

Hydroxamate based Selective Macrophage Elastase (MMP-12) Inhibitors and Radiotracers for Molecular Imaging

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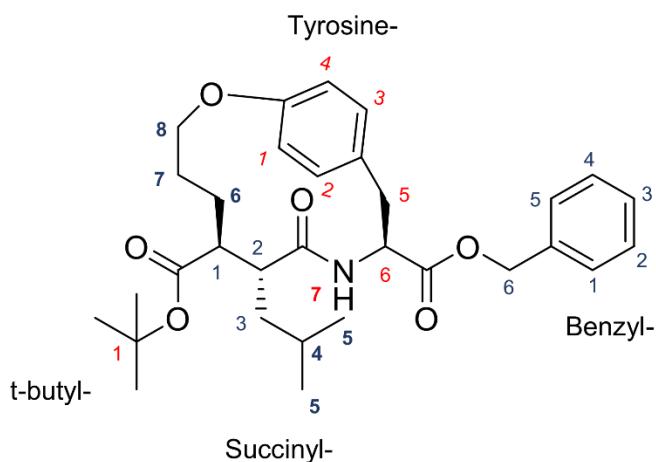
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Table S1. Inhibition constants (K_i) of CGA, CGA-1, RYM, AGA, AGA-1 and AGA-2 for recombinant murine (rm) MMP-9 and MMP-12.

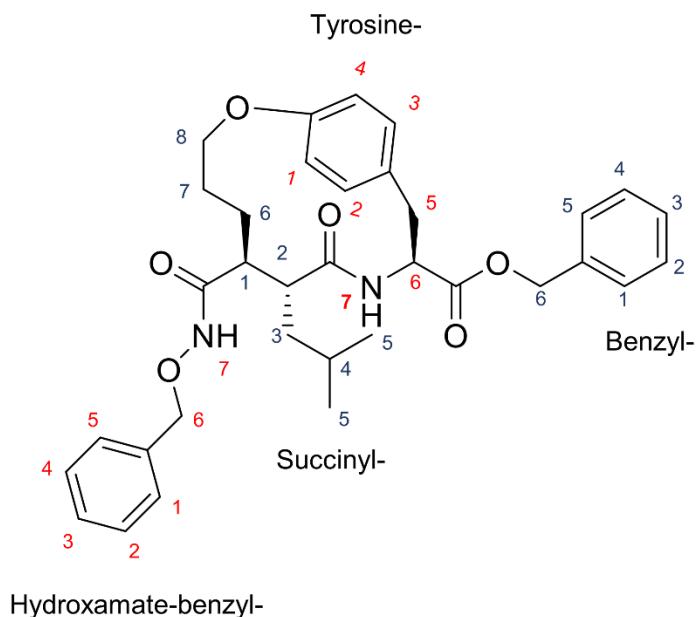
Ki (nM)	rmMMP-9	rmMMP-12
CGA	180	0.91
CGA-1	1497	1.5
RYM	7.2	11.3
AGA	261	2.1
AGA-1	869	1.7
AGA-2	1126	1.0

Table S2. ^1H NMR assignments of compound **6**



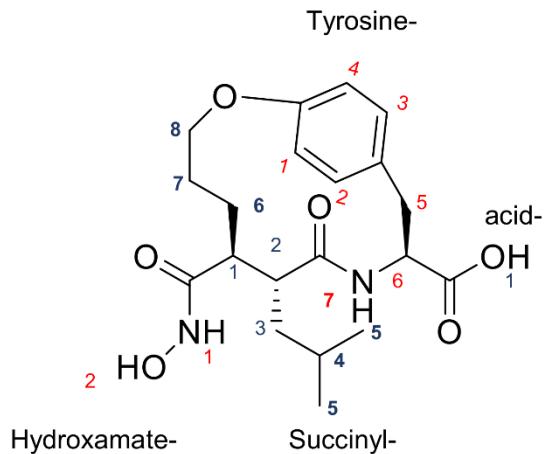
Group	Position	^1H (δ ppm)	Multiplicity ^1H (J Hz)
benzyl-	CH - 1-5 (5)	7.62-7.68	m
	CH ₂ - 6 (2)	5.24-4.93	m
tyrosine-	CH - 1-4 (4)	7.02-6.71	m
	CH ₂ - 5 (2)	4.47-4.26	m
	CH - 6 (1)	4.63	m
	NH - 7 (1)	7.99	dt
succinyl-	CH - 5 (6)	0.81	dd
	CH - 1-4 & 6-8 (11)	1.00-3.18	
t-butyl	CH ₃ - 1 (9)	1.42	s

Table S3. ^1H NMR assignments of compound 8



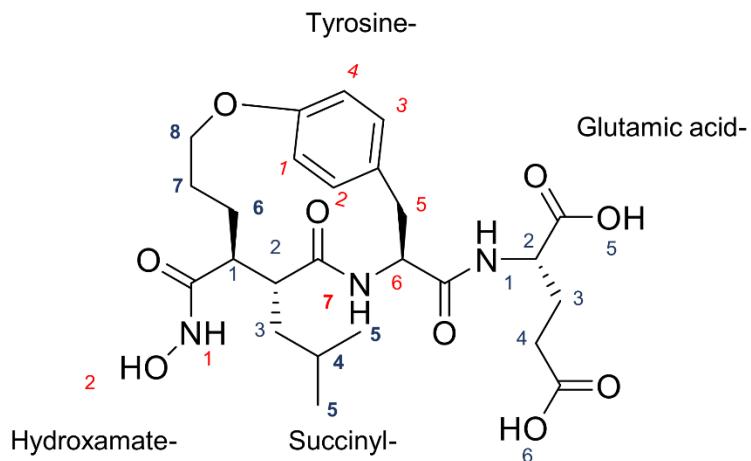
Group	Position	^1H (δ ppm)	Multiplicity ^1H (J Hz)
benzyl-	CH (5) 1-5	7.62-7.68	m
	CH ₂ (2) 6	5.24-4.93	m
tyrosine-	CH (4) 1-4	6.95-6.67	m
	CH ₂ (2) 5	3.53-3.32	m
succinyl-	CH (1) 6	4.51	m
	NH (1) 7	7.99	dt
hydroxamate-benzyl-	CH (6) 5	0.86	dd
	CH (11) 1-4 & 6-8	1.00-3.18	
hydroxamate-benzyl-	CH (5) 1-5	7.62-7.68	m
	CH ₂ (2) 6	5.24-4.93	m

Table S4. ^1H NMR assignments of compound CGA



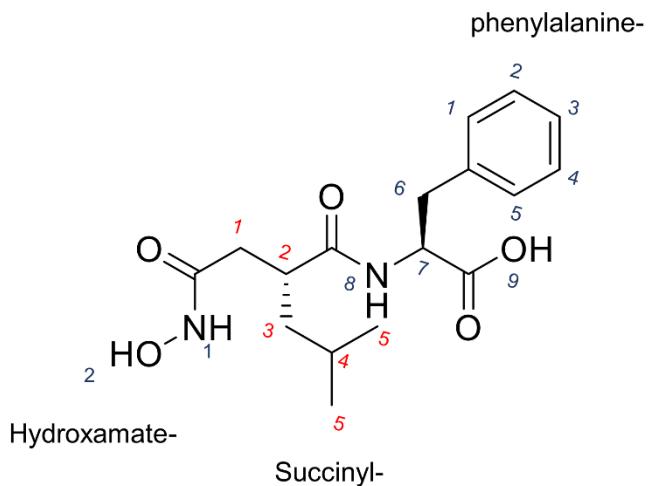
Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
hydroxamate-	OH - 2 (1)	10.33	s
	NH - 1(1)	8.67	s
tyrosine-	CH - 1-4 (4)	7.21-6.96	m
	CH ₂ - 5 (2)	3.53-3.32	m
	CH - 6 (1)	4.7	m
succinyl-	NH - 7 (1)	7.73	m
	CH - 5 (6)	0.89-0.66	dd
	CH - 1-4 & 6-8 (11)	1.00-3.18	

Table S5. ^1H NMR assignments of compound CGA-1



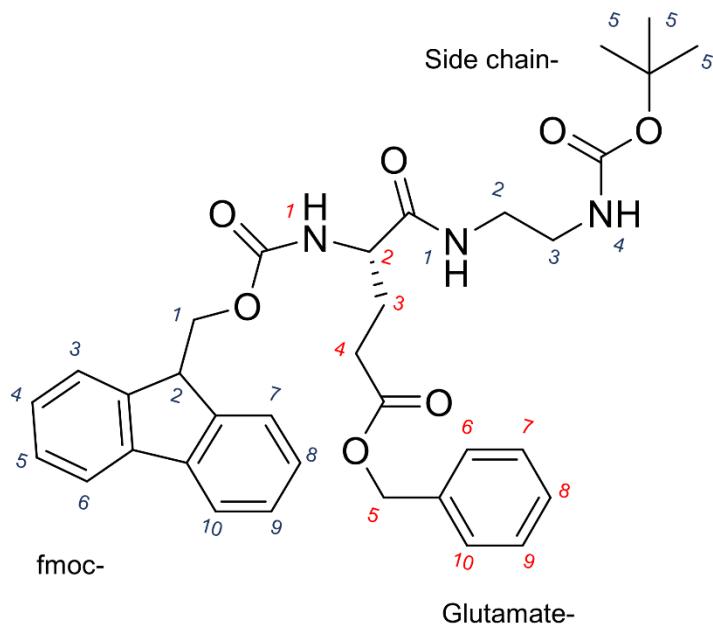
Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
glutamic acid	OH - 5 & 6 (2)	10.5	s
	NH - 1(1)	8.68	s
	CH & CH ₂ - 2-4 (5)	4.74, 2.27, 2.00	
hydroxamate-	OH - 2 (1)	7.92	s
	NH - 1(1)	7.51	s
tyrosine-	CH - 1-4 (4)	7.29-6.56	
	CH ₂ - 5 (2)	4.28-3.92	m
	CH - 6 (1)	4.74	m
succinyl-	NH - 7 (1)	7.5	m
	CH - 5 (6)	0.89-0.53	dd
	CH - 1-4 & 6-8 (11)	1.04-4.28	

Table S6. ^1H NMR assignments of compound AGA



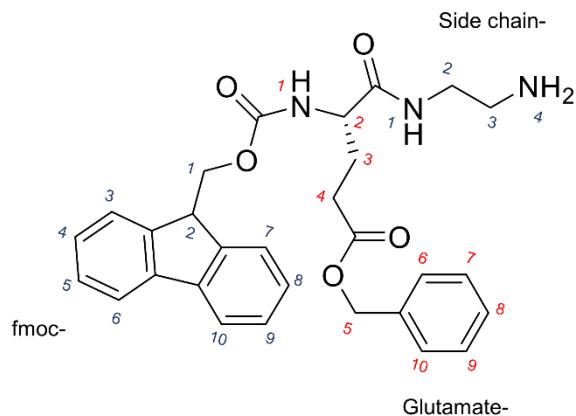
Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
phenyl alanine	CH - 1-5 (5)	7.35-7.16	
	CH - 7 (1)	4.75-4.62	m
	CH ₂ - 6 (2)	3.21-2.94	
succinyl-	CH - 5 (6)	0.88	dd
	CH & CH ₂ - 1-4 & 6-8 (6)	2.05-1.11	

Table S7. ^1H NMR assignments of compound intermediate **19**



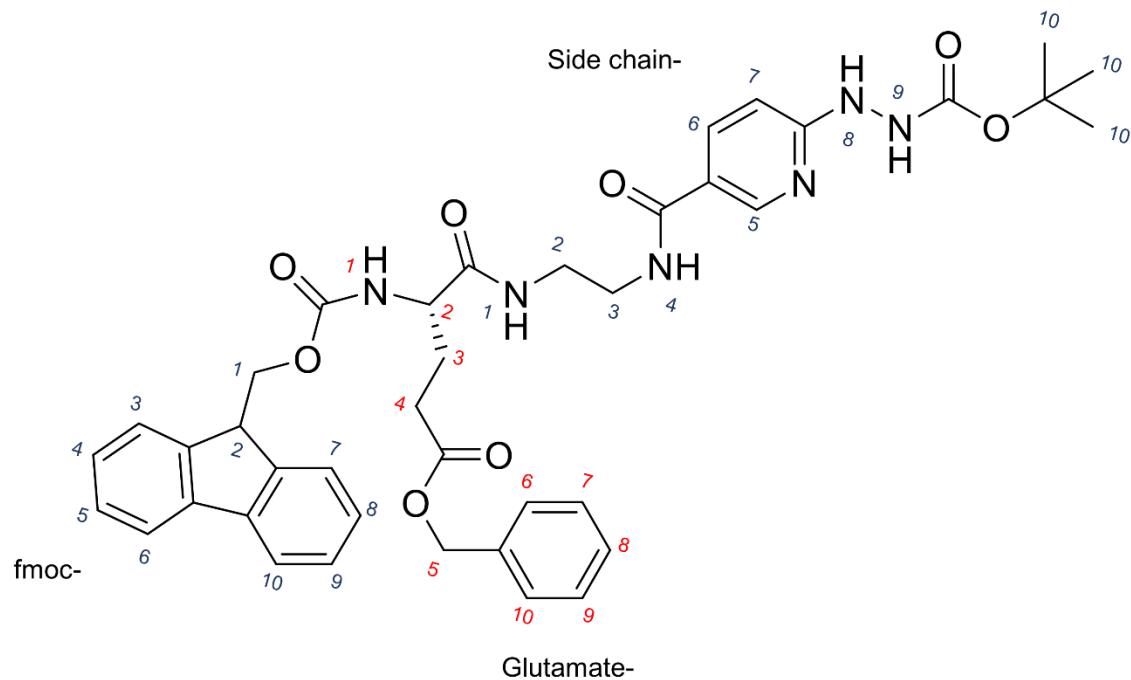
Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
side chain	t-Bu -5 (9)	1.34	s
	CH ₂ - 2 & 3 (4)	2.57-1.82	
glutamate-	CH - 6-10 (5)	7.91-7.53	m
	CH ₂ - 5 (2)	5.14	s
fmoc-	CH & CH ₂ - 2-4 (5)	3.21-2.94	
	CH ₂ - 1 (2)	4.37-4.24	
	CH - 2 (1)	4.53-4.40	m
	CH 3-10 (8)	7.49-7.20	m

Table S8. ^1H NMR assignments of compound intermediate **20**



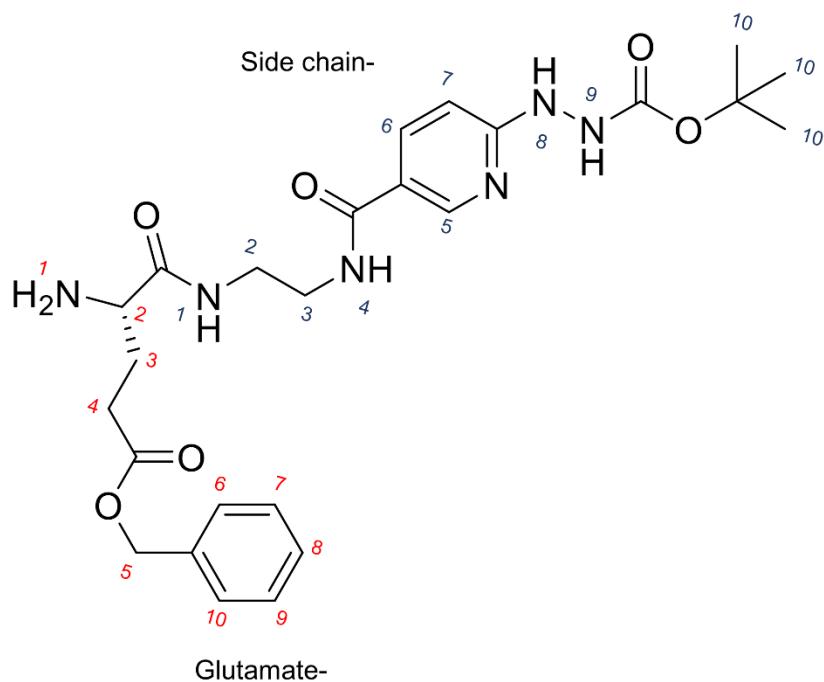
Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
side chain	CH_2 - 2 & 3 (4)	2.57-1.82	
glutamate-	CH - 6-10 (5)	7.81-7.38	m
	CH_2 - 5 (2)	5.11-4.97	s
	CH & CH_2 - 2-4 (5)	3.43-3.04	
fmoc-	CH_2 - 1 (2)	4.33	
	CH - 2 (1)	4.13	m
	CH 3-10 (8)	7.38-7.18	m

Table S9. ^1H NMR assignments of compound intermediate **21**



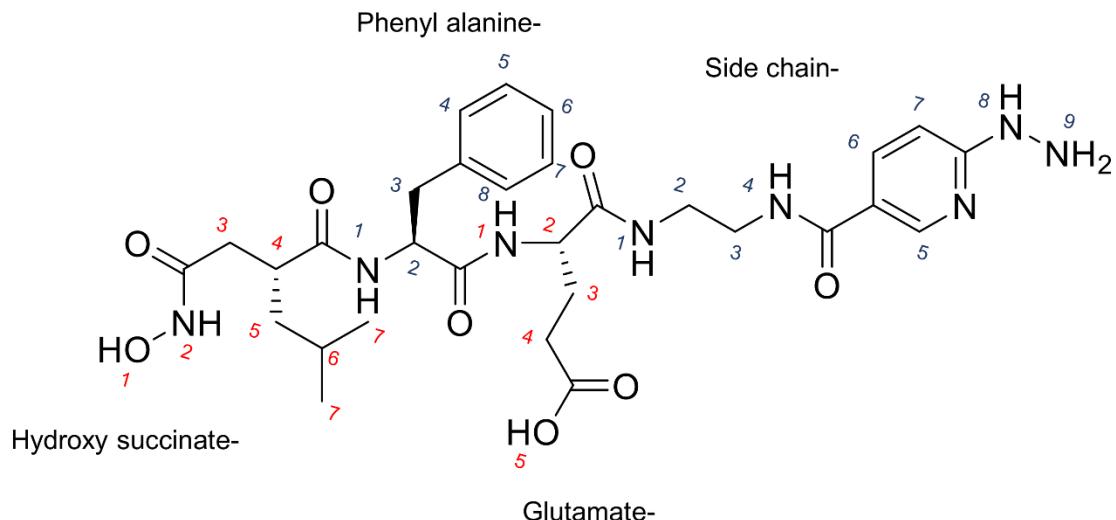
Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
side chain	t-Bu - 10 (9)	1.33	s
	NH - 1&4 (2)	8.00-7.84	m
	HYNIC - 5-7 (3)	8.99-8.35	
glutamate-	CH ₂ - 2 & 3 (4)	4.31-3.90	m
	CH - 6-10 (5)	7.79-7.38	m
	CH ₂ - 5 (2)	4.99	s
fmoc-	CH & CH ₂ - 2-4 (5)	3.43-3.04	
	CH ₂ - 1 (2)	4.33	
	CH - 2 (1)	4.06	m
	CH 3-10 (8)	7.37-7.15	m

Table S10. ^1H NMR assignments of compound intermediate **22**



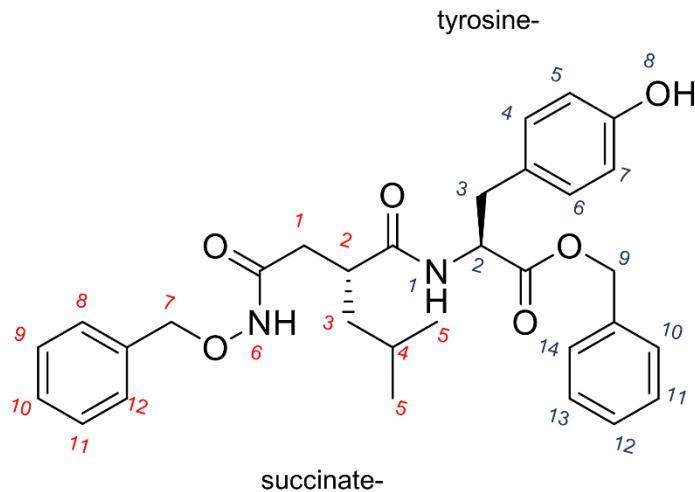
Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
side chain	t-Bu - 10 (9)	1.5	s
	HYNIC - 5-7 (3)	8.55-8.12	
	CH ₂ - 2 & 3 (4)	4.17 -3.72	m
glutamate-	CH - 6-10 (5)	7.79-7.38	m
	CH ₂ - 5 (2)	4.99	s
	CH & CH ₂ - 2-4 (5)	3.72-3.37	

Table S11. ^1H NMR assignments of compound AGA-1



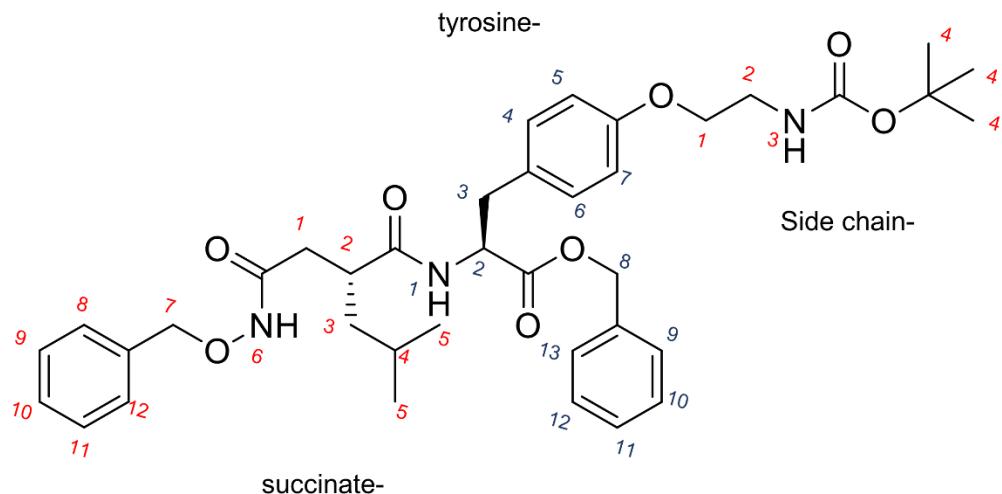
Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
side chain	HYNIC - 5-7 (3)	7.38-7.19	
	CH ₂ - 2 & 3 (4)	4.17 -3.72	m
glutamate-	CH - 2-4 (5)	5.20-4.74	m
phenyl alanine-	CH - 4-8 (5)	6.80-6.47	
	CH & CH ₂ - 2 & 3 (3)	2.65-2.11	m
Hydroxy succinate	CH ₃ - 7 (6)	0.71	m
	CH & CH ₂ - 3-6 (6)	2.99-2.65	
	CH ₃ - 6-10 (5)		
	CH & CH ₂ - 2-4 (5)	3.72-3.37	

Table S12. ^1H NMR assignments of compound intermediate **27**



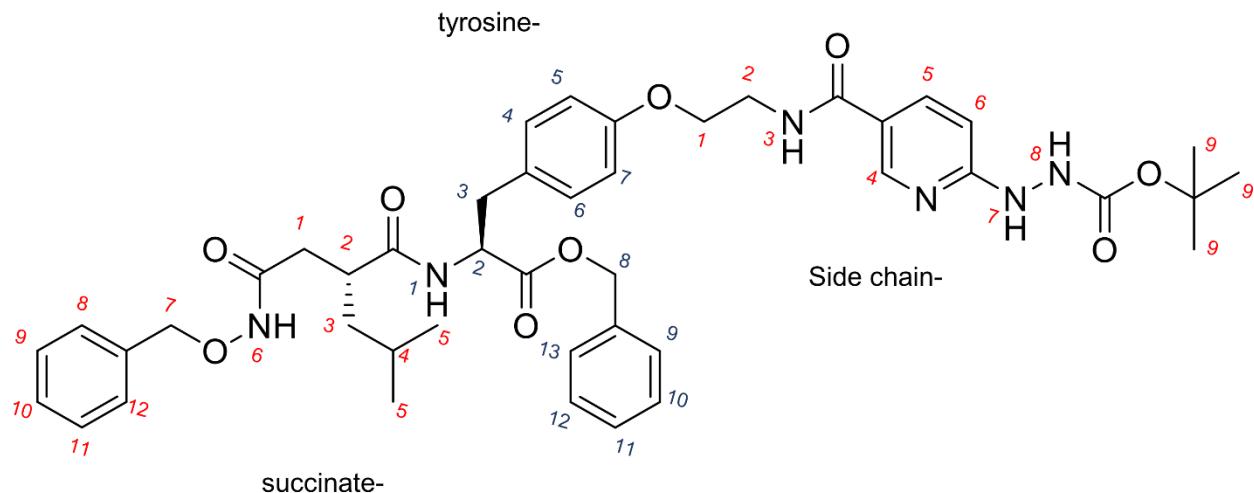
Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
Tyrosine-	CH - 5-7 (4)	7.08-6.59	
	CH - 10-14 (5)	7.47-7.25	
	CH ₂ - 9 (2)	5.1	
	CH & CH ₂ - 2 & 3 (3)	4.90-4.74	m
Succinate	CH - 8-12 (5)	7.47-7.25	m
	CH ₂ - 7 (2)	4.90-4.74	
	CH ₃ - 5 (6)	0.83	m
	CH & CH ₂ - 1-4 (6)	3.12-1.36	

Table S13. ^1H NMR assignments of compound intermediate **28**



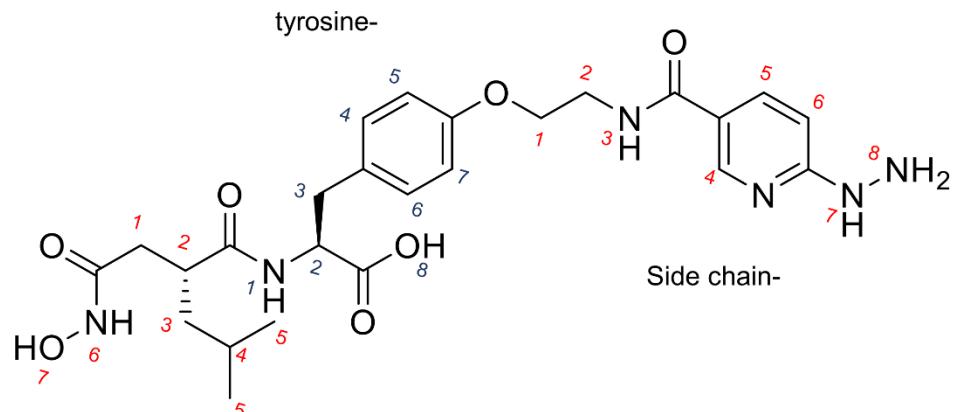
Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
Side chain	t-Bu - 4 (9)	1.38	s
	CH ₂ - 1-2 (4)	3.87-2.82	
Tyrosine-	CH - 4-7 (4)	7.15-6.62	
	CH - 9-13 (5)	7.47-7.18	m
	CH ₂ - 8 (2)	4.90-4.67	m
	CH - 2 (1)	4.51-4.37	m
Succinate	CH - 8-12 (5)	7.47-7.18	m
	CH ₂ - 7 (2)	5.18-4.96	m
	CH ₃ - 5 (6)	0.86-0.68	m
	CH & CH ₂ - 1-4 (6)	3.01-1.05	

Table S14. ^1H NMR assignments of compound intermediate **30**



Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
Side chain	t-Bu - 9 (9)	1.45-1.35	s
	NH- 3, 7 & 8	8.62-8.34	
	CH ₂ - 1-2 (4)	4.45-4.04	m
Tyrosine-	CH - 4-7 (4)	6.83-6.51	
	CH - 9-13 (5)	7.48-7.03	m
	CH ₂ - 8 (2)	4.7	
Succinate	CH ₂ - 3 (2)	2.06	t
	CH - 2 (1)	5.13-4.93	m
	CH - 8-12 (5)	7.48-7.03	m
	CH ₂ - 7 (2)	5.04	s
	CH ₃ - 5 (6)	0.85-0.60	m
	CH & CH ₂ - 1-4 (6)	3.01-2.09	

Table S15. ^1H NMR assignments of compound AGA-2



succinate-

Group	Position (number of ^1H)	^1H (δ ppm)	Multiplicity ^1H (J Hz)
Side chain	HYNIC-4-6 (3)	8.53-7.04	
	CH ₂ - 1-2 (4)	3.99-3.54	m
Tyrosine-	CH - 4-7 (4)	6.82-6.58	
	CH ₂ - 3 (2)	3.39	t
	CH - 2 (1)	3.78-3.54	m
Succinate	CH ₃ - 5 (6)	0.86-0.56	m
	CH & CH ₂ - 1-4 (6)	3.02-1.93	

Figure S1. ^{99m}Tc -AGA-1 and ^{99m}Tc -AGA-2 stability in blood. Representative radiochromatograms of $\text{Na}^{99m}\text{TcO}_4$ (green), ^{99m}Tc -AGA-1 (blue), ^{99m}Tc -AGA-2 (red) and radiochromatogram of ^{99m}Tc -co-ligand (black) after heating $\text{Na}^{99m}\text{TcO}_4^-$ with vehicle and without the precursor.

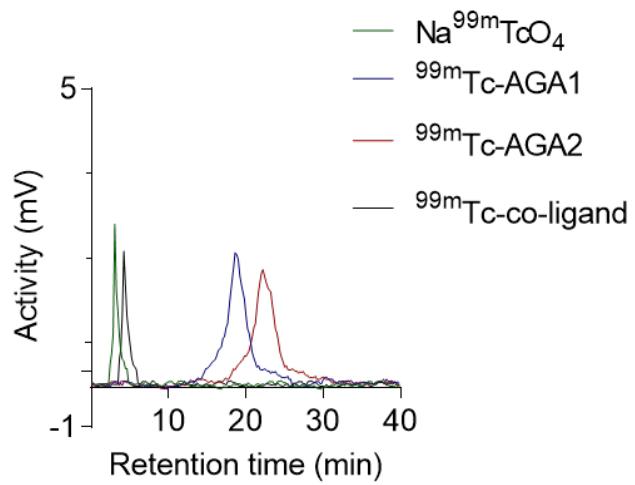


Figure S2. ^1H NMR spectra of intermediate **6**

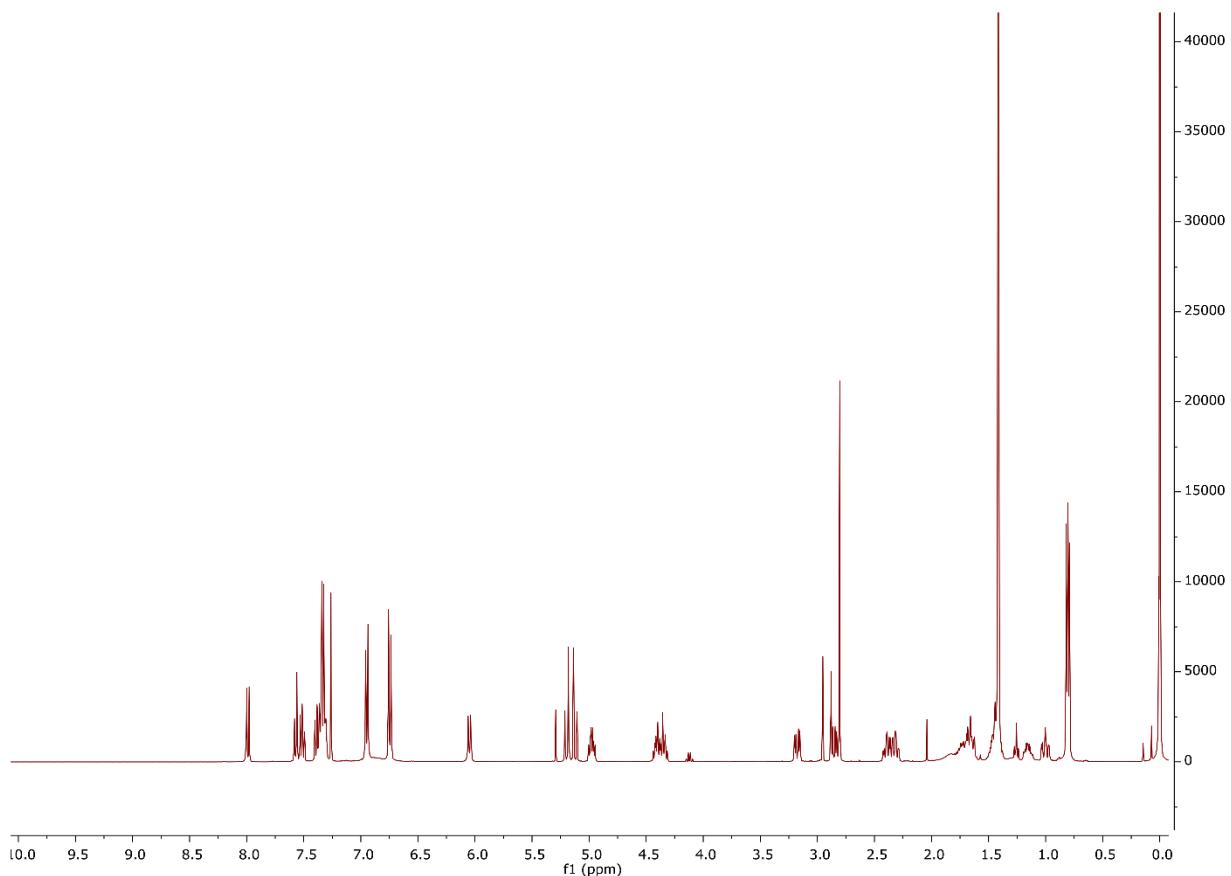


Figure S3. ^1H NMR spectra of intermediate **8**

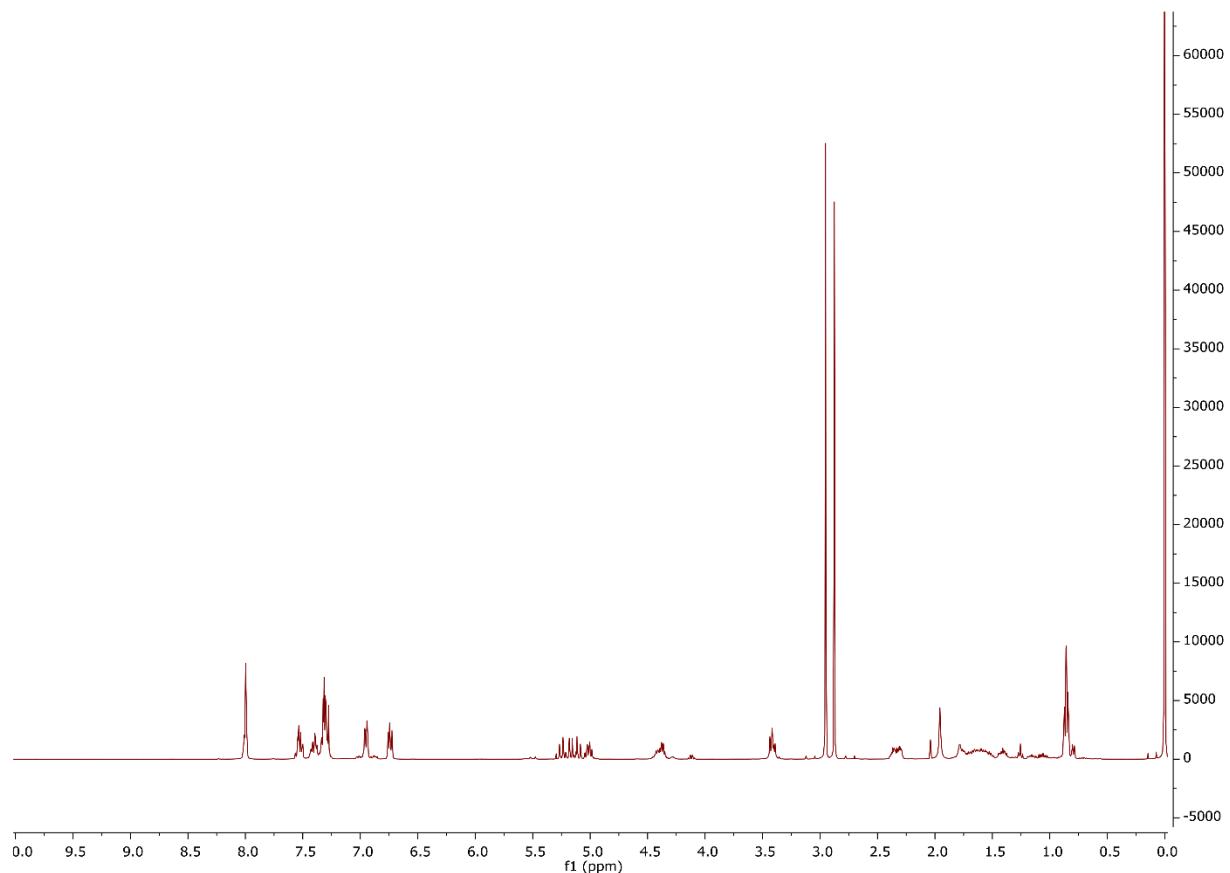


Figure S4. ^1H NMR spectra of CGA

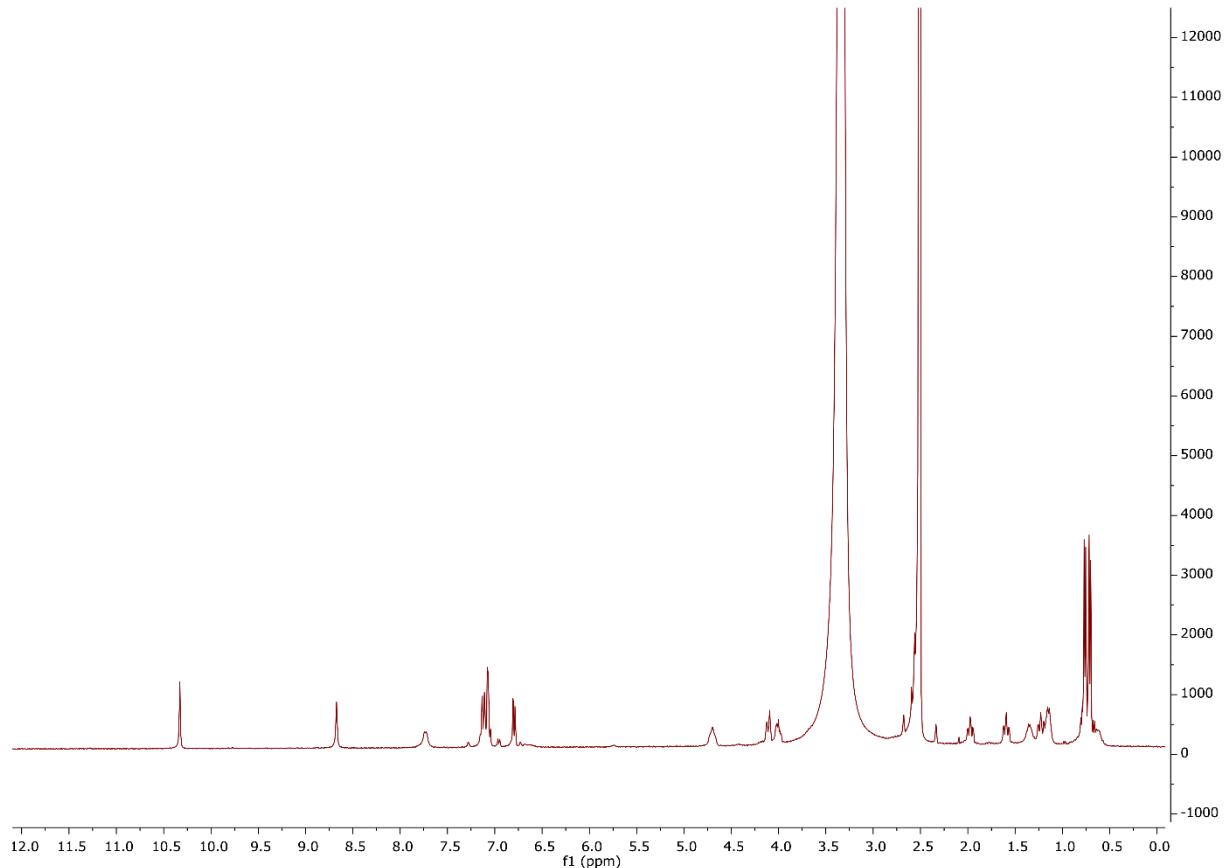
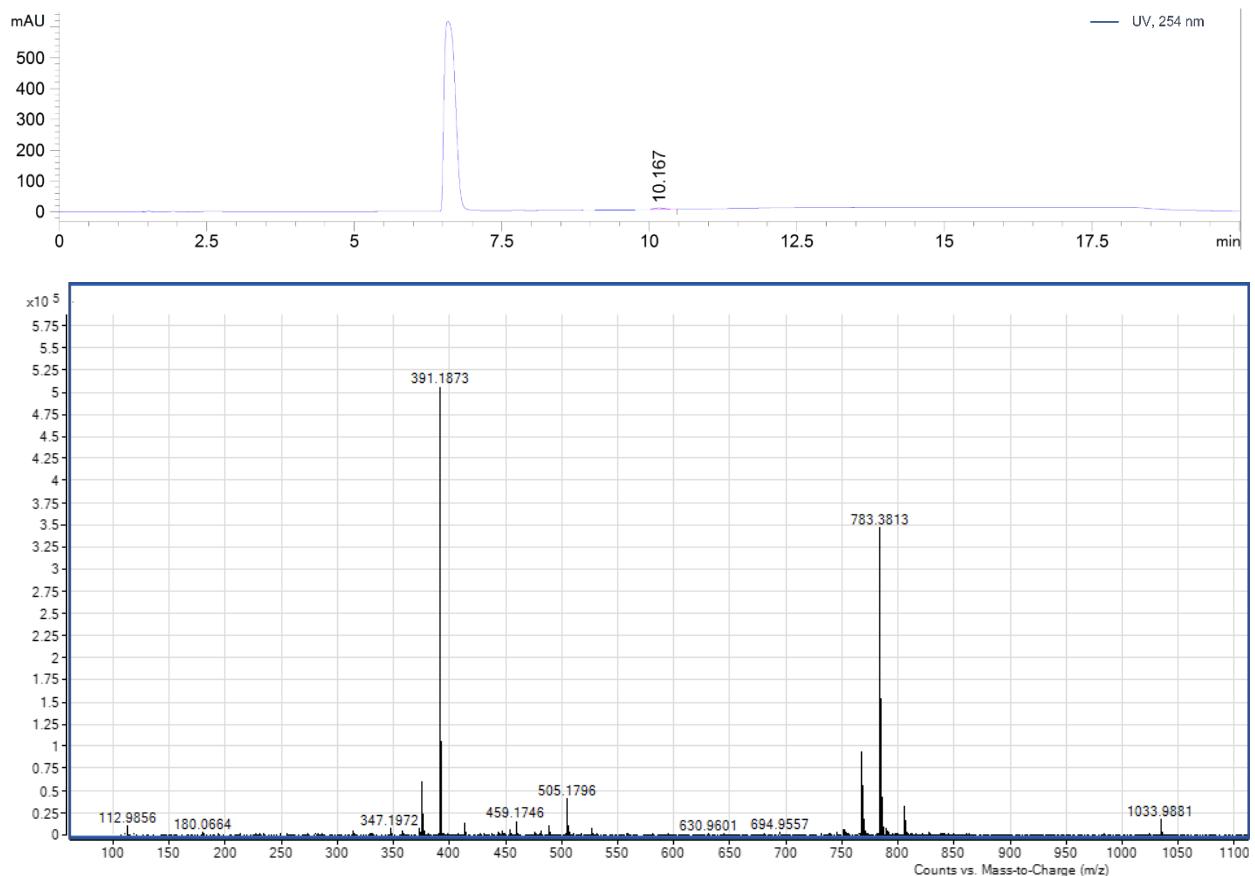


Figure S5. HPLC & HRMS spectra of CGA



Agilent QToF 6546 instrument (over 30,000 resolution at ~200mw): m/z of CGA calcd: 392.1947;
found: 391.1873 (negative mode)

Figure S6. ^1H NMR spectra of CGA-1

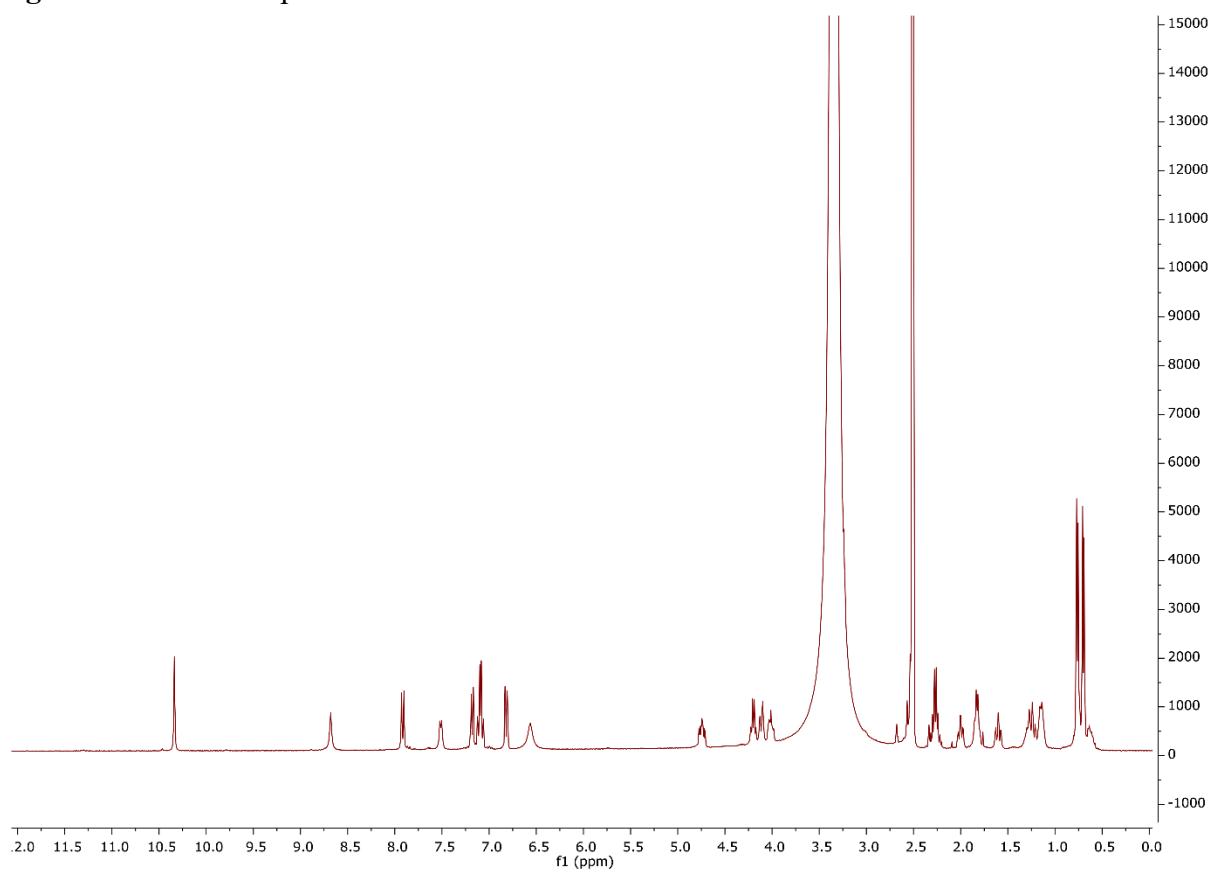
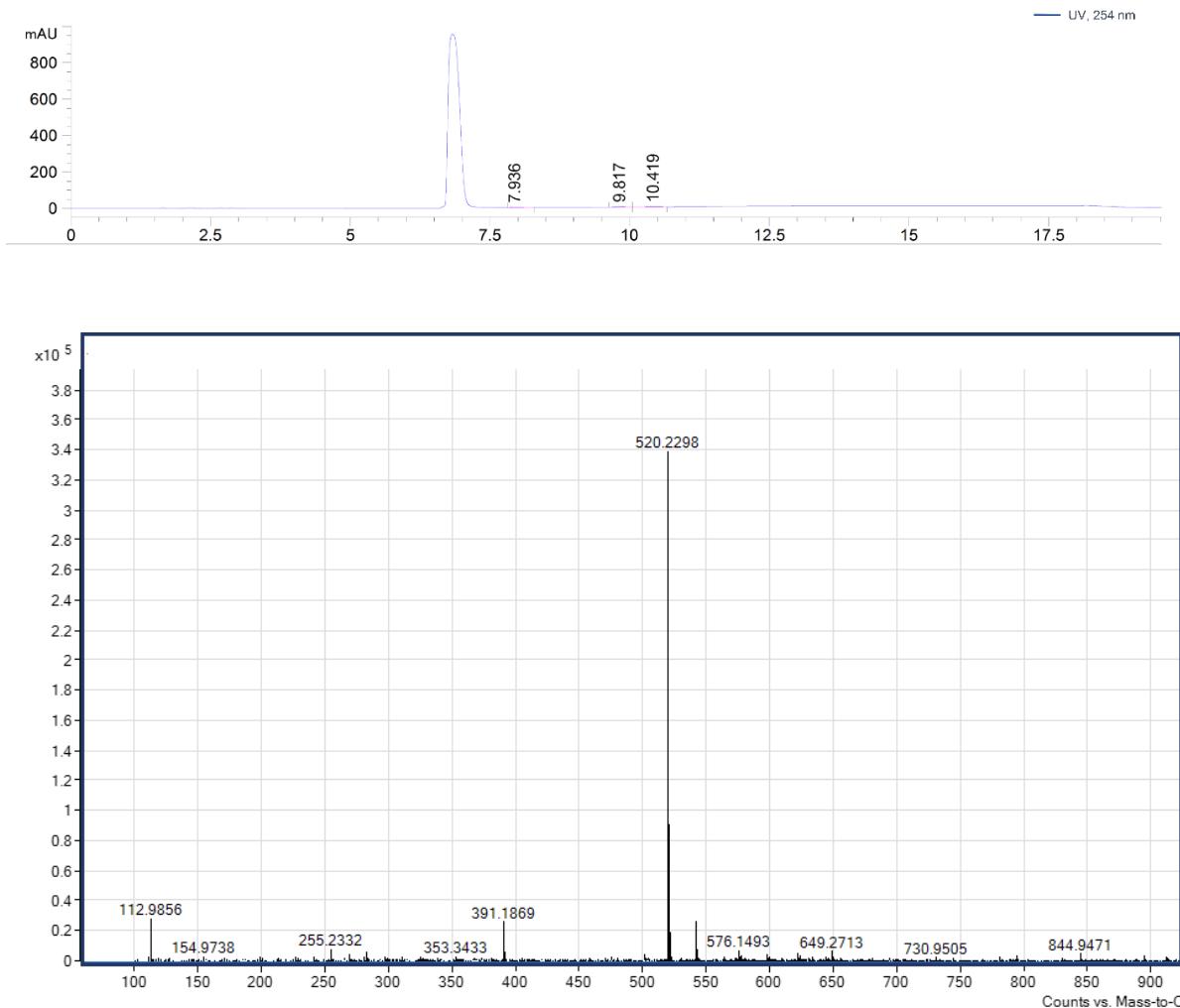


Figure S7. HPLC & HRMS spectra of CGA-1



Agilent QToF 6546 instrument (over 30,000 resolution at ~200mw): m/z of CGA-1 calcd: 521.2373; found: 520.2298 (negative mode)

Figure S8. ^1H NMR spectra of AGA

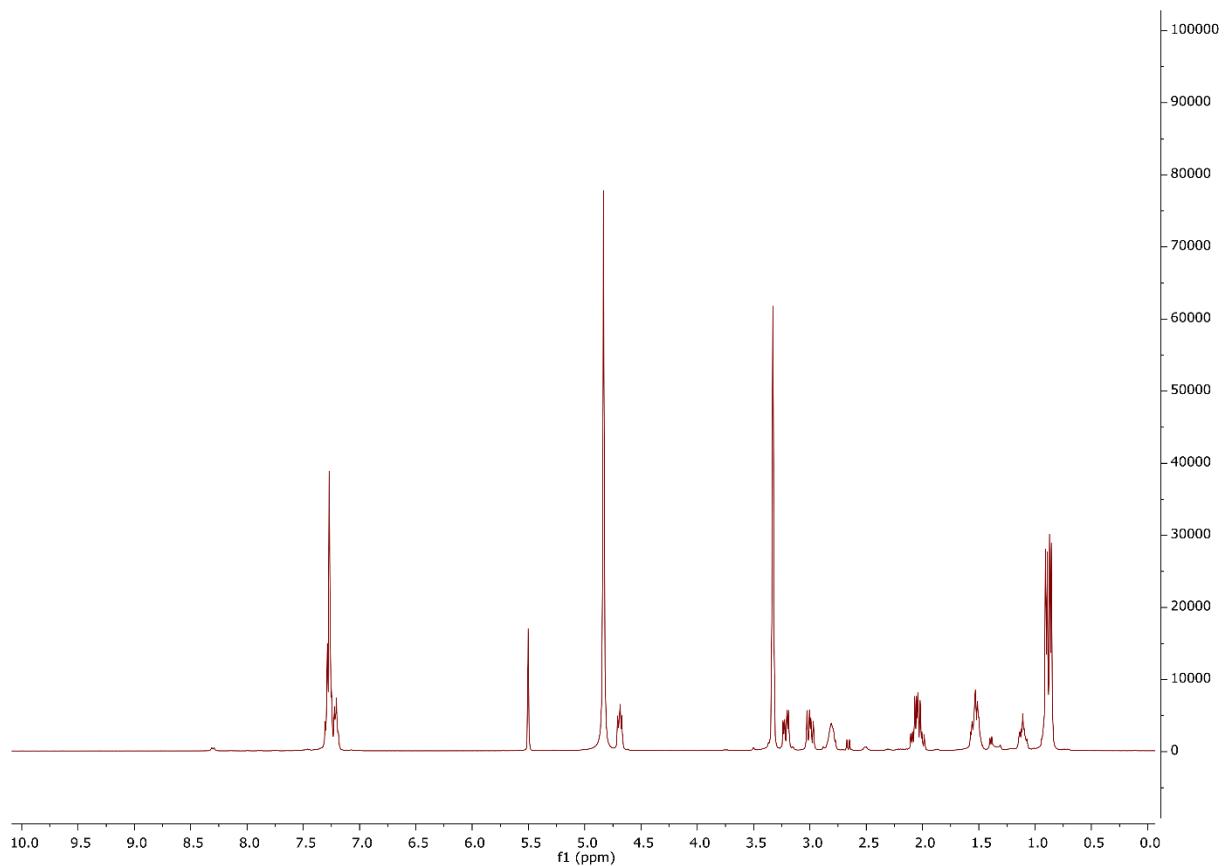
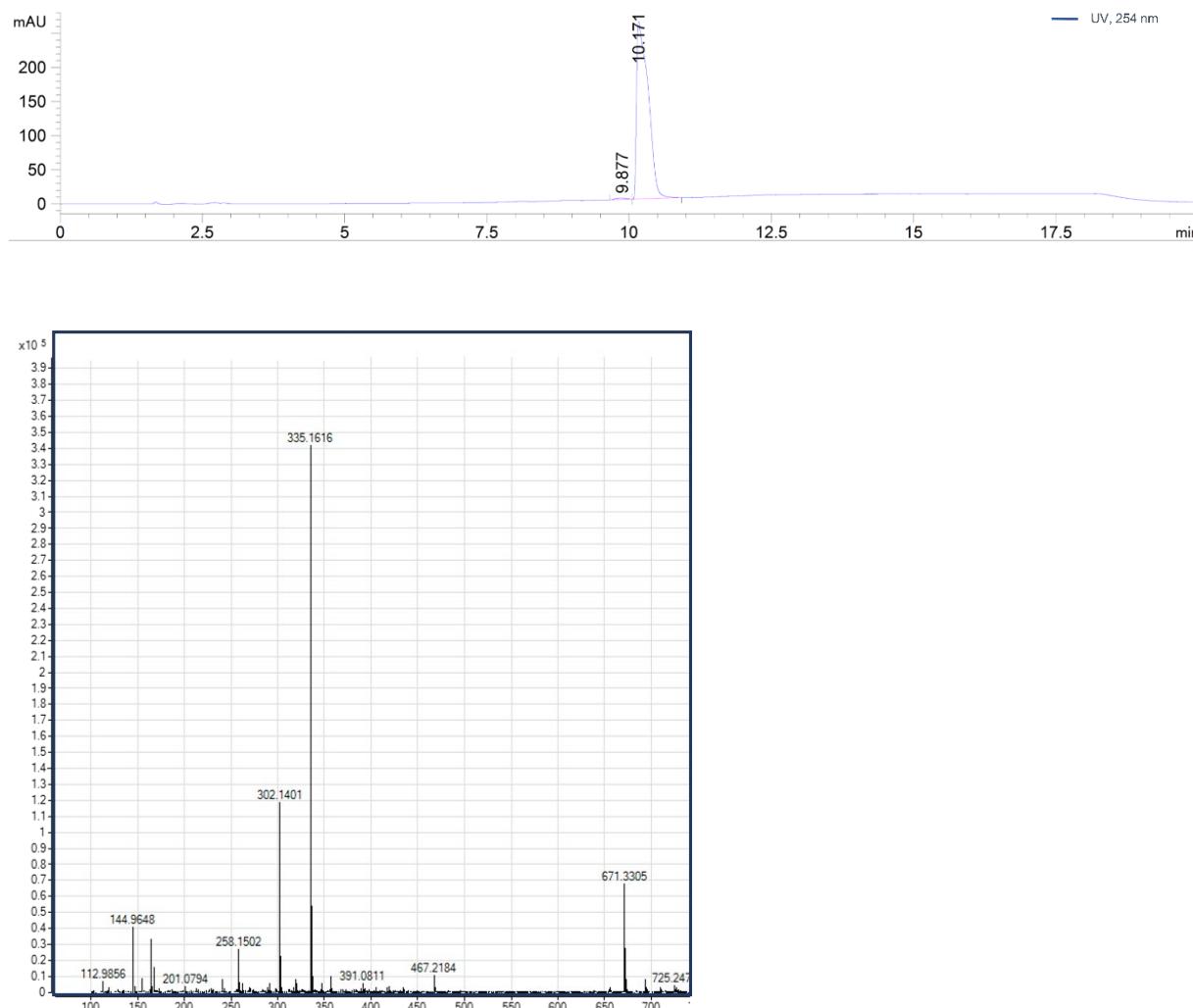


Figure S9. HPLC & HRMS spectra of AGA



Agilent QToF 6546 instrument (over 30,000 resolution at ~200mw): m/z of AGA calcd: 336.1685;
found: 335.1616 (negative mode)

Figure S10. ^1H NMR spectra of intermediate **19**

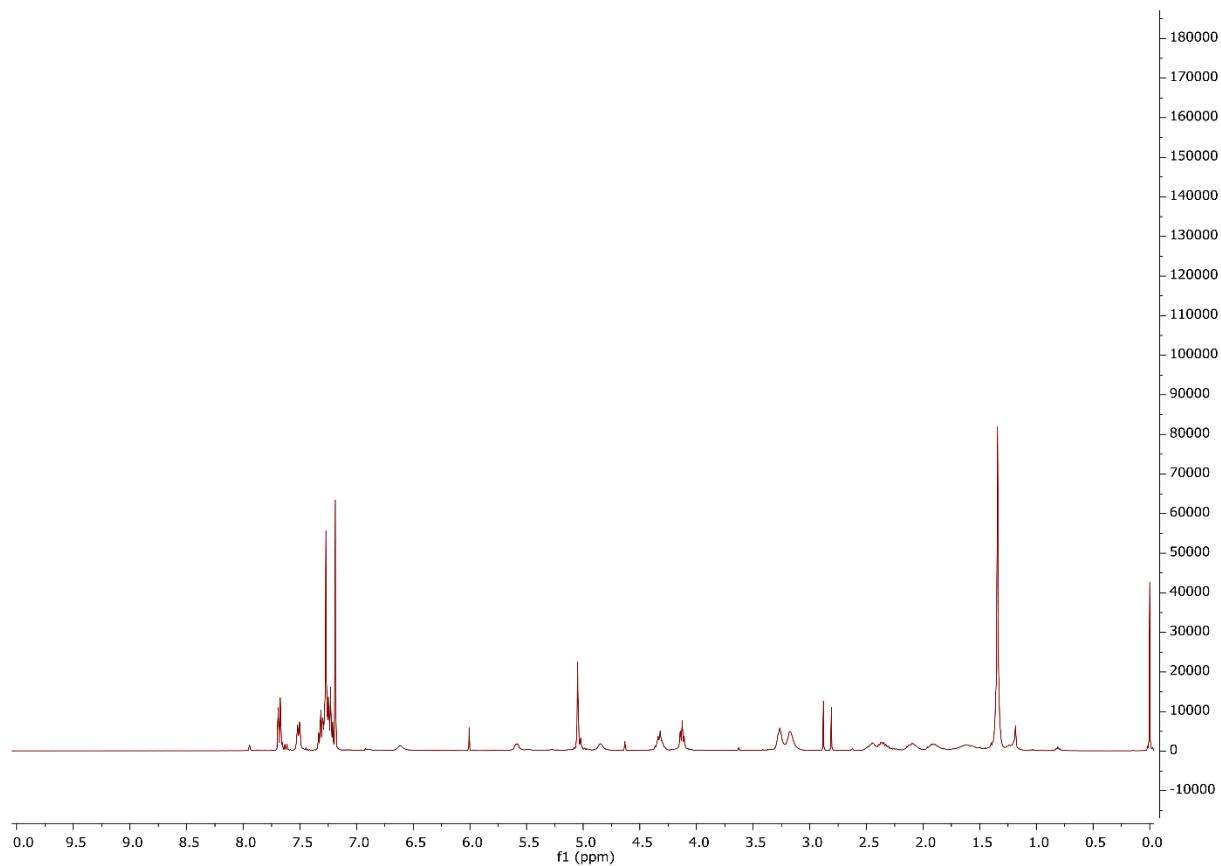


Figure S11. ^1H NMR spectra of intermediate **20**

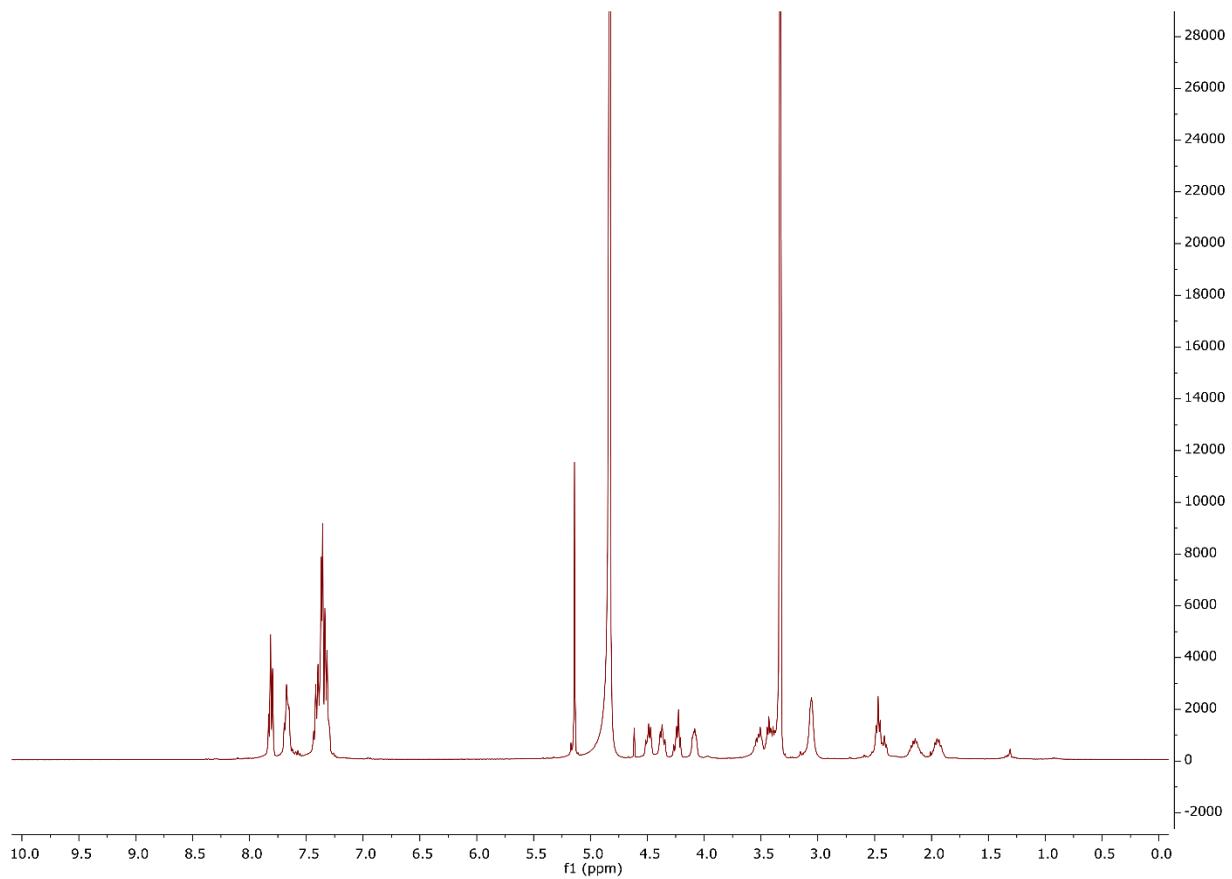


Figure S12. ^1H NMR spectra of intermediate **21**

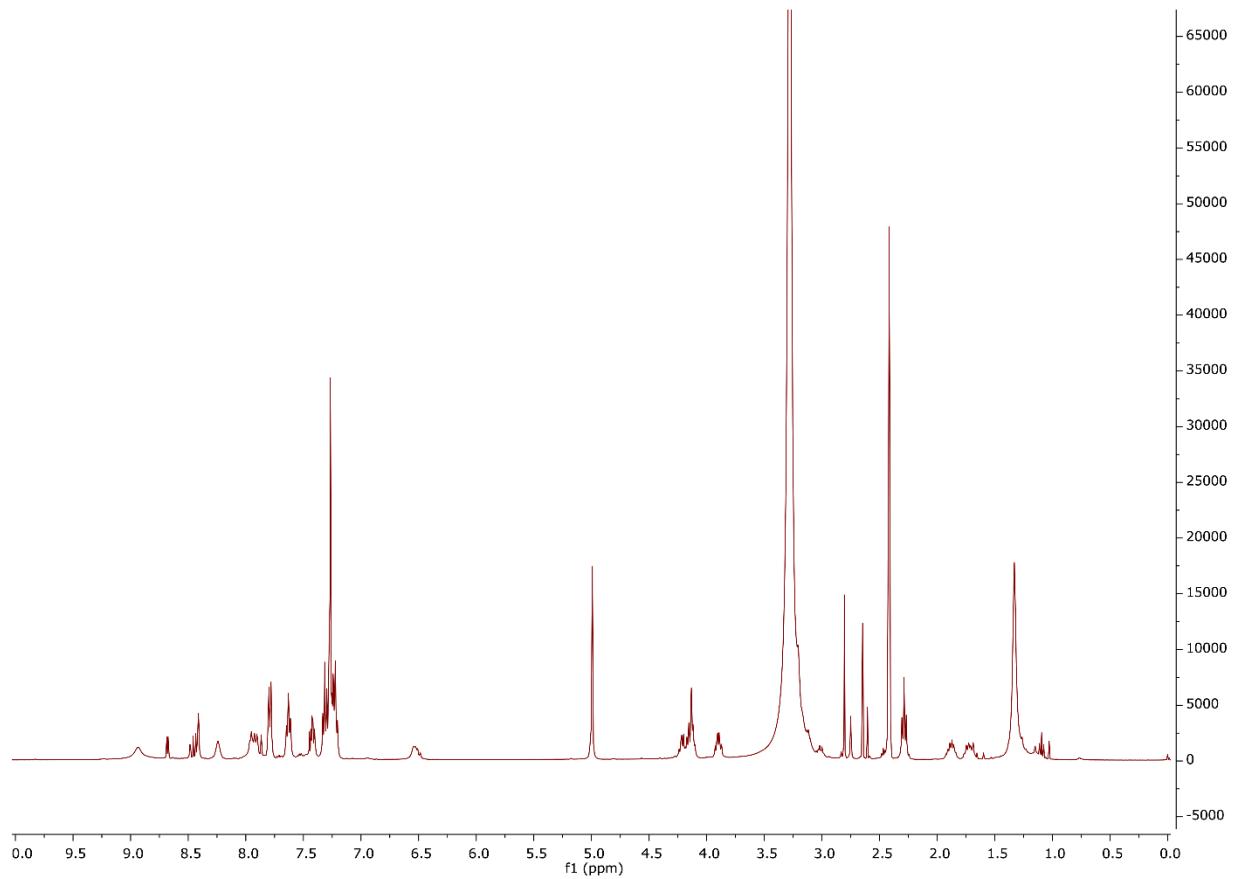


Figure S13. ^1H NMR spectra of intermediate **22**

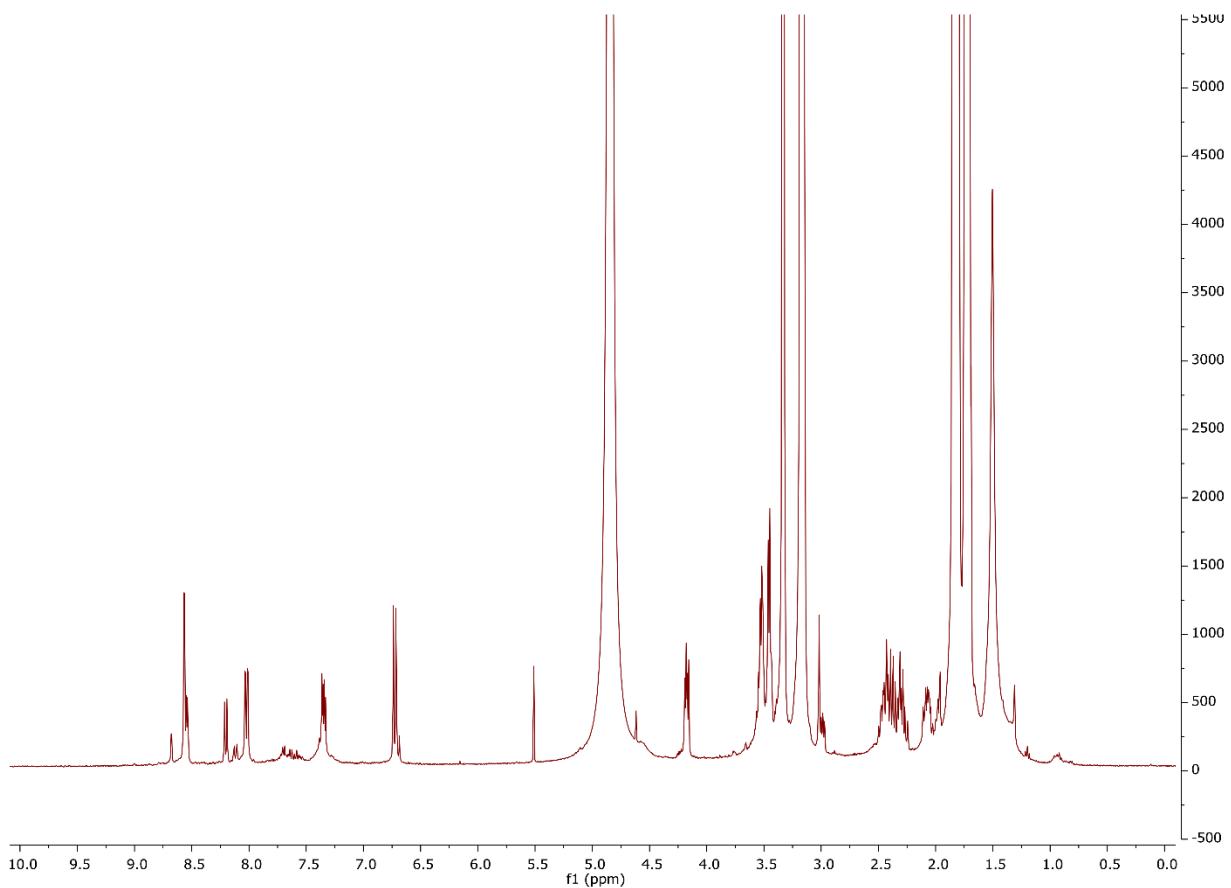


Figure S14. ^1H NMR spectra of intermediate **24** (AGA-1)

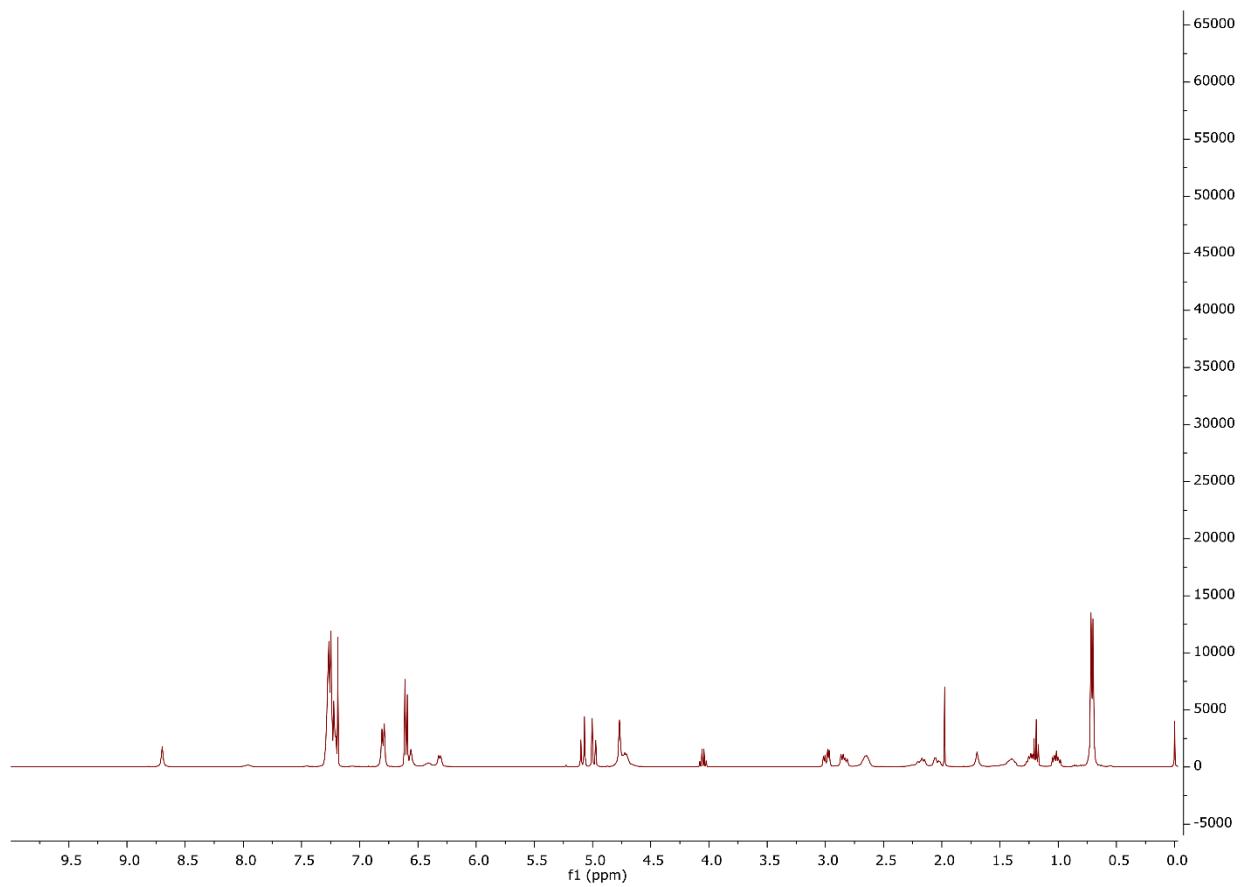
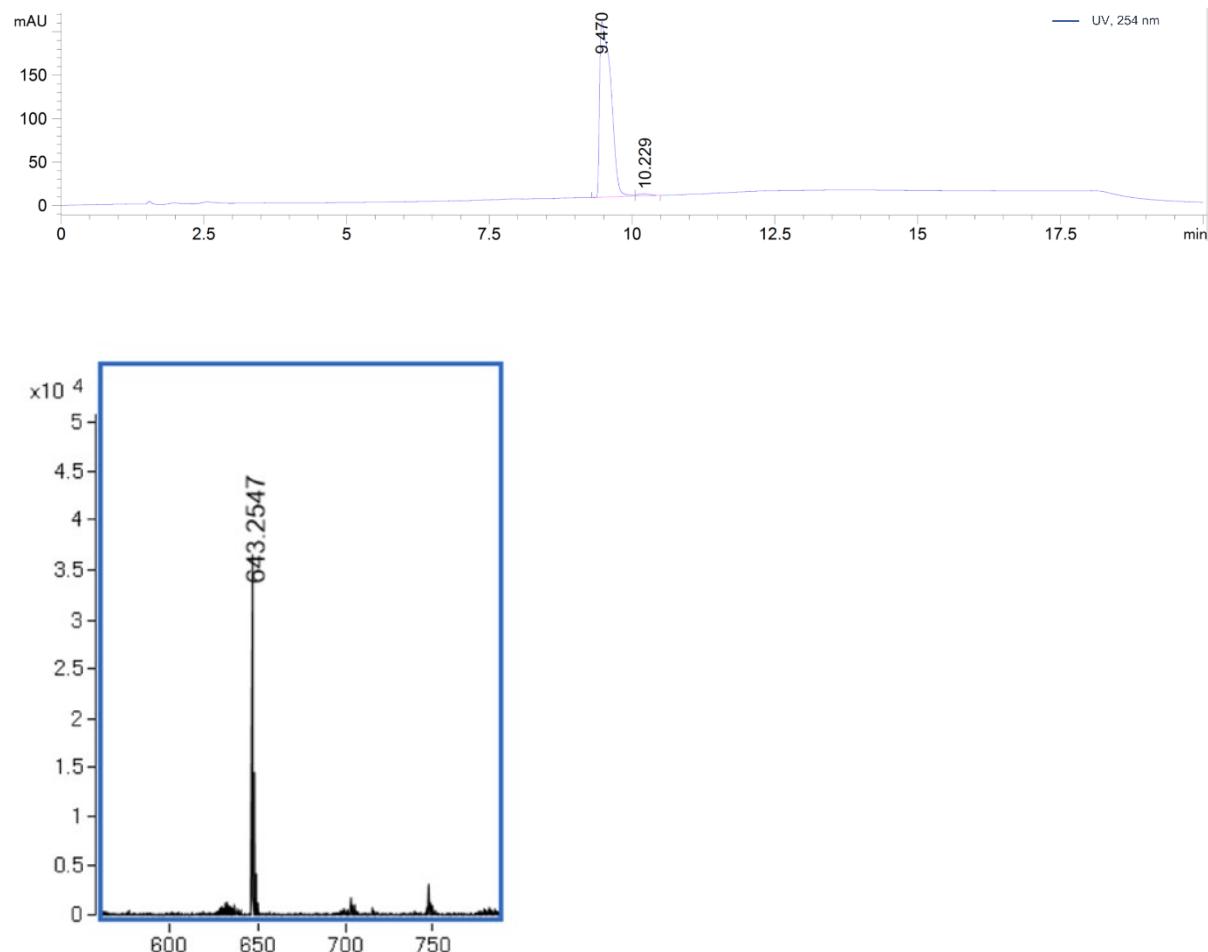


Figure S15. HPLC & HRMS spectra of AGA-1



Agilent QToF 6546 instrument (over 30,000 resolution at ~200mw): m/z of AGA-1 calcd: 642.3126; found: 643.2547 (positive mode)

Figure S16. ^1H NMR spectra of intermediate **27**

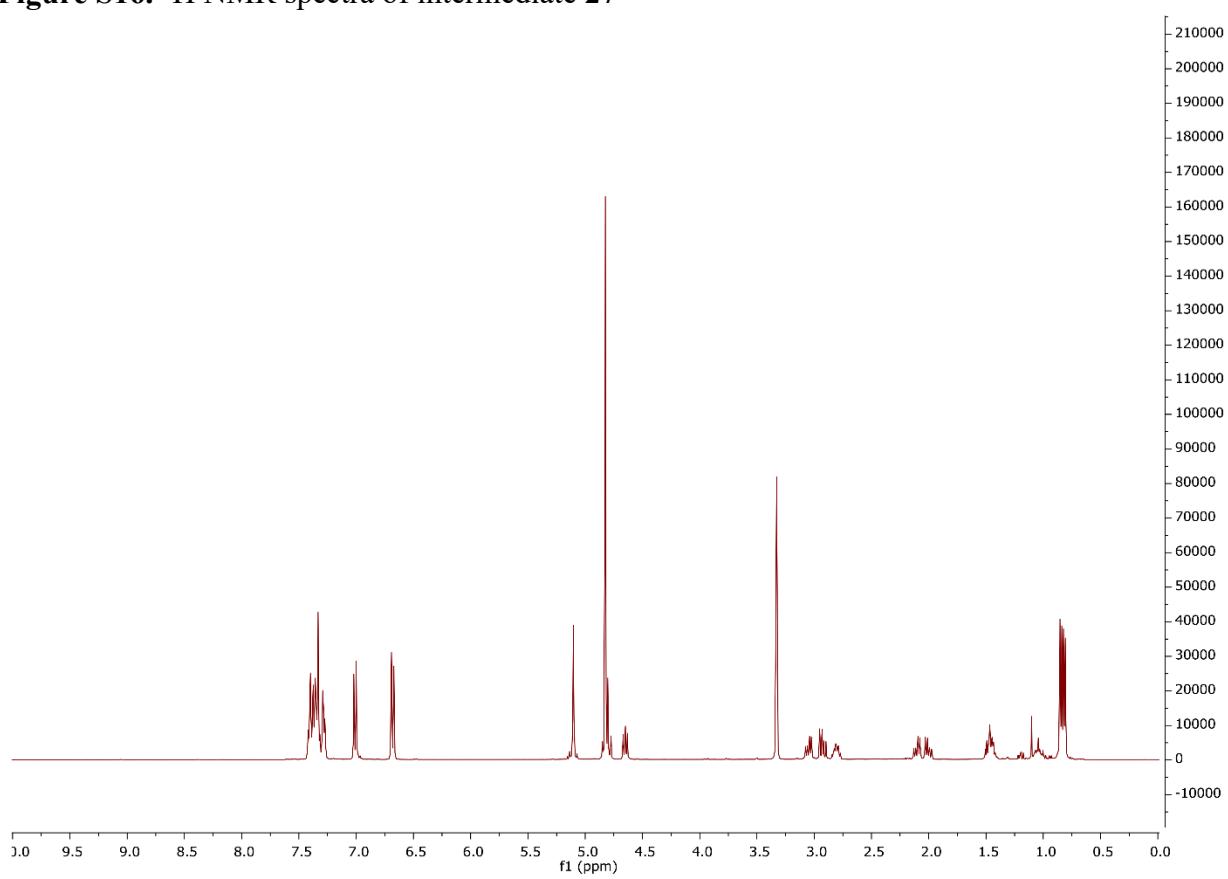


Figure S17. ^1H NMR spectra of intermediate **28**

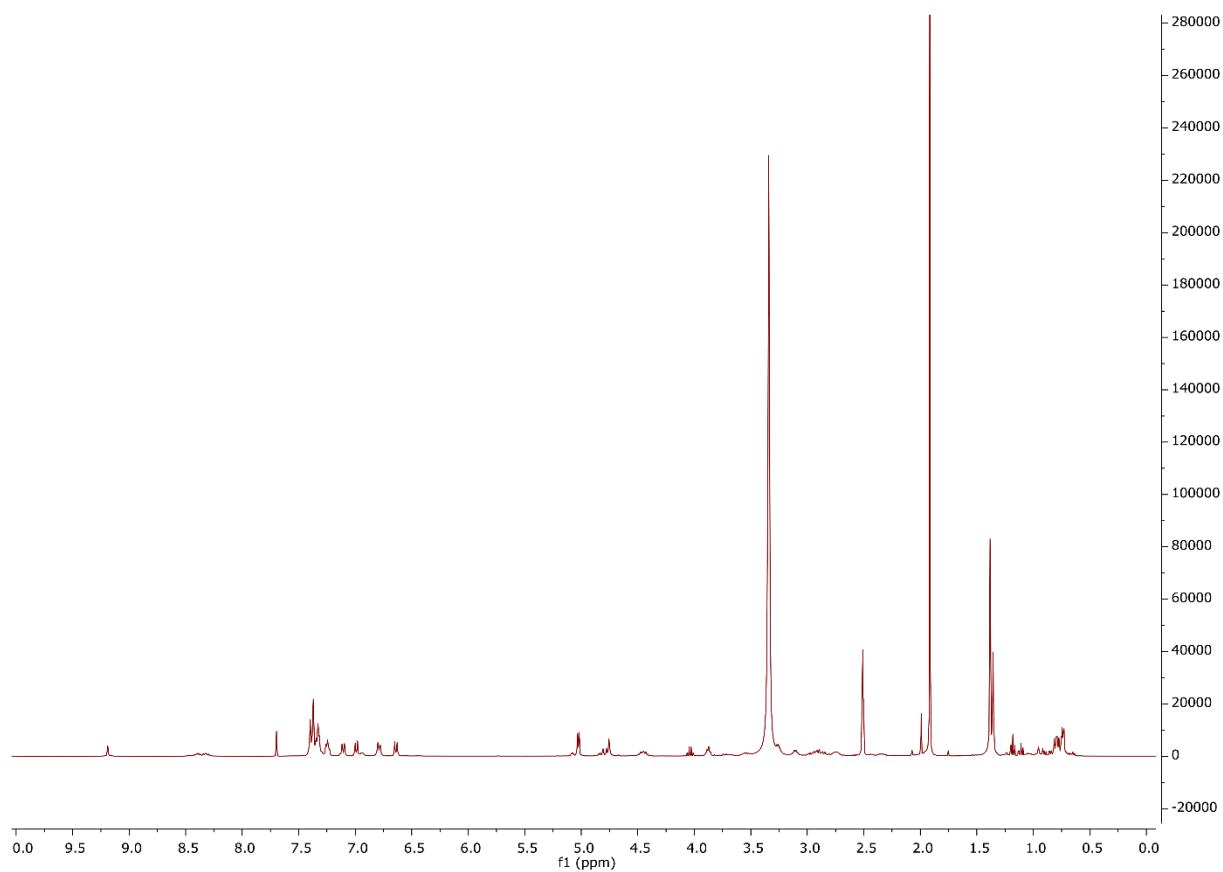


Figure S18. ^1H NMR spectra of intermediate **30**

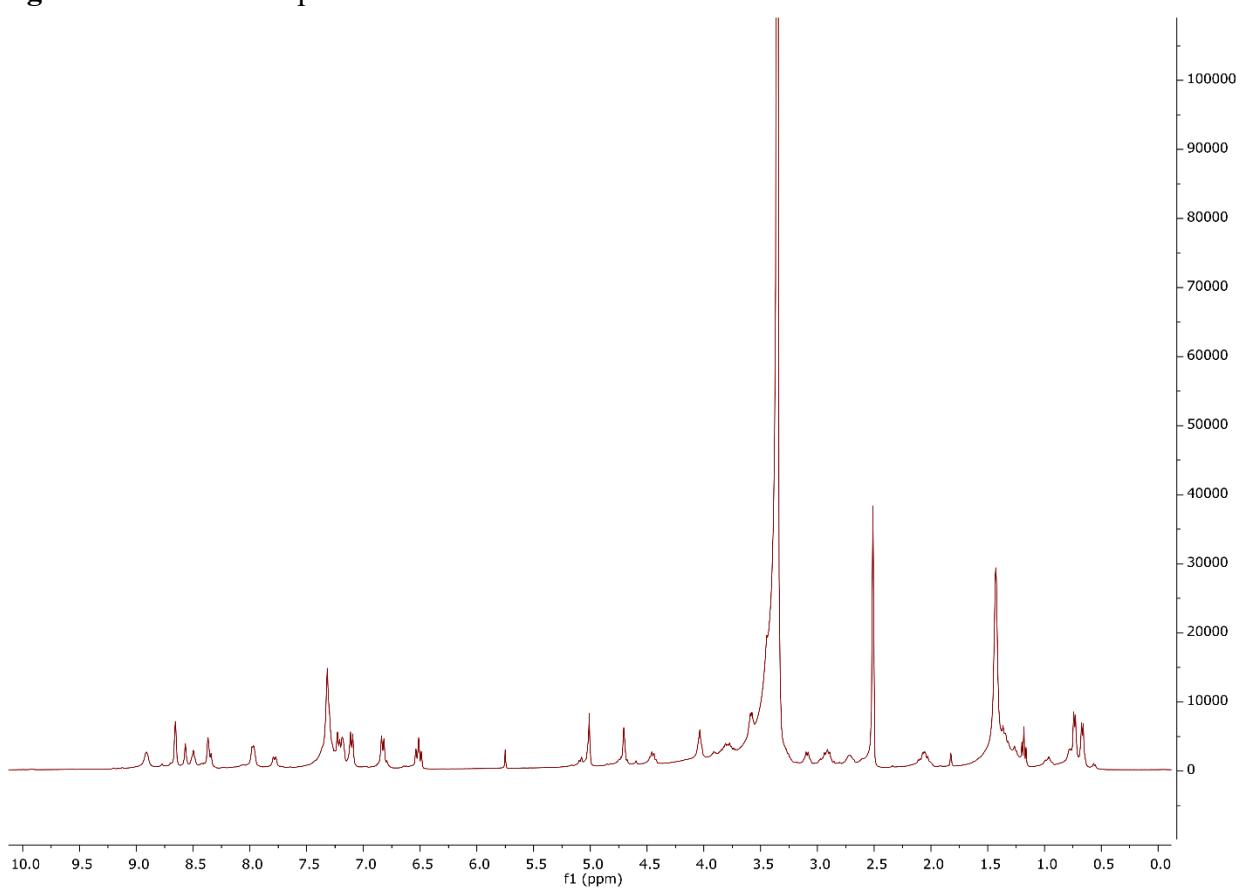


Figure S19. ^1H NMR spectra of intermediate 3.8 (AGA-2)

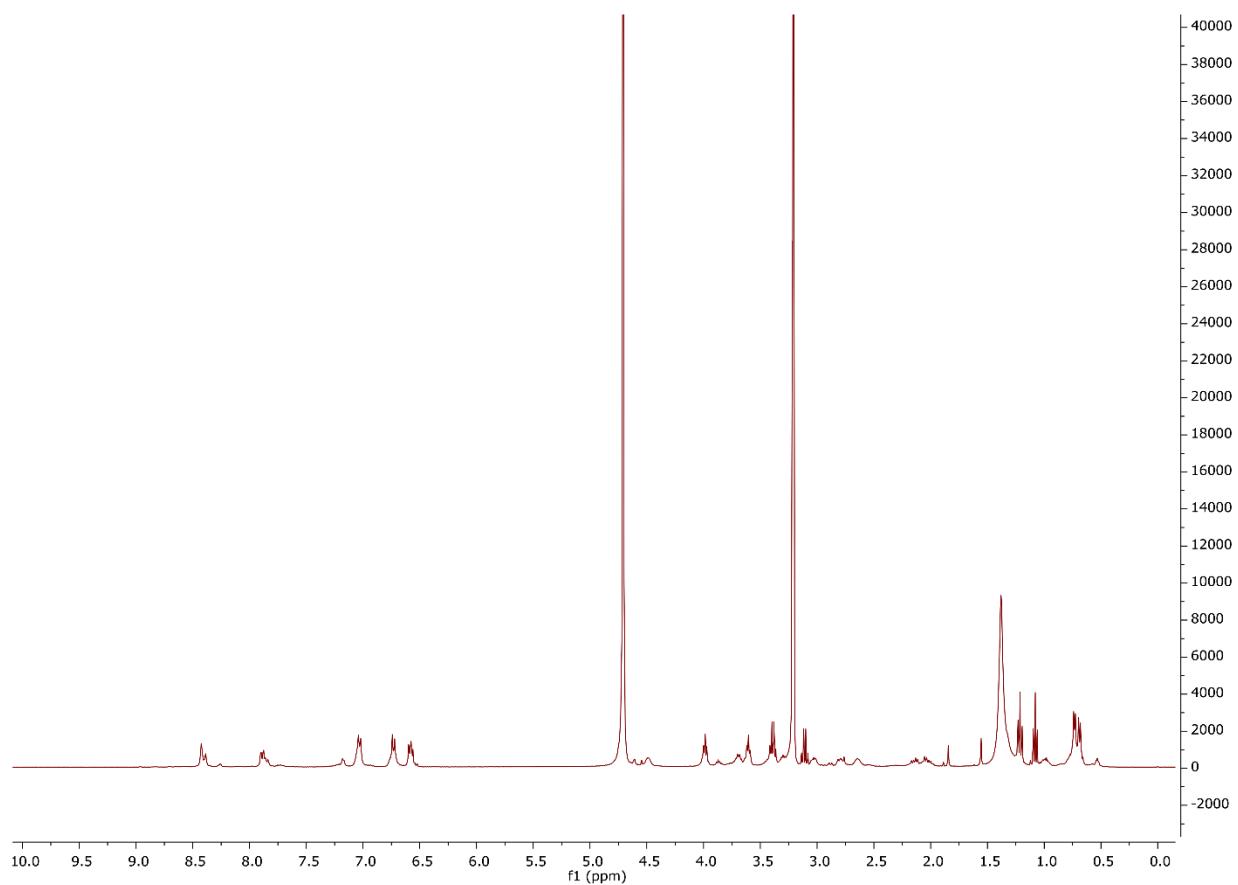
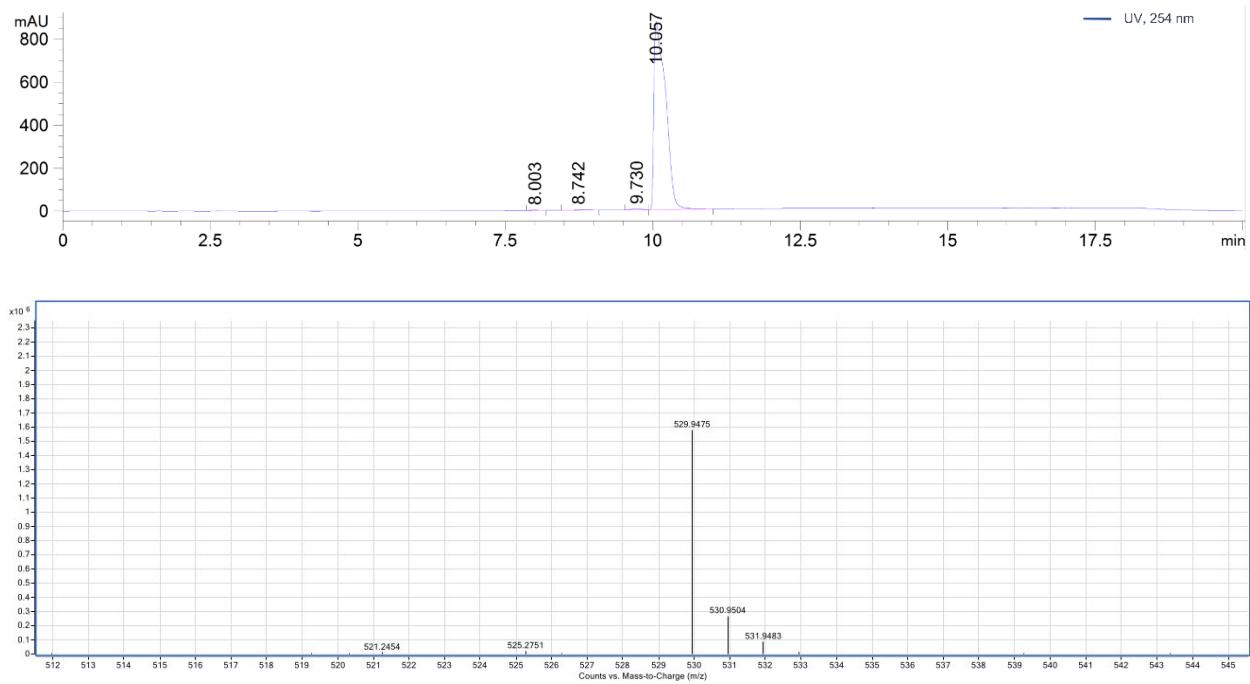


Figure S20. HPLC & HRMS spectra of AGA-2



Agilent QToF 6546 instrument (over 30,000 resolution at ~200mw): m/z of AGA-2 calcd: 529.2536; found: 529.9475 (positive mode)