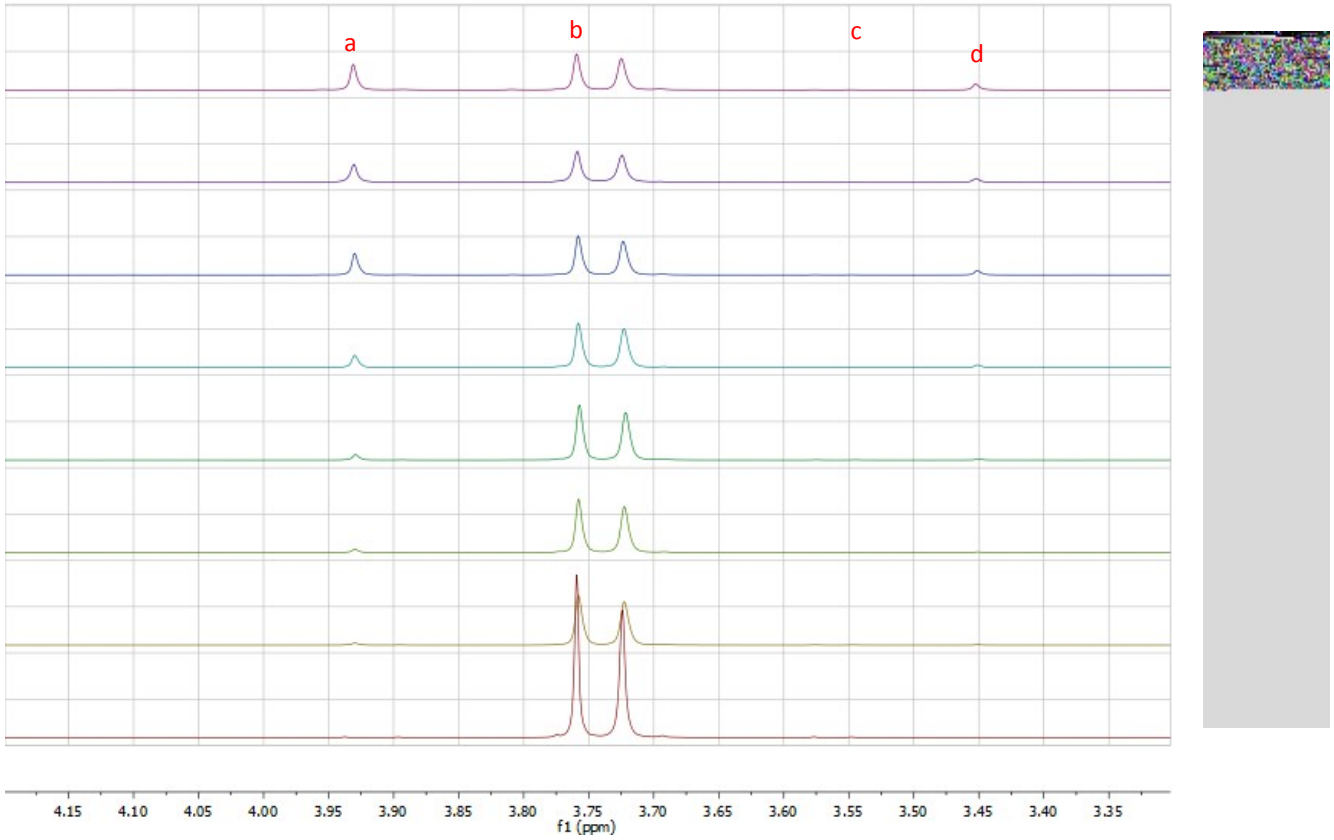
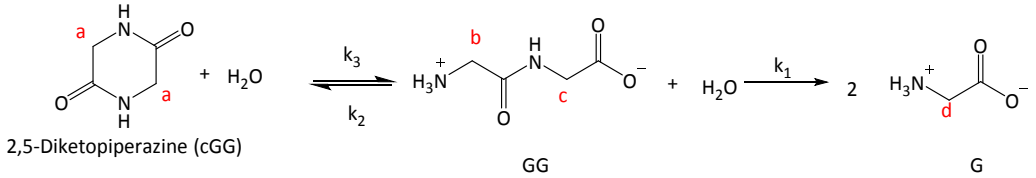


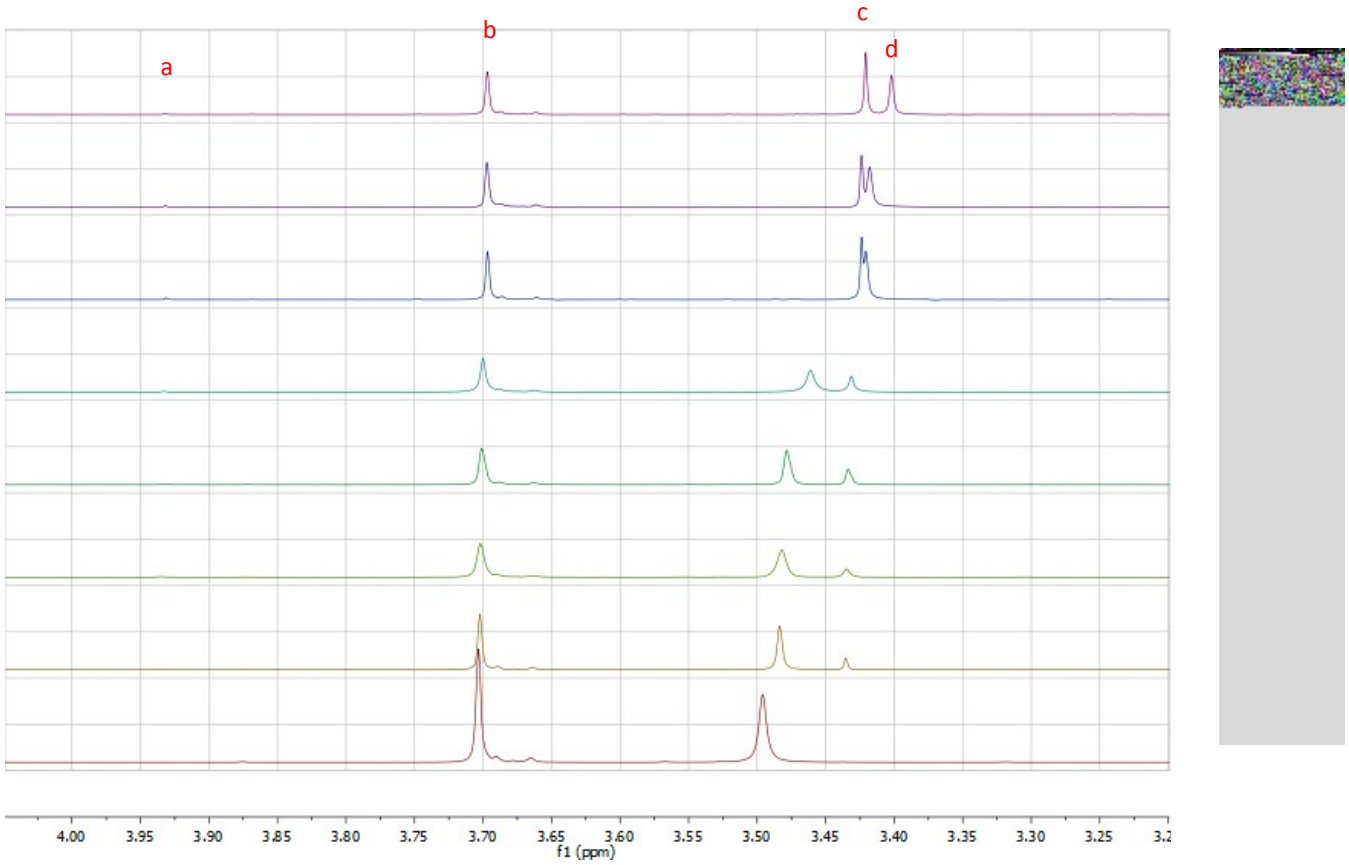
Table S3. GG degradation reactants and products amounts at varying reaction time under pH 7 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=7 GG analysis	NMR integrations				actual amount		
time(h)	KHP	G	GG	cGG	G(μmol)	GG(μmol)	cGG (μmol)
0	1	0	2.34	0	0	35.1	0
0	1	0	2.32	0	0	34.8	0
0	1	0	2.34	0	0	35.1	0
16	1	0.01	2.13	0.04	0.3	31.95	0.6
16	1	0.01	2.05	0.04	0.3	30.75	0.6
16	1	0.01	2.17	0.07	0.3	32.55	1.05
24	1	0.01	2.18	0.07	0.3	32.7	1.05
24	1	0.02	2.11	0.09	0.6	31.65	1.35
24	1	0.01	2.21	0.06	0.3	33.15	0.9
40	1	0.02	2.19	0.11	0.6	32.85	1.65
40	1	0.02	2.03	0.12	0.6	30.45	1.8
40	1	0.04	2	0.19	1.2	30	2.85
48	1	0.05	1.95	0.24	1.5	29.25	3.6
48	1	0.03	1.97	0.17	0.9	29.55	2.55
48	1	0.02	1.99	0.14	0.6	29.85	2.1
72	1	0.05	1.89	0.24	1.5	28.35	3.6
72	1	0.05	1.9	0.29	1.5	28.5	4.35
72	1	0.09	1.65	0.42	1.5	27.3	3.75
96	1	0.1	1.62	0.41	3	24.3	6.15
96	1	0.06	1.84	0.31	1.8	27.6	4.65
96	1	0.05	1.82	0.25	1.5	27.3	3.75
120	1	0.12	1.61	0.51	3.6	24.15	7.65
120	1	0.06	1.8	0.32	1.8	27	4.8
120	1	0.07	1.74	0.34	2.1	26.1	5.1

Figure S4. Stacked ^{1}H NMR spectra for GG decomposition at 95°C under pH 10. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.2ppm to 4.1ppm.



2,5-Diketopiperazine (cGG) GG G



The chemical shift over time for peak assignment is due to pH changes.

Table S4. GG degradation reactants and products amounts at varying reaction time under pH 10 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=10 GG analysis	NMR integrations				actual amount		
time(h)	KHP	G	GG	cGG	G(μmol)	GG(μmol)	cGG (μmol)
0	1	0	2.06	0	0	30.9	0
0	1	0	2	0	0	30	0
0	1	0	2.1	0	0	31.5	0
16	1	0.04	2.02	0.01	1.2	30.3	0.15
16	1	0.05	1.96	0.01	1.5	29.4	0.15
16	1	0.05	1.98	0.01	1.5	29.7	0.15
24	1	0.1	1.92	0.01	3	28.8	0.15
24	1	0.09	1.82	0.01	2.7	27.3	0.15
24	1	0.08	1.92	0.01	2.4	28.8	0.15
40	1	0.17	1.74	0.01	5.1	26.1	0.15
40	1	0.17	1.76	0.01	5.1	26.4	0.15
40	1	0.17	1.72	0.01	5.1	25.8	0.15
48	1	0.23	1.74	0.01	6.9	26.1	0.15
48	1	0.24	1.68	0.02	7.2	25.2	0.3
48	1	0.24	1.66	0.02	7.2	24.9	0.3
72	1	0.47	1.46	0.01	14.1	21.9	0.15
72	1	0.46	1.4	0.01	13.8	21	0.15
72	1	0.46	1.46	0.01	13.8	21.6	0.15
96	1	0.51	1.36	0.01	15.3	20.4	0.15
96	1	0.5	1.42	0.01	15	21.3	0.15
96	1	0.51	1.44	0.01	15.3	21.6	0.15
120	1	0.58	1.35	0.01	17.4	20.25	0.15
120	1	0.58	1.34	0.01	17.4	20.1	0.15
120	1	0.6	1.34	0.01	18	20.1	0.15

Figure S5. Stacked ^{1}H NMR spectra for cGG decomposition at 95°C under pH 3. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.35ppm to 4ppm.

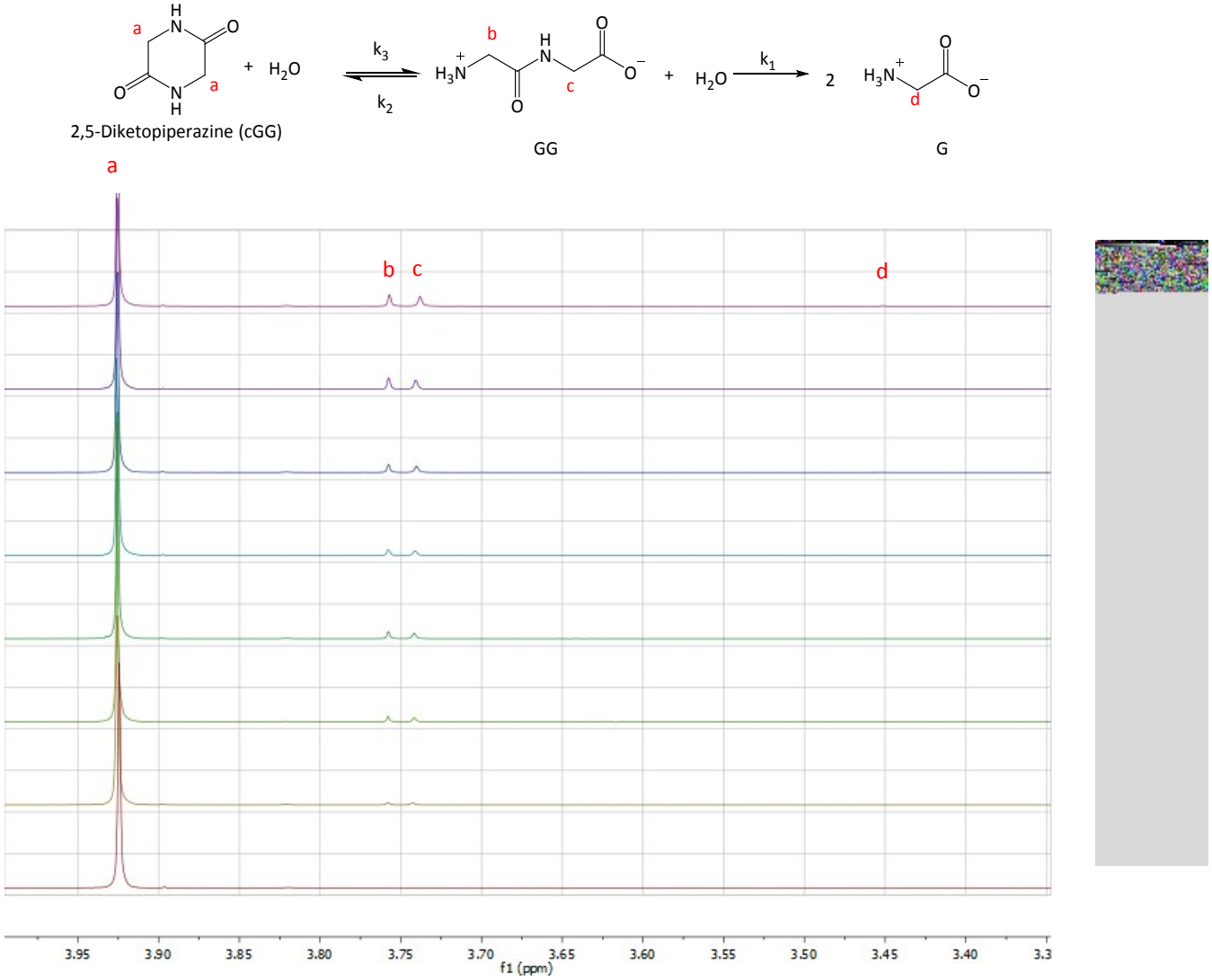
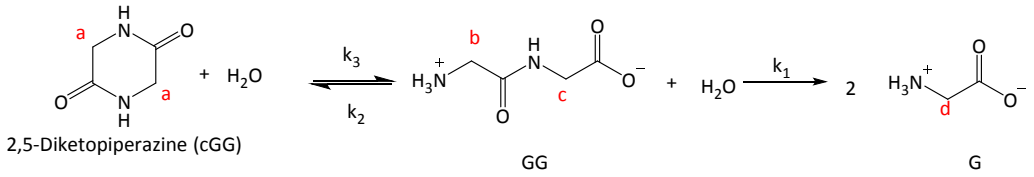


Table S5. cGG degradation reactants and products amounts at varying reaction time under pH 3 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=3 cGG analysis	NMR integrations				actual amount		
time(h)	KHP	G	GG	cGG	G(μmol)	GG(μmol)	cGG (μmol)
0	1	0	0	1.07	0	0	16.05
0	1	0	0	1.06	0	0	15.9
0	1	0	0	1.05	0	0	15.75
12	1	0	0.03	1.01	0	0.45	15.15
12	1	0	0.03	1	0	0.45	15
12	1	0	0.04	0.96	0	0.6	14.4
24	1	0	0.05	0.96	0	0.75	14.4
24	1	0	0.05	0.99	0	0.75	14.85
24	1	0	0.04	0.98	0	0.6	14.7
36	1	0	0.07	0.89	0	1.05	13.35
36	1	0	0.08	0.91	0	1.2	13.65
36	1	0	0.08	0.92	0	1.2	13.8
48	1	0	0.07	0.93	0	1.05	13.95
48	1	0	0.06	0.94	0	0.9	14.1
48	1	0	0.07	0.92	0	1.05	13.8
72	1	0	0.08	0.93	0	1.2	13.95
72	1	0	0.1	0.89	0	1.5	13.35
72	1	0	0.11	0.87	0	1.65	13.05
96	1	0	0.12	0.9	0	1.8	13.5
96	1	0	0.12	0.86	0	1.8	12.9
96	1	0	0.09	0.9	0	1.35	13.5
120	1	0	0.12	0.87	0	1.8	13.05
120	1	0	0.11	0.89	0	1.65	13.35
120	1	0	0.12	0.88	0	1.8	13.2

Figure S6. Stacked ^{1}H NMR spectra for cGG decomposition at 95°C under pH 5. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.3ppm to 4.1ppm.



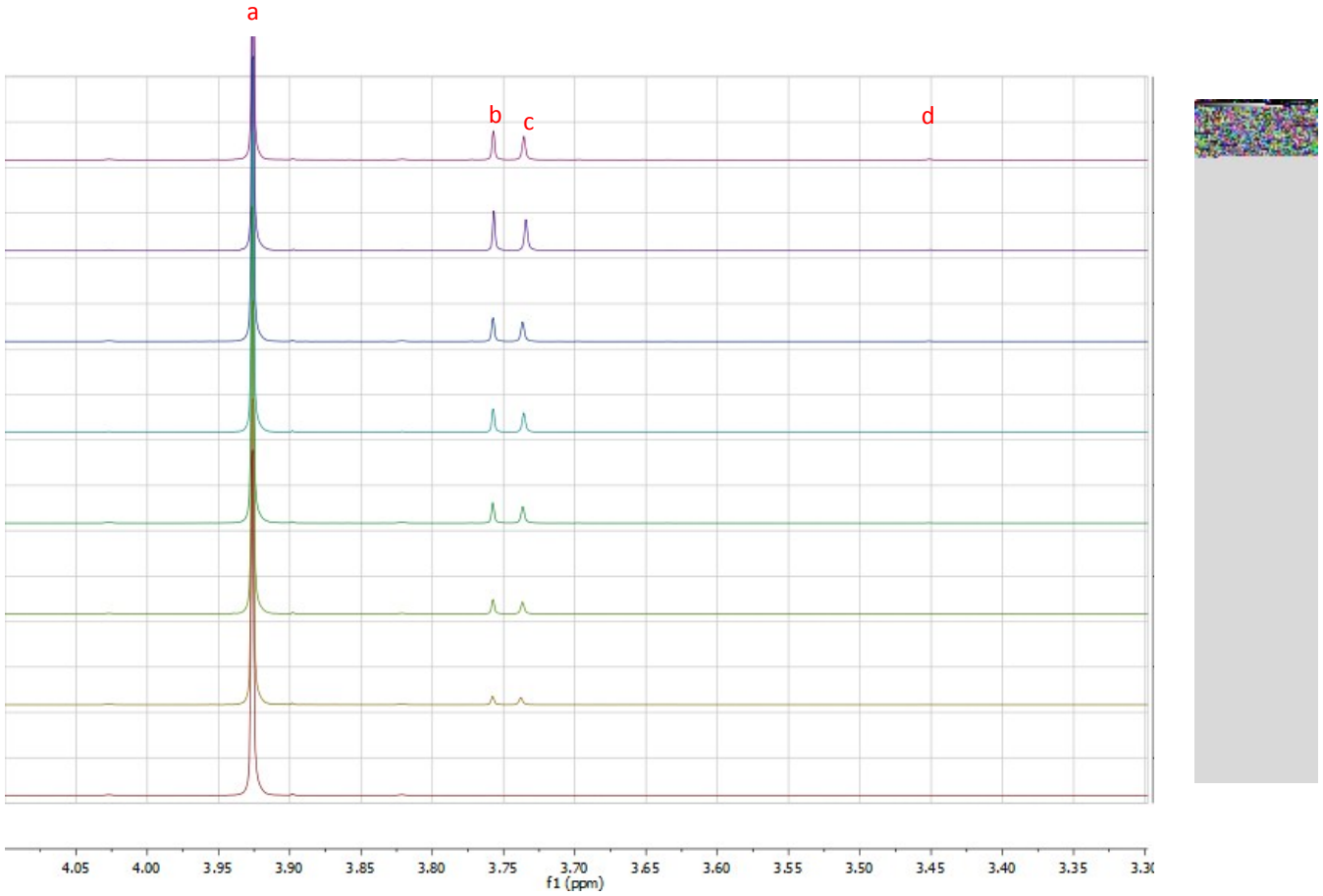
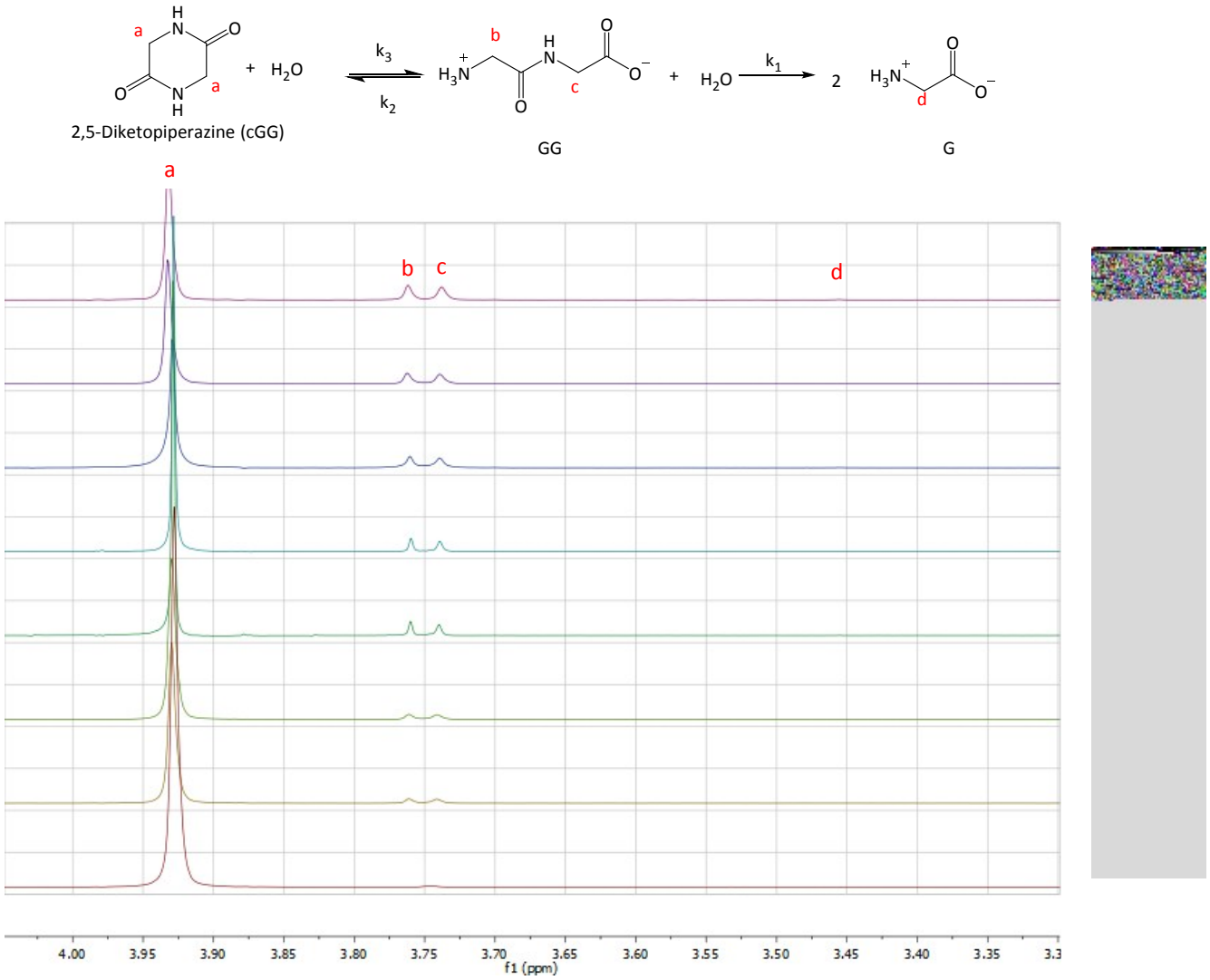


Table S6. cGG degradation reactants and products amounts at varying reaction time under pH 5 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=5 cGG analysis	NMR integrations				actual amount		
time(h)	KHP	G	GG	cGG	G(μmol)	GG(μmol)	cGG(μmol)
0	1	0	0	1.04	0	0	15.6
0	1	0	0	1.04	0	0	15.6
0	1	0	0	1.04	0	0	15.6
12	1	0	0.06	0.94	0	0.9	14.1
12	1	0	0.06	0.98	0	0.9	14.7
12	1	0	0.06	0.95	0	0.9	14.25
24	1	0	0.09	0.91	0	1.35	13.65
24	1	0	0.07	0.94	0	1.05	14.1
24	1	0	0.09	0.91	0	1.35	13.65
36	1	0	0.12	0.89	0	1.8	13.35
36	1	0	0.1	0.91	0	1.5	13.65
36	1	0	0.09	0.91	0	1.35	13.65
48	1	0	0.14	0.83	0	2.1	12.45
48	1	0	0.16	0.84	0	2.4	12.6
48	1	0	0.15	0.84	0	2.25	12.6
72	1	0	0.15	0.83	0	2.25	12.45
72	1	0	0.19	0.79	0	2.85	11.85
72	1	0	0.2	0.79	0	3	11.85
96	1	0	0.22	0.76	0	3.3	11.4
96	1	0	0.18	0.81	0	2.7	12.15
96	1	0	0.24	0.77	0	3.6	11.55
120	1	0	0.18	0.78	0	2.7	11.7
120	1	0	0.2	0.79	0	3	11.85
120	1	0	0.23	0.76	0	3.45	11.4

Figure S7. Stacked ^{1}H NMR spectra for cGG decomposition at 95°C under pH 7. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.3ppm to 4.05ppm.



2,5-Diketopiperazine (cGG)

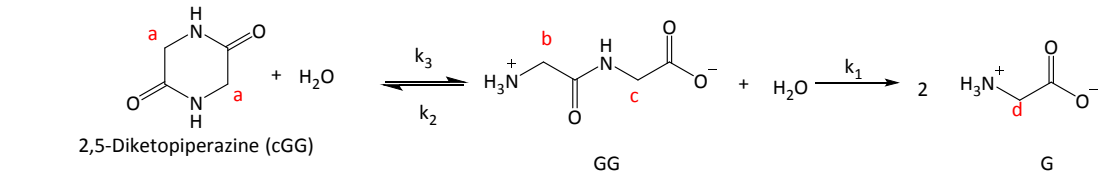
GG

G

Table S7. cGG degradation reactants and products amounts at varying reaction time under pH 7 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=7 cGG analysis	NMR integrations				actual amount		
time(h)	KHP	G	GG	cGG	G(µmol)	GG(µmol)	cGG (µmol)
0	1	0.01	0.01	1.3	0.3	0.15	19.5
0	1	0.01	0.01	1.3	0.3	0.15	19.5
0	1	0.01	0.01	1.26	0.3	0.15	18.9
16	1	0.01	0.07	1.19	0.3	1.05	17.85
16	1	0.01	0.07	1.12	0.3	1.05	16.8
16	1	0.01	0.05	1.15	0.3	0.75	17.25
24	1	0.01	0.08	1.14	0.3	1.2	17.1
24	1	0.02	0.07	1.12	0.6	1.05	16.8
24	1	0.01	0.07	1.17	0.3	1.05	17.55
40	1	0	0.12	1.08	0	1.8	16.2
40	1	0.01	0.11	1.1	0.3	1.65	16.5
40	1	0.01	0.11	1.07	0.3	1.65	16.05
48	1	0.01	0.11	1.07	0.3	1.65	16.05
48	1	0.01	0.11	1.07	0.3	1.65	16.05
48	1	0.01	0.13	1.07	0.3	1.95	16.05
72	1	0	0.19	0.98	0	2.85	14.7
72	1	0.01	0.2	1.25	0.3	3	18.75
72	1	0	0.17	1	0	2.85	15.15
96	1	0.01	0.2	1.06	0.3	3	15.9
96	1	0.01	0.2	1.21	0.3	3	18.15
96	1	0.01	0.19	1.01	0.3	2.85	15.15
120	1	0	0.22	1.02	0	3.3	15.3
120	1	0.01	0.28	1.16	0.3	4.2	17.4
120	1	0.01	0.26	1.15	0.3	3.9	17.25

Figure S8. Stacked ^{1}H NMR spectra for cGG decomposition at 95°C under pH 10. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.3ppm to 4.1ppm.



2,5-Diketopiperazine (cGG)

GG

G

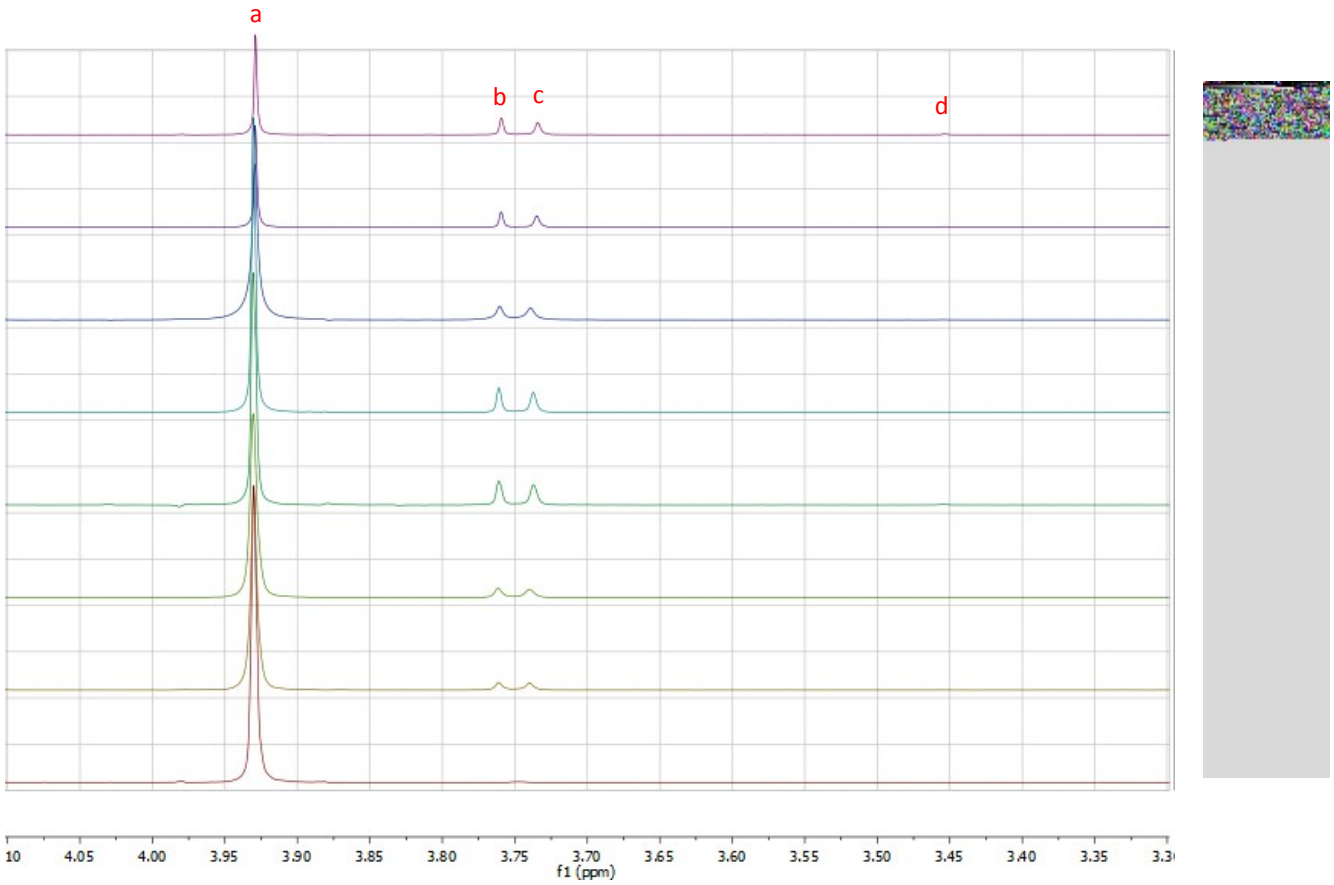
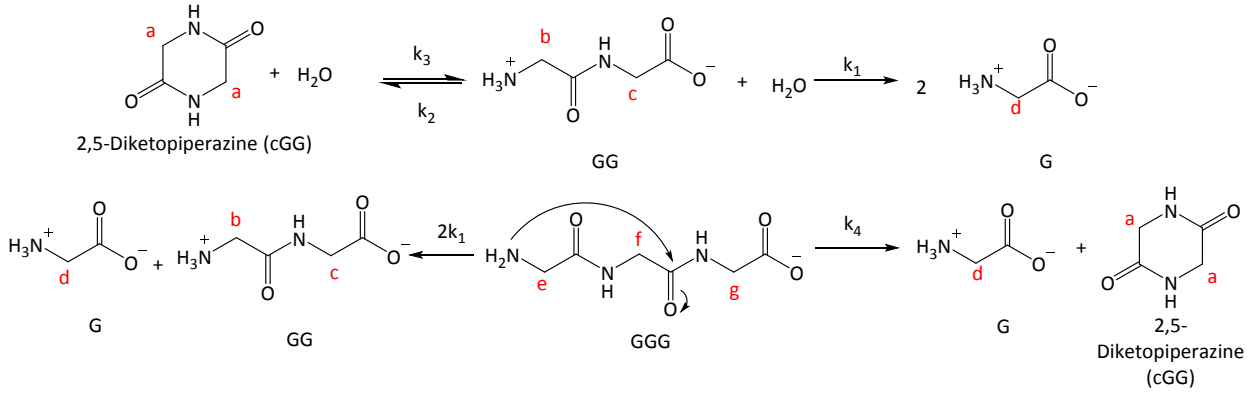


Table S8. cGG degradation reactants and products amounts at varying reaction time under pH 10 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=10 cGG analysis	NMR integrations				actual amount		
time(h)	KHP	G	GG	cGG	G(μmol)	GG(μmol)	cGG (μmol)
0	1	0	0	1.3	0	0	19.5
0	1	0	0	1.25	0	0	18.75
0	1	0	0	1.25	0	0	18.75
16	1	0	0.09	1.1	0	1.35	16.5
16	1	0	0.09	1.07	0	1.35	16.05
16	1	0	0.08	1.12	0	1.2	16.8
24	1	0	0.11	1.03	0	1.65	15.45
24	1	0	0.11	1.09	0	1.65	16.35
24	1	0	0.11	1.08	0	1.65	16.2
40	1	0	0.22	0.98	0	3.3	14.7
40	1	0	0.21	0.96	0	3.15	14.4
40	1	0	0.19	0.95	0	2.85	14.25
48	1	0	0.2	1.02	0	3	15.3
48	1	0	0.19	0.92	0	2.85	13.8
48	1	0	0.19	0.94	0	2.85	14.1
72	1	0	0.19	0.92	0	2.85	13.8
72	1	0	0.17	0.99	0	2.55	14.85
72	1	0	0.17	0.99	0	2.55	14.85
96	1	0.01	0.3	0.8	0.3	4.5	12
96	1	0.01	0.29	0.84	0.3	4.35	12.6
96	1	0.01	0.29	0.87	0.3	4.35	13.05
120	1	0.01	0.33	0.76	0.3	4.95	11.4
120	1	0.01	0.38	0.83	0.3	5.7	12.45
120	1	0.01	0.35	0.76	0.3	5.25	11.4

Figure S9. Stacked ^{1}H NMR spectra for GGG decomposition at 95°C under pH 3. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.3ppm to 4.1ppm.



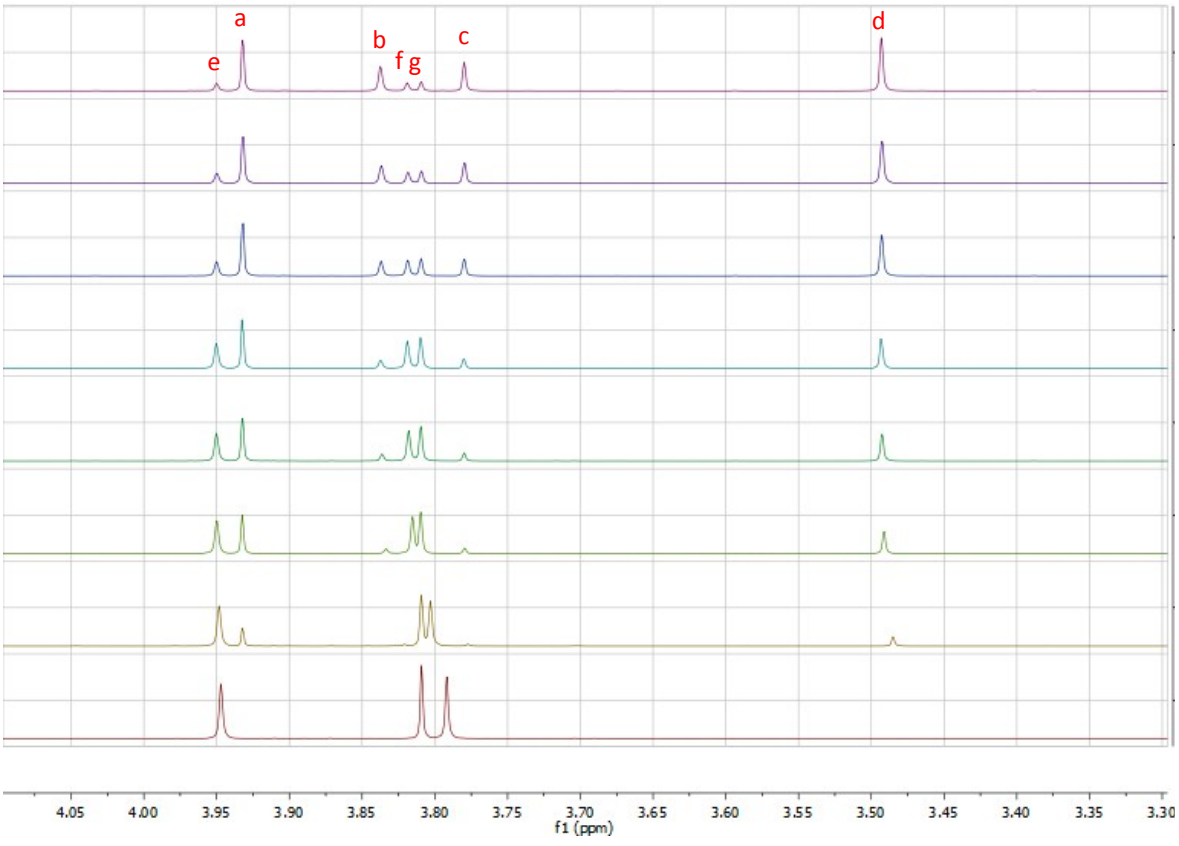


Table S9. GGG degradation reactants and products amounts at varying reaction time under pH 3 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=3 GGG analysis	NMR peak integrations					Actual amount			
time(h)	KHP	G	GG	cGG	GGG	G (µmol)	GG (µmol)	cGG (µmol)	GGG (µmol)
0	1	0	0.02	0	3.18	0	0.3	0	31.8
0	1	0	0.02	0	3.24	0	0.3	0	32.4
0	1	0	0.02	0	3.3	0	0.3	0	33
12	1	0.15	0.08	0.28	2.52	4.5	1.2	4.2	25.2
12	1	0.15	0.06	0.23	2.61	4.5	0.9	3.45	26.1
12	1	0.14	0.08	0.23	2.76	4.2	1.2	3.45	27.6
24	1	0.37	0.18	0.57	1.98	11.1	2.7	8.55	19.8
24	1	0.35	0.16	0.53	2.07	10.5	2.4	7.95	20.7
24	1	0.34	0.18	0.53	2.19	10.2	2.7	7.95	21.9
36	1	0.47	0.28	0.67	1.77	14.1	4.2	10.05	17.7
36	1	0.44	0.24	0.64	1.86	13.2	3.6	9.6	18.6
36	1	0.43	0.24	0.63	1.92	12.9	3.6	9.45	19.2
48	1	0.53	0.34	0.71	1.65	15.9	5.1	10.65	16.5
48	1	0.52	0.32	0.71	1.71	15.6	4.8	10.65	17.1
48	1	0.46	0.3	0.66	1.74	13.8	4.5	9.9	17.4
72	1	0.73	0.58	0.84	1.02	21.9	8.7	12.6	10.2
72	1	0.69	0.54	0.82	1.17	20.7	8.1	12.3	11.7
72	1	0.7	0.54	0.84	1.14	21	8.1	12.6	11.4
96	1	0.8	0.74	0.79	0.66	24	11.1	11.85	6.6
96	1	0.82	0.74	0.85	0.78	24.6	11.1	12.75	7.8
96	1	0.78	0.68	0.82	0.87	23.4	10.2	12.3	8.7
120	1	0.94	0.96	0.78	0.45	28.2	14.4	11.7	4.5
120	1	0.98	0.96	0.88	0.33	29.4	14.4	13.2	3.3
120	1	0.95	0.88	0.9	0.48	28.5	13.2	13.5	4.8

Figure S10. Stacked ^{1}H NMR spectra for GGG decomposition at 95°C under pH 5. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.2ppm to 4.1ppm.

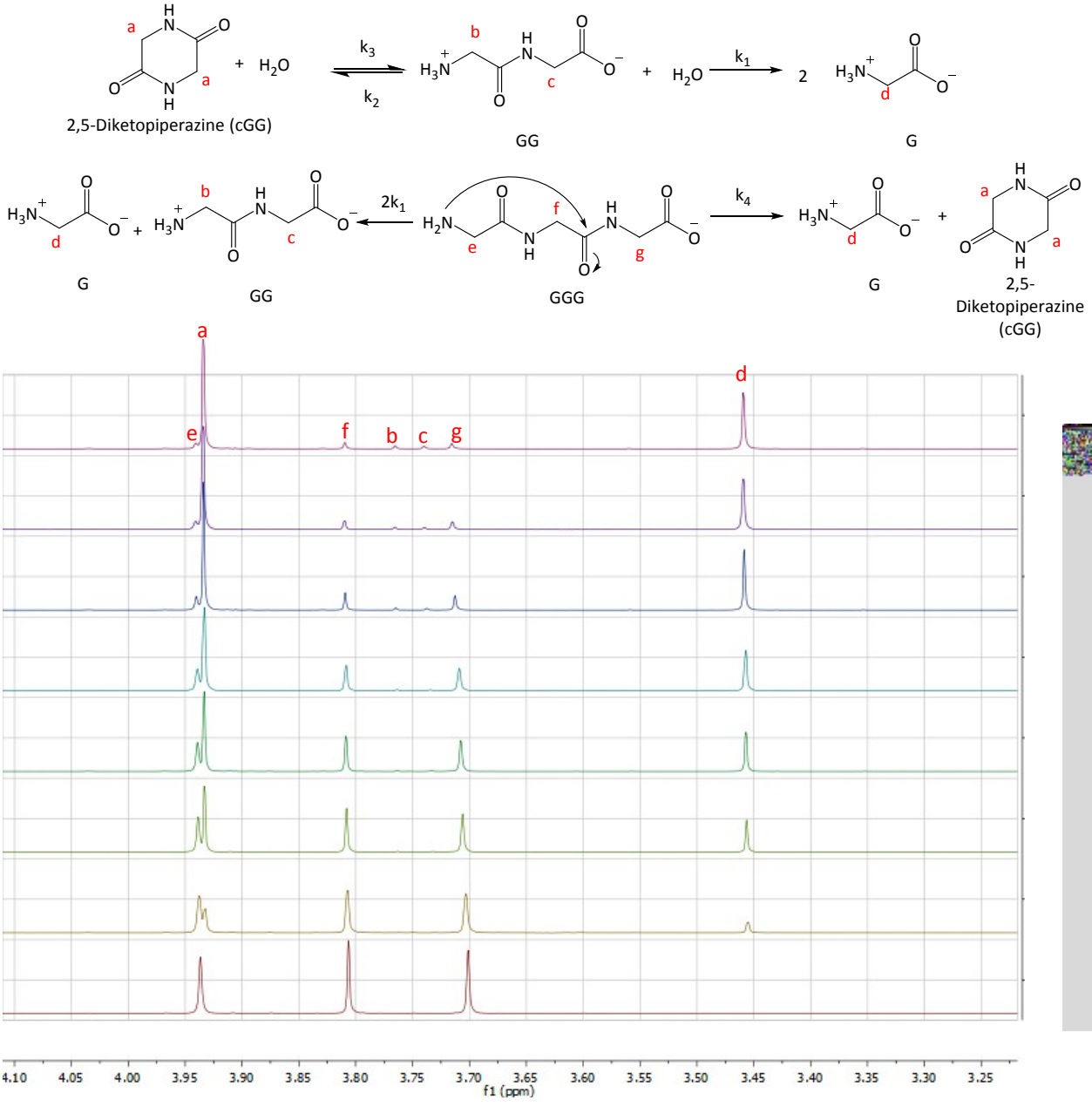
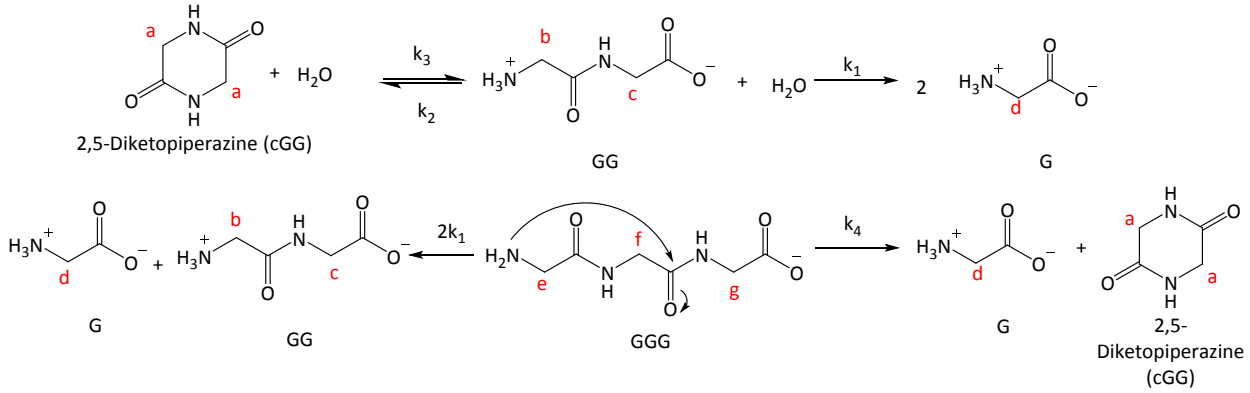


Table S10. GGG degradation reactants and products amounts at varying reaction time under pH 5 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=5 GGG analysis	NMR peak integrations					Actual amount (µmol)			
time(h)	KHP	G	GG	cGG	GGG	G (µmol)	GG (µmol)	cGG (µmol)	GGG (µmol)
0	1	0	0	0	3.81	0	0	0	38.1
0	1	0	0	0	3.84	0	0	0	38.4
0	1	0	0	0	3.84	0	0	0	38.4
12	1	0.24	0.02	0.44	3.03	7.2	0.3	6.6	30.3
12	1	0.22	0.02	0.43	3.09	6.6	0.3	6.45	30.9
12	1	0.21	0.02	0.4	3.12	6.3	0.3	6	31.2
24	1	0.51	0.03	0.98	2.16	15.3	0.45	14.7	21.6
24	1	0.47	0.03	0.9	2.37	14.1	0.45	13.5	23.7
24	1	0.42	0.03	0.81	2.46	12.6	0.45	12.15	24.6
36	1	0.63	0.04	1.23	1.77	18.9	0.6	18.45	17.7
36	1	0.6	0.04	1.18	1.92	18	0.6	17.7	19.2
36	1	0.54	0.04	1.2	1.62	16.2	0.6	18	16.2
48	1	0.76	0.05	1.47	1.47	22.8	0.75	22.05	14.7
48	1	0.65	0.04	1.25	1.77	19.5	0.6	18.75	17.7
48	1	0.57	0.04	1.11	1.95	17.1	0.6	16.65	19.5
72	1	0.96	0.07	1.81	0.84	28.8	1.05	27.15	8.4
72	1	0.9	0.06	1.67	0.96	27	0.9	25.05	9.6
72	1	0.82	0.06	1.5	1.17	24.6	0.9	22.5	11.7
96	1	1.05	0.09	1.99	0.54	31.5	1.35	29.85	5.4
96	1	1	0.08	1.87	0.66	30	1.2	28.05	6.6
96	1	0.95	0.08	1.79	0.84	28.5	1.2	26.85	8.4
120	1	1.08	0.11	1.95	0.36	32.4	1.65	29.25	3.6
120	1	1.13	0.12	2.03	0.3	33.9	1.8	30.45	3
120	1	1.07	0.13	1.94	0.39	32.1	1.95	29.1	3.9

Figure S11. Stacked ^{1}H NMR spectra for GGG decomposition at 95^{o}C under pH 7. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.2ppm to 4.20ppm.



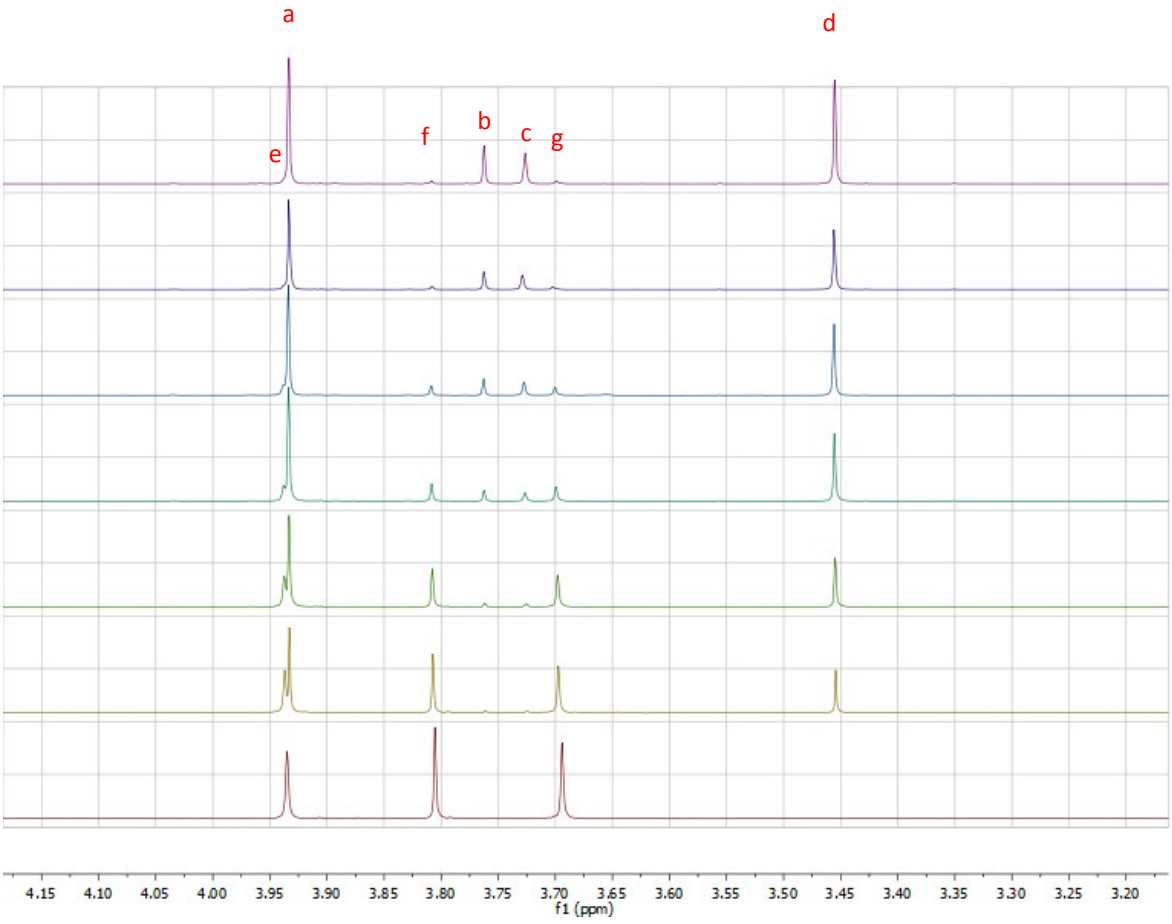
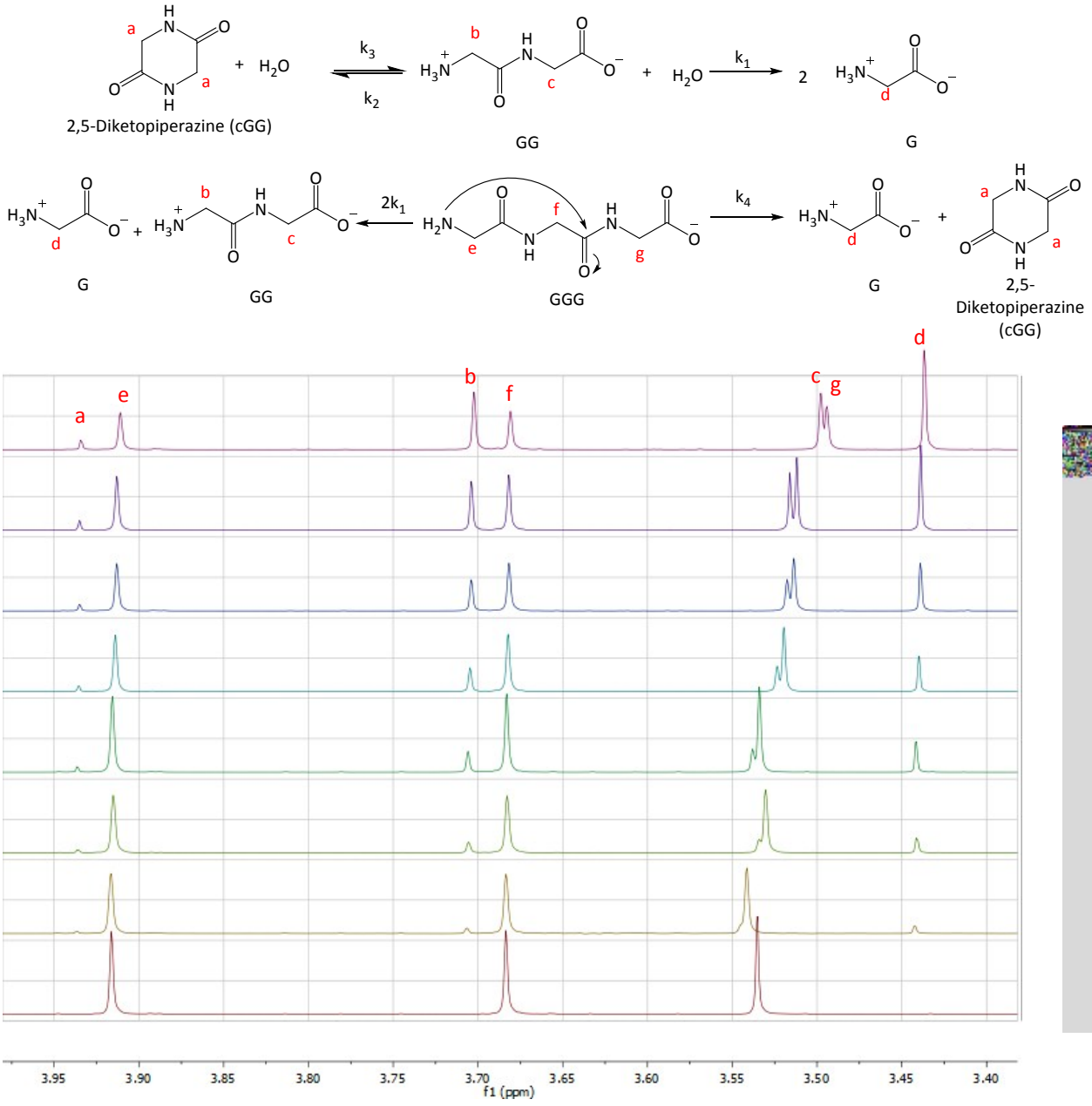


Table S11. GGG degradation reactants and products amounts at varying reaction time under pH 7 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=7 GGG analysis	NMR peak integrations					Actual amount (µmol)			
time(h)	KHP	G	GG	cGG	GGG	G (µmol)	GG (µmol)	cGG (µmol)	GGG (µmol)
0	1	0	0.02	0.01	3.51	0	0.32	0.16	37.44
0	1	0	0.02	0.01	3.54	0	0.32	0.16	37.76
0	1	0	0.01	0.02	3.42	0	0.16	0.32	36.48
12	1	0.41	0.06	0.78	1.98	13.12	0.96	12.48	21.12
12	1	0.4	0.05	0.76	2.07	12.8	0.8	12.16	22.08
12	1	0.37	0.05	0.69	2.07	11.84	0.8	11.04	22.08
24	1	0.63	0.11	1.15	1.59	20.16	1.76	18.4	16.96
24	1	0.53	0.09	0.96	1.59	16.96	1.44	15.36	16.96
24	1	0.58	0.1	1.05	1.62	18.56	1.6	16.8	17.28
36	1	0.81	0.19	1.42	1.05	25.92	3.04	22.72	11.2
36	1	0.72	0.16	1.26	1.05	23.04	2.56	20.16	11.2
36	1	0.67	0.14	1.2	1.23	21.44	2.24	19.2	13.12
48	1	0.9	0.29	1.47	0.72	28.8	4.64	23.52	7.68
48	1	0.84	0.25	1.41	0.78	26.88	4	22.56	8.32
48	1	0.75	0.2	1.3	0.93	24	3.2	20.8	9.92
72	1	0.96	0.44	1.43	0.45	30.72	7.04	22.88	4.8
72	1	0.92	0.4	1.4	0.48	29.44	6.4	22.4	5.12
72	1	0.88	0.33	1.39	0.57	28.16	5.28	22.24	6.08
96	1	1.05	0.54	1.6	0.36	31.5	8.1	24	3.6
96	1	1.04	0.6	1.41	0.27	33.28	9.6	22.56	2.88
96	1	1.01	0.55	1.39	0.33	32.32	8.8	22.24	3.52
120	1	1.14	0.83	1.33	0.15	34.2	12.45	19.95	1.6
120	1	1.17	0.83	1.4	0.18	35.1	12.45	21	1.92
120	1	1.1	0.76	1.36	0.18	33	11.4	20.4	1.92

Figure S12. Stacked ^{1}H NMR spectra for GGG decomposition at 95°C under pH 10. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.35ppm to 4.0ppm.



Peak shifts for GGG and GG are observed comparing day0 and day5 spectra due to pH variation over reaction time.

Table S12. GGG degradation reactants and products amounts at varying reaction time under pH 10 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=10 GGG analysis	NMR peak integrations					Actual amount (µmol)			
time(h)	KHP	G	GG	cGG	GGG	G (µmol)	GG (µmol)	cGG (µmol)	GGG (µmol)
0	1	0.01	0.02	0	3.39	0.3	0.3	0	33.9
0	1	0.01	0.02	0.01	3.39	0.3	0.3	0.15	33.9
0	1	0.01	0.02	0.01	3.36	0.3	0.3	0.15	33.6
12	1	0.1	0.16	0.03	2.94	3	2.4	0.45	29.4
12	1	0.1	0.18	0.03	3.09	3	2.7	0.45	30.9
12	1	0.09	0.16	0.03	3.03	2.7	2.4	0.45	30.3
24	1	0.18	0.32	0.04	2.79	5.4	4.8	0.6	27.9
24	1	0.17	0.3	0.04	2.82	5.1	4.5	0.6	28.2
24	1	0.16	0.28	0.04	2.88	4.8	4.2	0.6	28.8
36	1	0.24	0.42	0.05	2.58	7.2	6.3	0.75	25.8
36	1	0.27	0.3	0.06	2.61	8.1	4.5	0.9	26.1
36	1	0.22	0.36	0.05	2.58	6.6	5.4	0.75	25.8
48	1	0.35	0.58	0.06	2.4	10.5	8.7	0.9	24
48	1	0.31	0.52	0.05	2.43	9.3	7.8	0.75	24.3
48	1	0.28	0.48	0.05	2.52	8.4	7.2	0.75	25.2
72	1	0.5	0.76	0.07	2.07	15	11.4	1.05	20.7
72	1	0.45	0.72	0.07	2.13	13.5	10.8	1.05	21.3
72	1	0.42	0.66	0.06	2.22	12.6	9.9	0.9	22.2
96	1	0.64	0.9	0.08	1.62	19.2	13.5	1.2	16.2
96	1	0.6	0.88	0.07	1.74	18	13.2	1.05	17.4
96	1	0.54	0.8	0.07	1.86	16.2	12	1.05	18.6
120	1	0.87	1.12	0.08	1.35	26.1	16.8	1.2	13.5
120	1	0.8	1.08	0.08	1.47	24	16.2	1.2	14.7
120	1	0.74	1.02	0.07	1.62	22.2	15.3	1.05	16.2

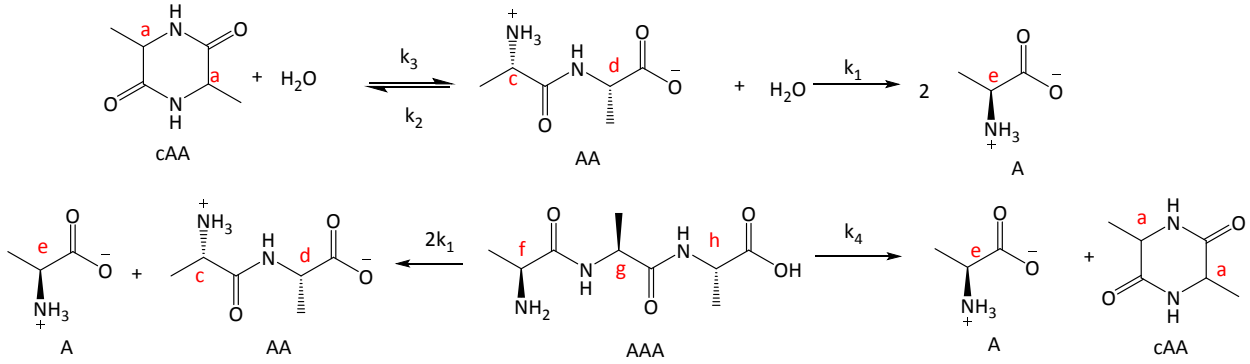
Table S13. The estimated rate constants based on joint fitting results for GG, cGG and GGG kinetics, under pH 3, 5, 7 and 10, at 95°C. The confidence intervals follow the chi-squared method at the 95% confidence level.

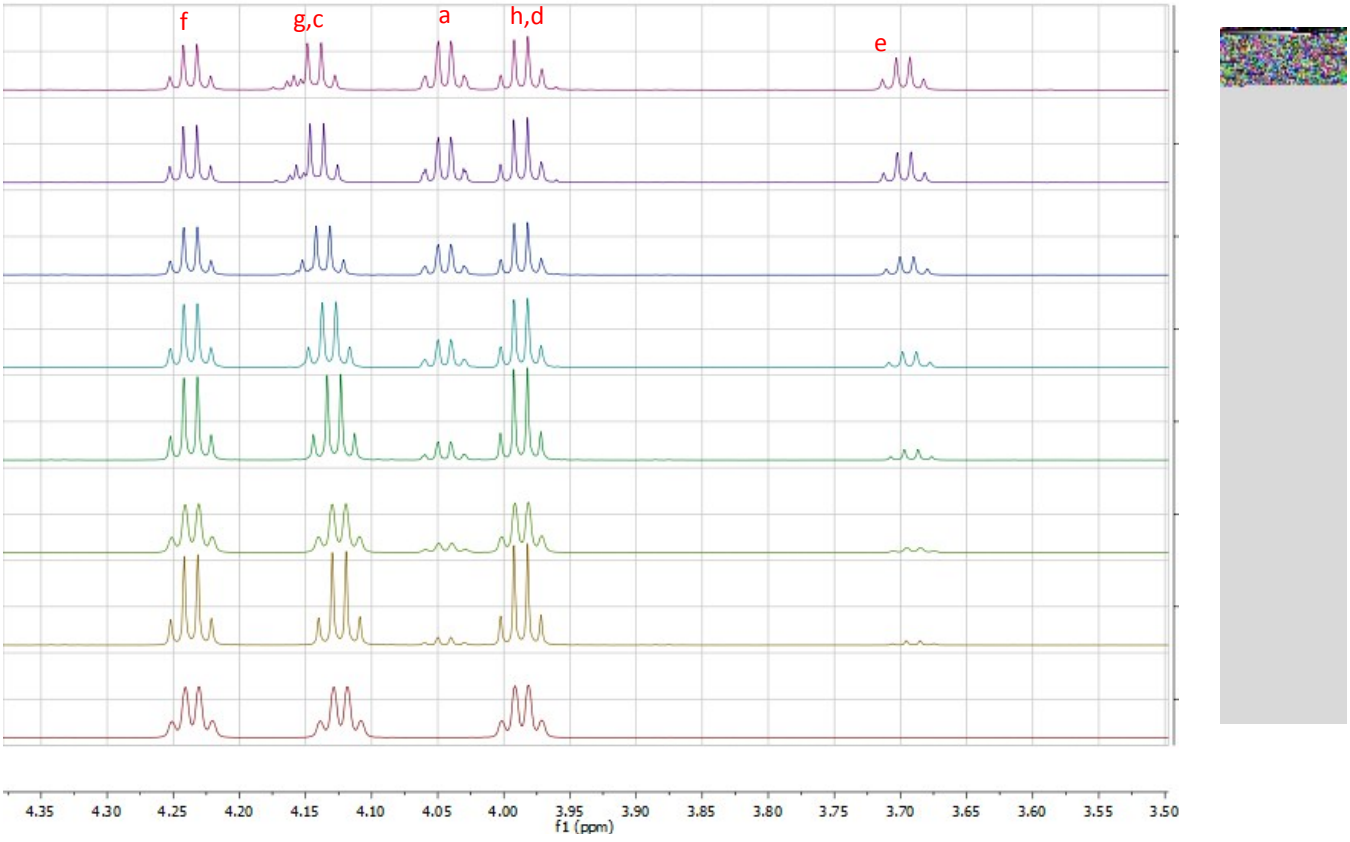
	k_{sc}x10^6 (s^{-1})	k_{rc}x10^6 (s^{-1})	$2k_{ro}$ x10^6 (s^{-1})	k_{bb} x10^6 (s^{-1})
pH=3	0.48(±0.183)	0.60(±0.205)	0.92(±0.255)	2.98(±0.458)
pH=5	0.06(±0.009)	0.27(±0.020)	0.35(±0.023)	5.03(±0.087)
pH=7	0.14(±0.004)	0.60(±0.008)	1.11(±0.012)	8.24(±0.031)
pH=10	0.84(±0.011)	0.07(±0.003)	0.96(±0.012)	0.09(±0.004)

Table S14. The estimated initial amount of GG, cGG and GGG based on joint fitting results, under pH 3, 5, 7 and 10, at 95°C. The confidence intervals are calculated following the chi-squared method at the 95% confidence level.

	GG_0 (μmol)	cGG_0 (μmol)	GGG_0 (μmol)
pH=3	30.15(±1.458)	15.98(±1.061)	31.23(±1.484)
pH=5	37.78(±0.240)	14.09(±0.146)	37.41(±0.239)
pH=7	33.76(±0.064)	19.63(±0.049)	34.17(±0.064)
pH=10	29.92(±0.068)	17.67(±0.053)	33.08(±0.072)

Figure S13. Stacked ^{1}H NMR spectra for AAA decomposition at 95^{o}C under pH 3. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.5ppm to 4.4ppm.



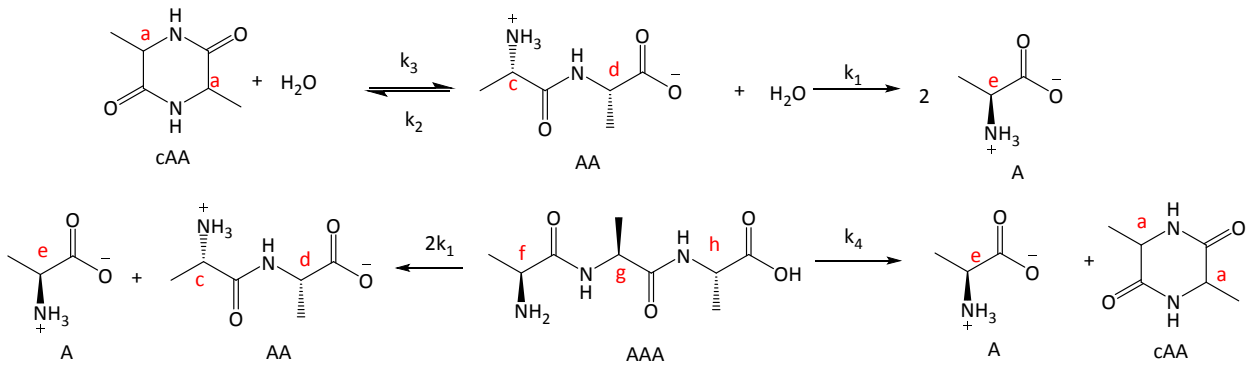


For all the quantitative analysis of AAA degradation studies under different pH conditions, the calculations are based on the assignment of methylene protons on AAA.

Table S15. AAA degradation reactants and products amounts at varying reaction time under pH 3 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=3 AAA analysis	NMR peak integrations					Actual amount (µmol)			
time(h)	KHP	A	AA	cAA	AAA	A (µmol)	AA (µmol)	cAA (µmol)	AAA (µmol)
0	1	0	0	0	0.9	0	0	0	18
0	1	0	0	0	0.9	0	0	0	18
0	1	0	0	0	0.9	0	0	0	18
12	1	0.02	0	0.03	0.81	1.2	0	0.9	16.2
12	1	0.02	0	0.03	0.81	1.2	0	0.9	16.2
12	1	0.02	0	0.03	0.84	1.2	0	0.9	16.8
24	1	0.03	0	0.05	0.81	1.8	0	1.5	16.2
24	1	0.03	0	0.06	0.78	1.8	0	1.8	15.6
24	1	0.03	0	0.06	0.81	1.8	0	1.8	16.2
36	1	0.04	0	0.07	0.78	2.4	0	2.1	15.6
36	1	0.04	0	0.08	0.75	2.4	0	2.4	15
36	1	0.04	0	0.08	0.78	2.4	0	2.4	15.6
48	1	0.06	0	0.11	0.69	3.6	0	3.3	13.8
48	1	0.06	0.02	0.1	0.72	3.6	0.6	3	14.4
48	1	0.06	0.02	0.1	0.72	3.6	0.6	3	14.4
72	1	0.09	0.04	0.15	0.6	5.4	1.2	4.5	12
72	1	0.08	0.04	0.14	0.63	4.8	1.2	4.2	12.6
72	1	0.07	0.02	0.13	0.66	4.2	0.6	3.9	13.2
96	1	0.11	0.04	0.17	0.54	6.6	1.2	5.1	10.8
96	1	0.1	0.04	0.16	0.54	6	1.2	4.8	10.8
96	1	0.1	0.04	0.16	0.57	6	1.2	4.8	11.4
120	1	0.13	0.06	0.2	0.48	7.8	1.8	6	9.6
120	1	0.12	0.06	0.19	0.51	7.2	1.8	5.7	10.2
120	1	0.12	0.06	0.18	0.51	7.2	1.8	5.4	10.2

Figure S14. Stacked ^{1}H NMR spectra for AAA decomposition at 95^{o}C under pH 5. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.4ppm to 4.3ppm.



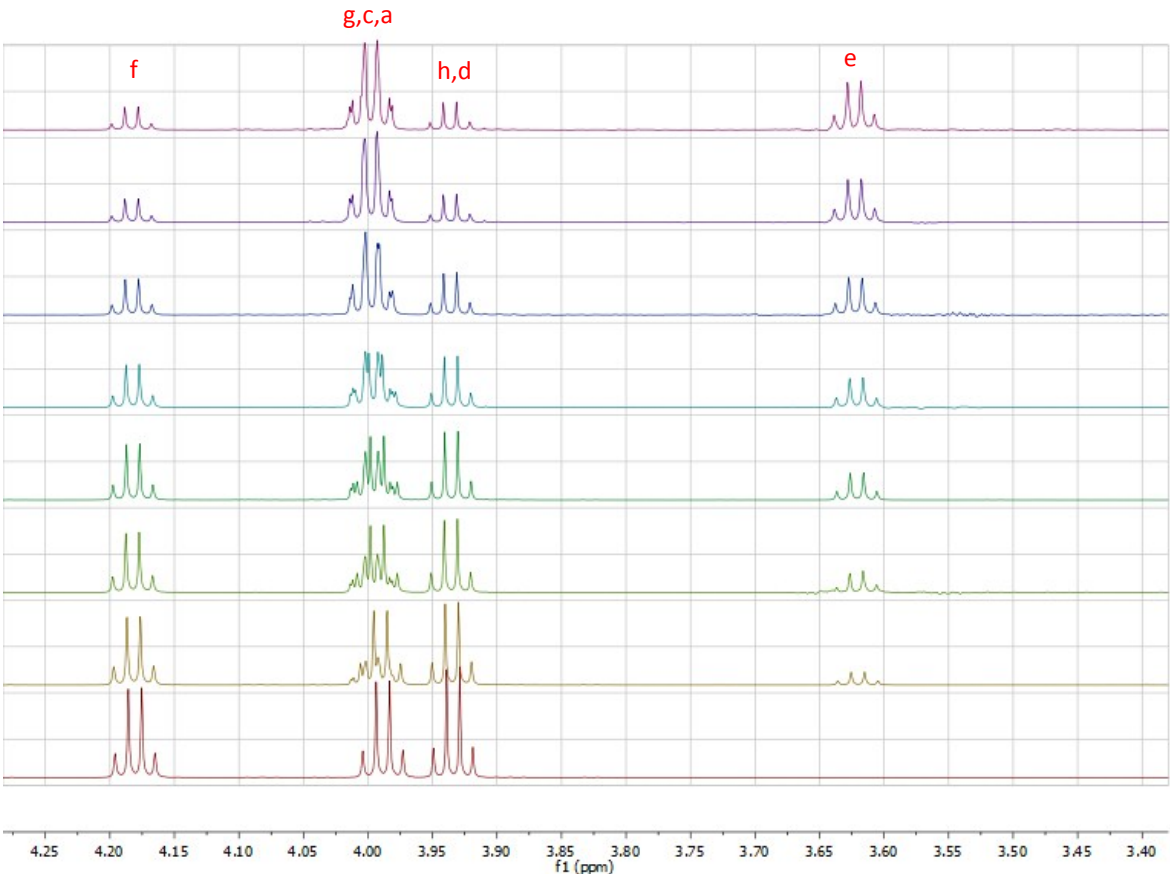
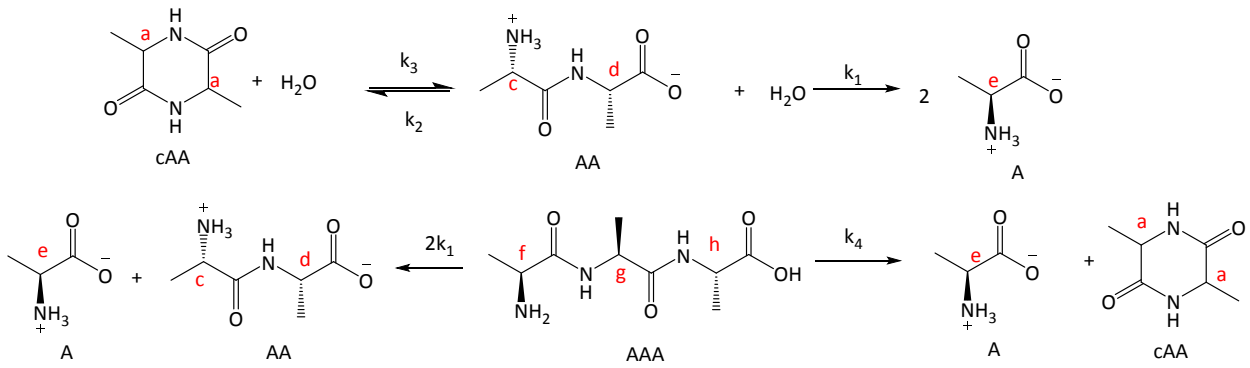


Table S16. AAA degradation reactants and products amounts at varying reaction time under pH 5 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=5 AAA analysis	NMR peak integrations					Actual amount (µmol)			
time(h)	KHP	A	AA	cAA	AAA	A (µmol)	AA (µmol)	cAA (µmol)	AAA (µmol)
0	1	0	0	0	0.96	0	0	0	19.2
0	1	0	0	0	0.96	0	0	0	19.2
0	1	0	0	0	0.93	0	0	0	18.6
12	1	0.05	0	0.11	0.78	3	0	3.3	15.6
12	1	0.05	0	0.11	0.75	3	0	3.3	15
12	1	0.05	0	0.1	0.81	3	0	3	16.2
24	1	0.09	0	0.17	0.66	5.4	0	5.1	13.2
24	1	0.08	0	0.16	0.66	4.8	0	4.8	13.2
24	1	0.08	0	0.16	0.72	4.8	0	4.8	14.4
36	1	0.11	0	0.22	0.57	6.6	0	6.6	11.4
36	1	0.11	0	0.21	0.6	6.6	0	6.3	12
36	1	0.11	0	0.21	0.63	6.6	0	6.3	12.6
48	1	0.14	0.02	0.26	0.51	8.4	0.6	7.8	10.2
48	1	0.12	0	0.24	0.54	7.2	0	7.2	10.8
48	1	0.12	0	0.25	0.57	7.2	0	7.5	11.4
72	1	0.17	0.02	0.33	0.45	10.2	0.6	9.9	9
72	1	0.17	0	0.34	0.39	10.2	0	10.2	7.8
72	1	0.16	0	0.32	0.45	9.6	0	9.6	9
96	1	0.21	0.02	0.41	0.27	12.6	0.6	12.3	5.4
96	1	0.2	0	0.39	0.3	12	0	11.7	6
96	1	0.2	0	0.38	0.36	12	0	11.4	7.2
120	1	0.22	0.02	0.42	0.27	13.2	0.6	12.6	5.4
120	1	0.23	0.02	0.45	0.21	13.8	0.6	13.5	4.2
120	1	0.22	0	0.44	0.27	13.2	0	13.2	5.4

Figure S15. Stacked ^{1}H NMR spectra for AAA decomposition at 95^{o}C under pH 7. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.5ppm to 4.4ppm.



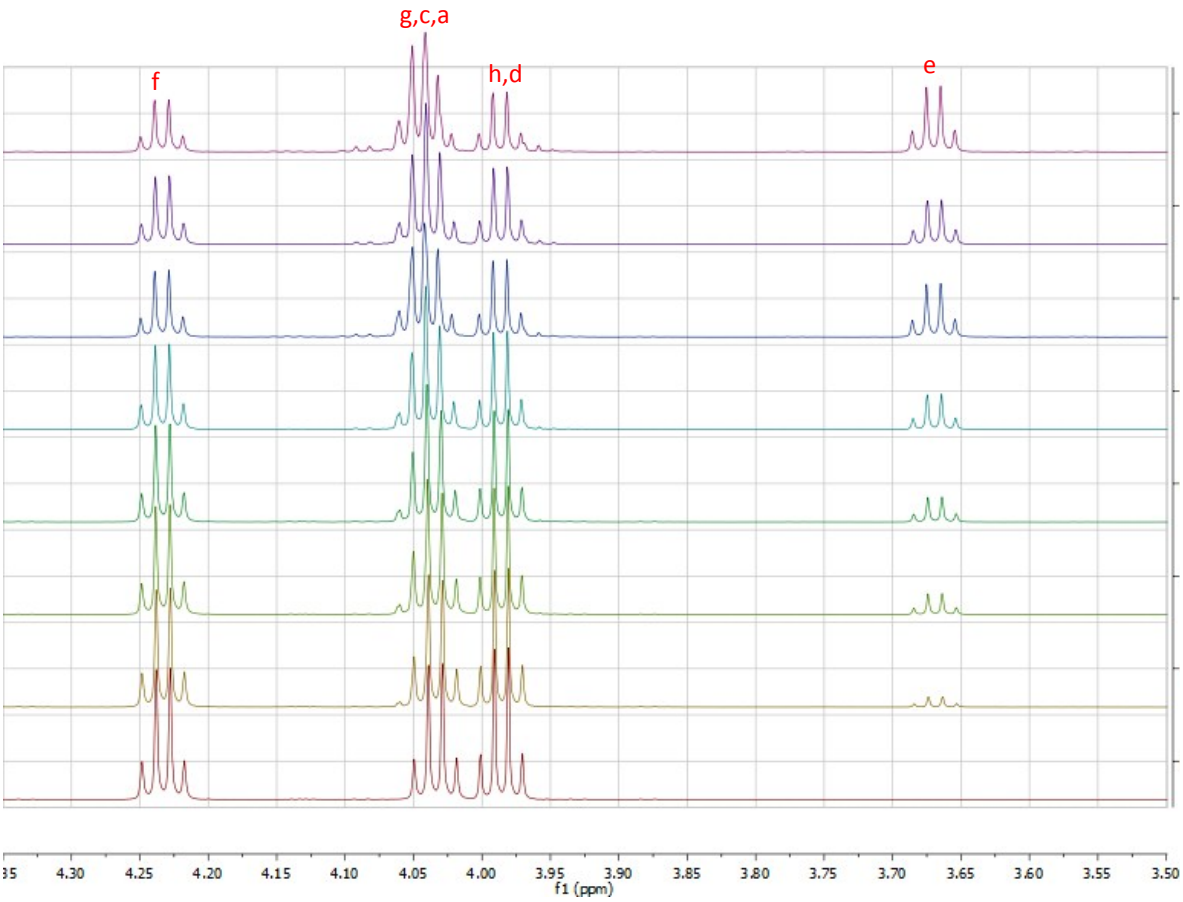
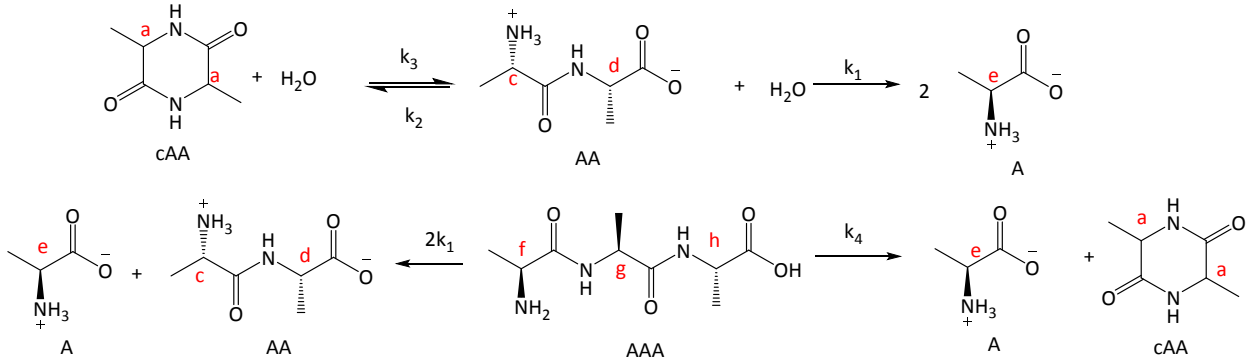


Table S17. AAA degradation reactants and products amounts at varying reaction time under pH 7 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=7 AAA analysis	NMR peak integrations					Actual amount (µmol)			
time(h)	KHP	A	AA	cAA	AAA	A (µmol)	AA (µmol)	cAA (µmol)	AAA (µmol)
0	1	0	0	0	0.96	0	0	0	19.2
0	1	0	0	0	0.99	0	0	0	19.8
0	1	0	0	0	0.99	0	0	0	19.8
12	1	0.02	0	0.05	0.87	1.2	0	1.5	17.4
12	1	0.03	0.02	0.04	0.87	1.8	0.6	1.2	17.4
12	1	0.02	0	0.03	0.9	1.2	0	0.9	18
24	1	0.05	0	0.09	0.81	3	0	2.7	16.2
24	1	0.04	0	0.08	0.84	2.4	0	2.4	16.8
24	1	0.03	0	0.06	0.84	1.8	0	1.8	16.8
36	1	0.06	0	0.12	0.75	3.6	0	3.6	15
36	1	0.07	0	0.13	0.75	4.2	0	3.9	15
36	1	0.04	0	0.11	0.81	2.4	0	3.3	16.2
48	1	0.09	0.02	0.17	0.63	5.4	0.6	5.1	12.6
48	1	0.1	0.02	0.18	0.63	6	0.6	5.4	12.6
48	1	0.06	0	0.12	0.72	3.6	0	3.6	14.4
72	1	0.14	0.02	0.27	0.51	8.4	0.6	8.1	10.2
72	1	0.09	0.02	0.18	0.66	5.4	0.6	5.4	13.2
72	1	0.11	0	0.23	0.6	6.6	0	6.9	12
96	1	0.12	0.02	0.24	0.57	7.2	0.6	7.2	11.4
96	1	0.15	0.02	0.27	0.51	9	0.6	8.1	10.2
96	1	0.15	0.04	0.29	0.42	9	1.2	8.7	8.4
120	1	0.17	0.02	0.32	0.42	10.2	0.6	9.6	8.4
120	1	0.18	0.04	0.34	0.36	10.8	1.2	10.2	7.2
120	1	0.19	0.04	0.36	0.33	11.4	1.2	10.8	6.6

Figure S16. Stacked ^{1}H NMR spectra for AAA decomposition at 95°C under pH 10. Each spectrum corresponds to a different sampling time. From bottom to top spectrum, indicate increasing sampling times of 0, 12, 24, 36, 48, 72, 96 and 120 h. All the NMR spectra are zoomed in from 3.5ppm to 4.4ppm.



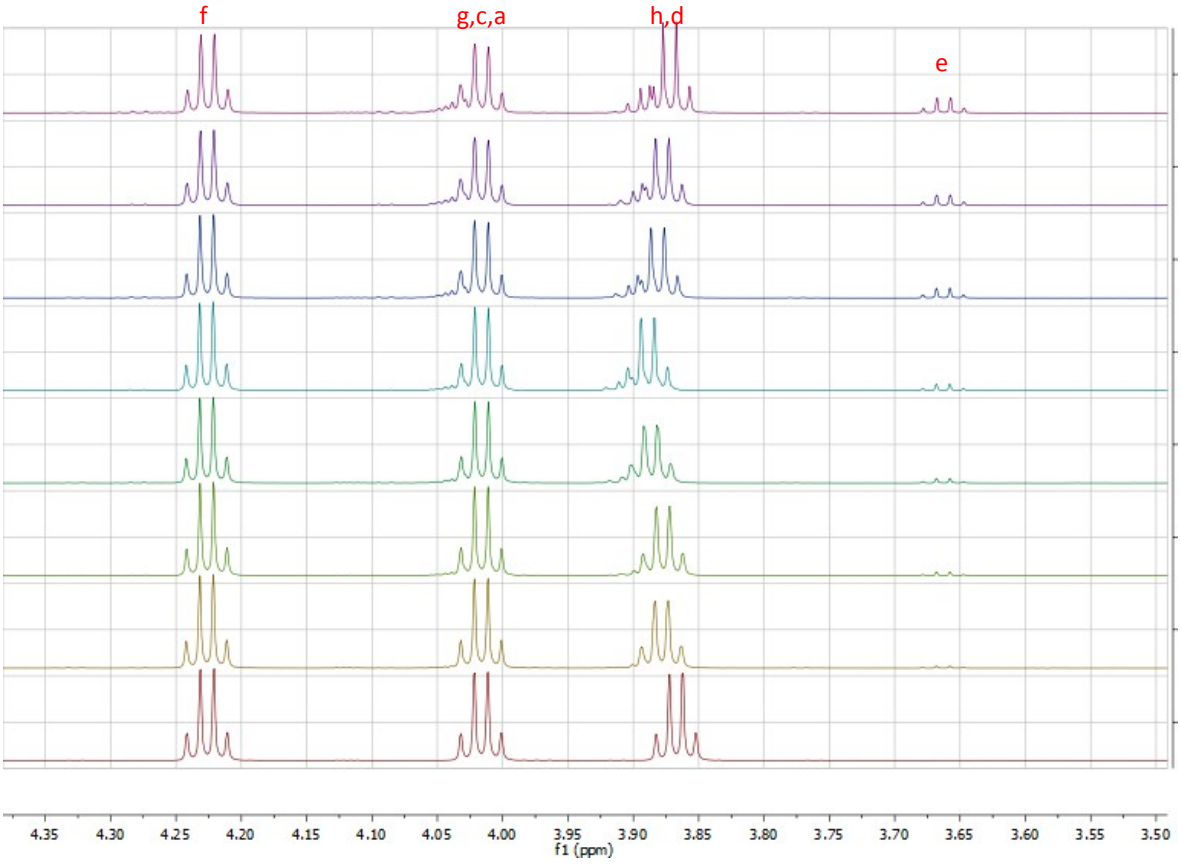


Table S18. AAA degradation reactants and products amounts at varying reaction time under pH 10 at 95°C. The table also includes the NMR integration peak areas for each species.

pH=10 AAA analysis	NMR peak integrations					Actual amount (µmol)			
time(h)	KHP	A	AA	cAA	AAA	A (µmol)	AA (µmol)	cAA (µmol)	AAA (µmol)
0	1	0	0	0	0.93	0	0	0	18.6
0	1	0	0	0	0.93	0	0	0	18.6
0	1	0	0	0	0.93	0	0	0	18.6
12	1	0.01	0	0	0.9	0.6	0	0	18
12	1	0.01	0	0	0.93	0.6	0	0	18.6
12	1	0	0.01	0	0.87	0	0.3	0	17.4
24	1	0.01	0.02	0	0.87	0.6	0.6	0	17.4
24	1	0.01	0	0.01	0.9	0.6	0	0.3	18
24	1	0.01	0	0.01	0.87	0.6	0	0.3	17.4
36	1	0.01	0.02	0.01	0.84	0.6	0.6	0.3	16.8
36	1	0.02	0.02	0.01	0.87	1.2	0.6	0.3	17.4
36	1	0.01	0.02	0.01	0.87	0.6	0.6	0.3	17.4
48	1	0.02	0.04	0	0.81	1.2	1.2	0	16.2
48	1	0.02	0.02	0.01	0.84	1.2	0.6	0.3	16.8
48	1	0.01	0.02	0.01	0.84	0.6	0.6	0.3	16.8
72	1	0.02	0.06	0.01	0.78	1.2	1.8	0.3	15.6
72	1	0.02	0.04	0.01	0.84	1.2	1.2	0.3	16.8
72	1	0.02	0.04	0	0.84	1.2	1.2	0	16.8
96	1	0.03	0.06	0.01	0.78	1.8	1.8	0.3	15.6
96	1	0.03	0.06	0.01	0.75	1.8	1.8	0.3	15
96	1	0.02	0.04	0	0.84	1.2	1.2	0	16.8
120	1	0.04	0.08	0	0.75	2.4	2.4	0	15
120	1	0.04	0.08	0.01	0.75	2.4	2.4	0.3	15
120	1	0.03	0.06	0.01	0.81	1.8	1.8	0.3	16.2

Table S19. The estimated rate constants for AAA kinetics, under pH 3, 5, 7 and 10, at 95°C.

	k_{sc} x10^6 (s^{-1})	k_{rc}x10^6 (s^{-1})	$2k_{ro}$ x10^6 (s^{-1})	k_{bb} x10^6 (s^{-1})
pH=3	0.046	0.0	0.82	1.2
pH=5	0.0	2.0	0.32	3.2
pH=7	0.030	0.0	0.17	1.7
pH=10	0.0	2.2	63	0.37

The initial guesses for all AAA rate constants are from the GGG fitting results. The values of the rate constants are not unique due to the limited data for AAA, which does not include dimer degradation experiments.

Table S20. The estimated initial amount of AAA based on model fitting, under pH 3, 5, 7 and 10, at 95°C.

	AAA_0 (μmol)
pH=3	17.75
pH=5	18.57
pH=7	18.81
pH=10	17.83

III. Reference:

1. S. M. Miller and J. B. Rawlings, *AIChE Journal*, 1994, **40**, 1312-1327.