Degradation of Rapamycin and its Ring-Opened Isomer: Role of Base Catalysis

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Supplementary Information

Table S1. Results of fitting of the kinetic data (A1, t1, y0), first-order rate constants (k), and half-lives ($t_{1/2}$).

Data	Fitting Equation	A1 (%)	t1 (h)	y0 (%)	k (h^{-1})	$t_{1/2}$ (h)
Rapamycin 237 mM ammonium acetate						
Rapamycin Isomer Peak 1 (RT 20.3 min) [a]	y = A1*exp(-x/t1) + y0	9.1	304	0	3.3E-03	210
Rapamycin Isomer Peak 2 (RT 21.1 min)[a]	y = A1*exp(-x/t1) + y0	87.0	289	0	3.5E-03	200
Sum of two rapamycin isomer peaks (RT 20.3 and 21.1 min) [a]	y = A1*exp(-x/t1) + y0	96.1	290	0	3.4E-03	201
Product Peak 1 (RT 18.7 min)	y = A1*exp(-x/t1) + y0	-22.1	184	23.2	5.4E-03	127
Product Peak 1 (RT 18.7 min)	y = A1*(1-exp(-x/t1))	22.4	162	NA	6.2E-03	112
Product Peak 2 (RT 23.7 min)	y = A1*exp(-x/t1) + y0	-40.6	169	41.1	5.9E-03	117
Product Peak 2 (RT 23.7 min)	y = A1*(1-exp(-x/t1))	40.8	163	NA	6.1E-03	113
Sum of two product peaks (RT 18.7 and 23.7 min)	y = A1*exp(-x/t1) + y0	-62.7	174	64.3	5.8E-03	121
Sum of two product peaks (RT 18.7 and 23.7 min) [b, c]	y = A1*(1-exp(-x/t1))	97	285	NA	3.5E-03	197
Secorapamycin 237 mM ammonium acetate						
Sum of two secorapamycin isomer peaks (RT 23.7 and 24.5 min) [a]	y = A1*exp(-x/t1) + y0	77.0	1913	0	5.2E-04	1326
Rapamycin 23.7 mM ammonium acetate						
Rapamycin Isomer Peak 1 (RT 20.3 min) [a]	y = A1*exp(-x/t1) + y0	9.1	1321	0	7.6E-04	916
Rapamycin Isomer Peak 2 (RT 21.1 min)[a]	y = A1*exp(-x/t1) + y0	87.4	1274	0	7.9E-04	883
Sum of two rapamycin isomer peaks (RT 20.3 and 21.1 min) [a]	y = A1*exp(-x/t1) + y0	96.6	1278	0	7.8E-04	886
Product Peak 1 (RT 18.7 min)	y = A1*exp(-x/t1) + y0	-29.2	559	30.1	1.8E-03	388
Product Peak 1 (RT 18.7 min)	y = A1*(1-exp(-x/t1))	24.7	392	NA	2.5E-03	272
Product Peak 2 (RT 23.7 min)	y = A1*exp(-x/t1) + y0	-17.8	594	18.3	1.7E-03	412
Product Peak 2 (RT 23.7 min)	y = A1*(1-exp(-x/t1))	15.0	424	NA	2.4E-03	294
Sum of two product peaks (RT 18.7 and 23.7 min)	y = A1*exp(-x/t1) + y0	-47.0	572	48.3	1.7E-03	396
Sum of two product peaks (RT 18.7 and 23.7 min) [b]	y = A1*(1-exp(-x/t1))	97	1266	NA	7.9E-04	878
Secorapamycin 23.7 mM ammonium acetate						
Sum of two secorapamycin isomer peaks (RT 23.7 and 24.5 min) [a]	y = A1*exp(-x/t1) + y0	78.7	5620	0	1.8E-04	3896

[a] Parameter y0 was kept fixed at 0;

[b] Parameter A1 was kept fixed at 97, which corresponds to the average percentage of the total area for the two isomers of rapamycin found in non-degraded samples;

[c] The first six time points were used for fitting; For these data points, the sum area for the two isomers of rapamycin and two products accounted for >94% of the total area under the curve.

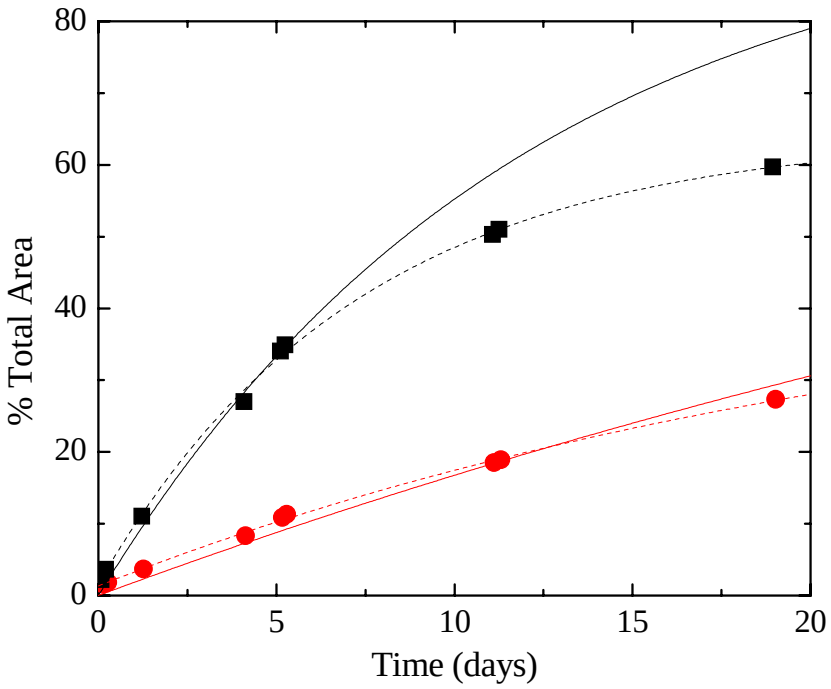
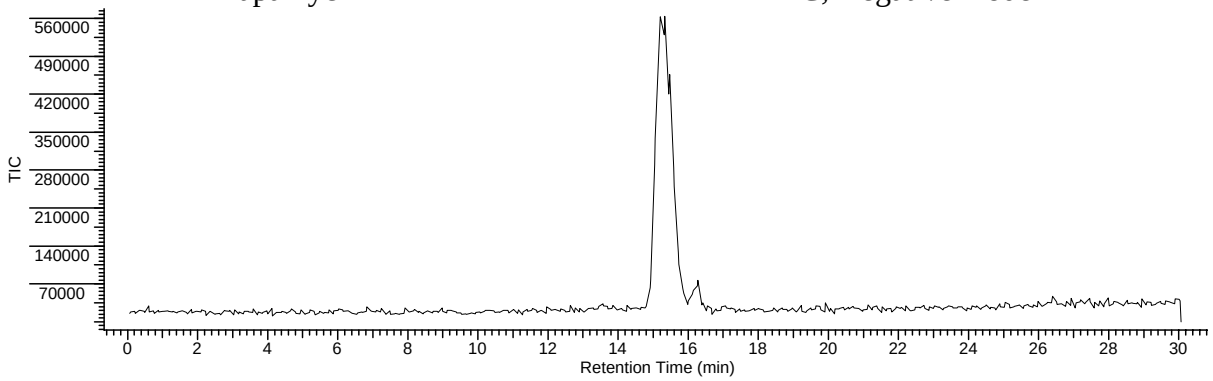


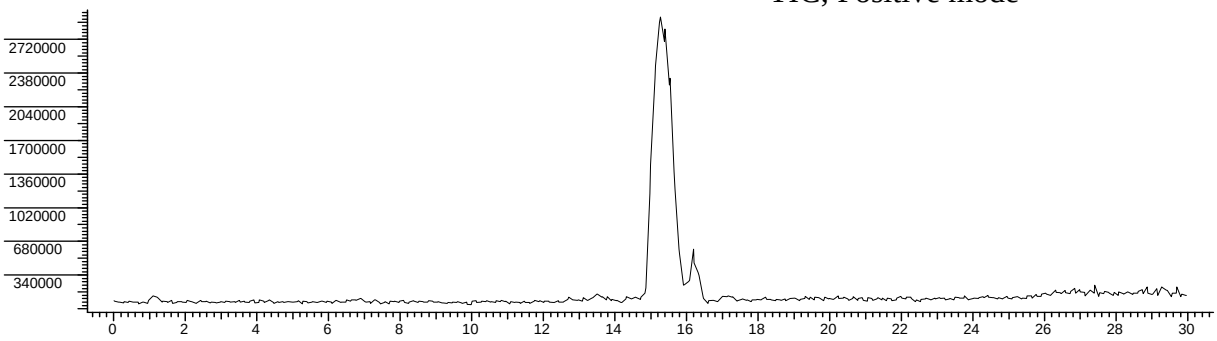
Figure S1. %Total area-time profiles showing accumulation of the two degradation products of rapamycin. Rapamycin solutions in 30/70 v/v MeCN/H_2O mixture contained 23.7 mM (red circles) and 237 mM ammonium acetate (black squares). % Total area was calculated by summing the area under the chromatographic curve for two peaks with RT 18.7 min and RT 23.7 min. Dashed lines show results of fitting with equation y = A1*exp(-x/t1) + y0. Solid lines correspond to results of fitting with equation y = A1*(1 - exp(-x/t1), where parameter t1 was varied and parameter A1 was kept constant and equal to 97.0%.

Rapamycin

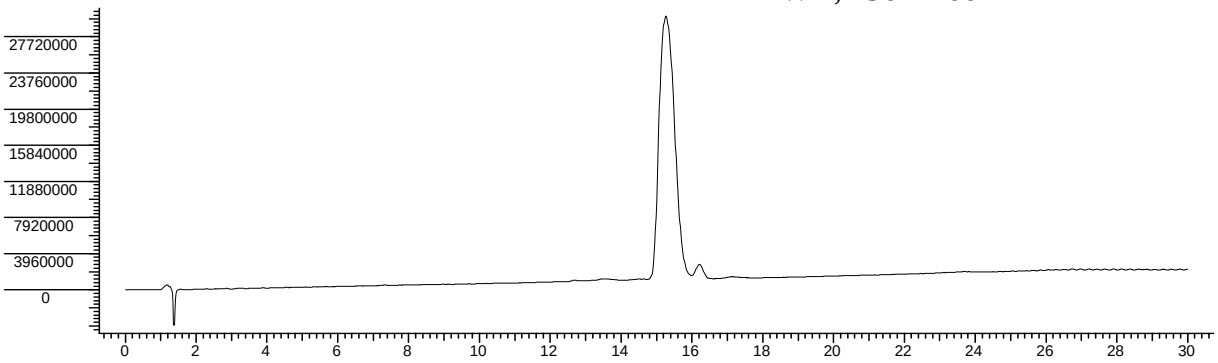
TIC; Negative mode



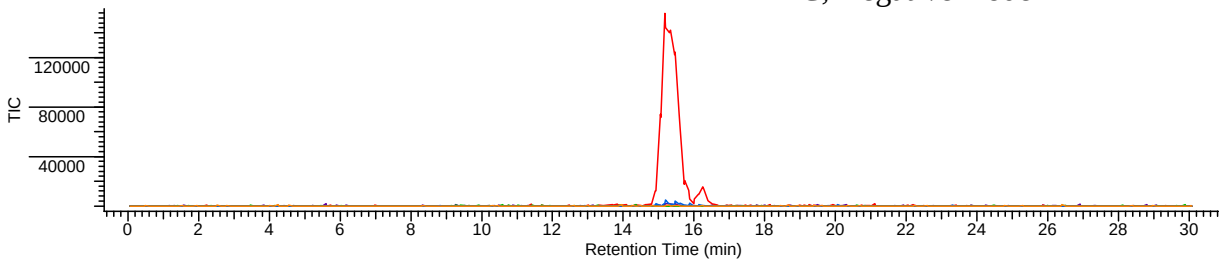
TIC; Positive mode



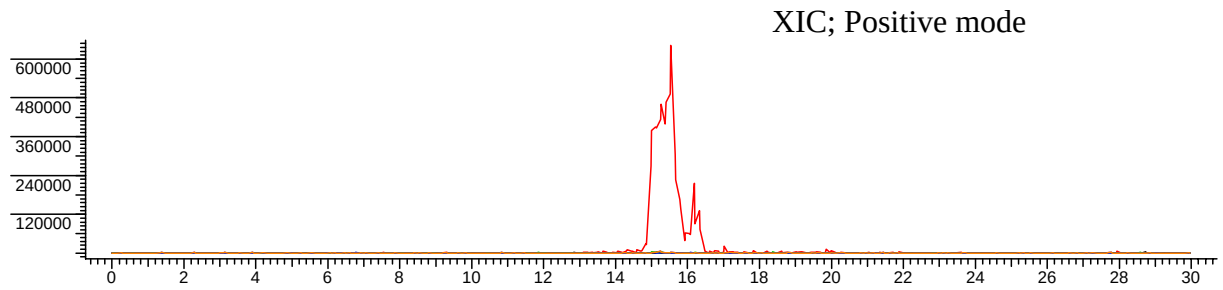
TWA; 190 – 400 nm



XIC; Negative mode



No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	321.20	-	-	-	-	-	-	-	-	-	-		Manual
2	590.50	-	-	-	-	-	-	-	-	-	-		Manual
3	608.50	-	-	-	-	-	-	-	-	-	-		Manual
4	912.50	1193267.88	15.179	-	225	155564.016	6.000	79.106	0.267	1	912.601		Manual
5	930.50	-	-	-	-	-	-	-	-	-	-		Manual
6	948.50	-	-	-	-	-	-	-	-	-	-		Manual

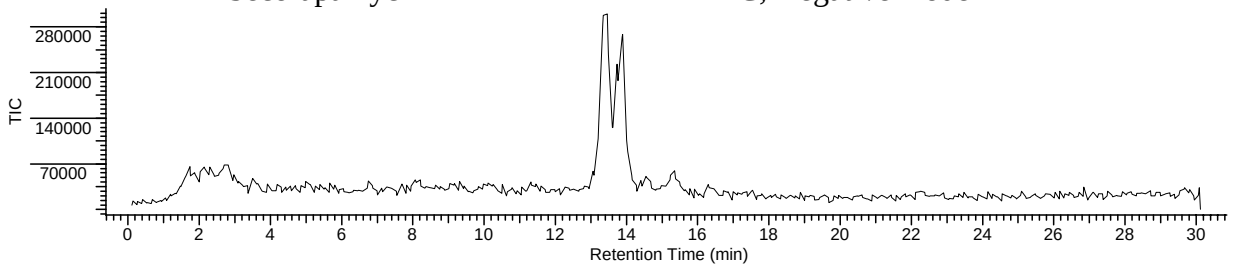


No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	323.20	15488.50	15.246	-	227	5179.001	2.000	4.474	1.686	1	864.503		Manual
2	323.20	15488.50	15.246	-	227	5179.001	2.000	4.474	1.686	1	864.503		Manual
3	632.50	-	-	-	-	-	-	-	-	-	-		Manual
4	936.50	5165959.00	15.536	-	232	641794.938	9.000	66.912	5.020	1	936.614		Manual
5	954.50	-	-	-	-	-	-	-	-	-	-		Manual
6	972.50	-	-	-	-	-	-	-	-	-	-		Manual

Figure S2. RP-HPLC-UV and –ESI-MS traces of rapamycin solution in 30/70 MeCN-water, no other additives.

Secorapamycin

TIC; Negative mode



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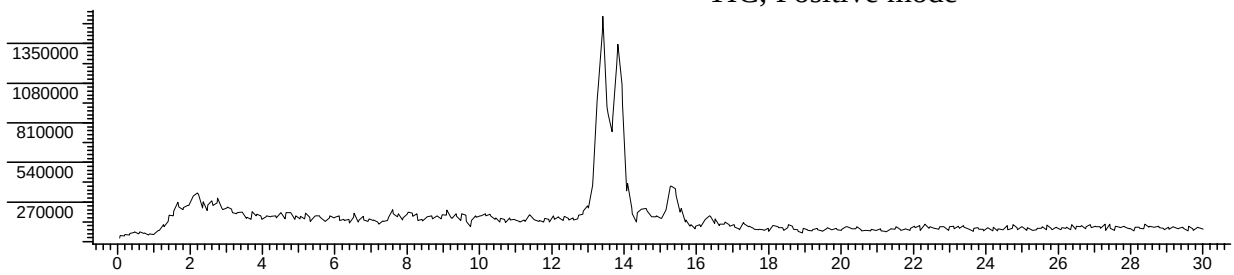
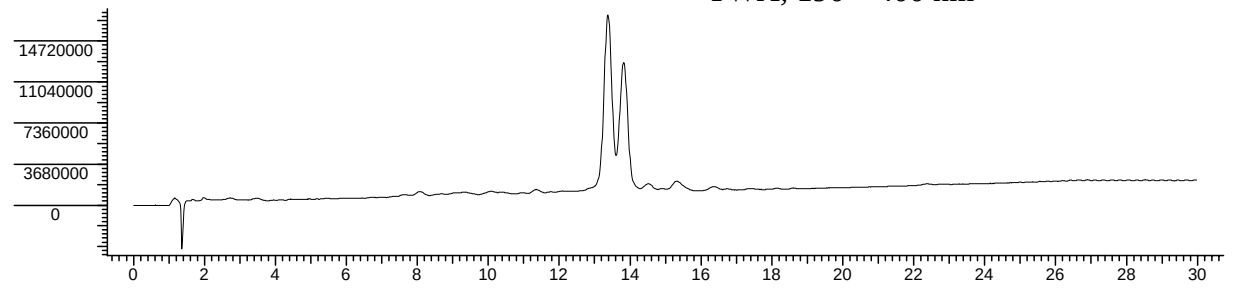


TWA; 190 – 400 nm



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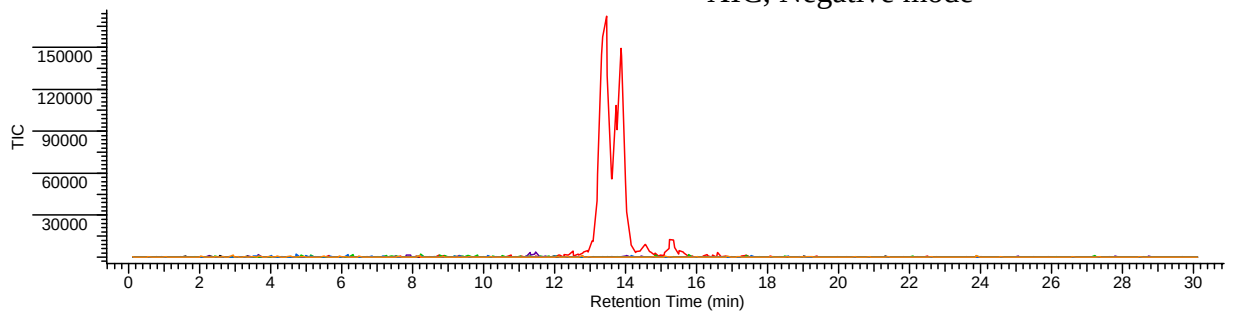
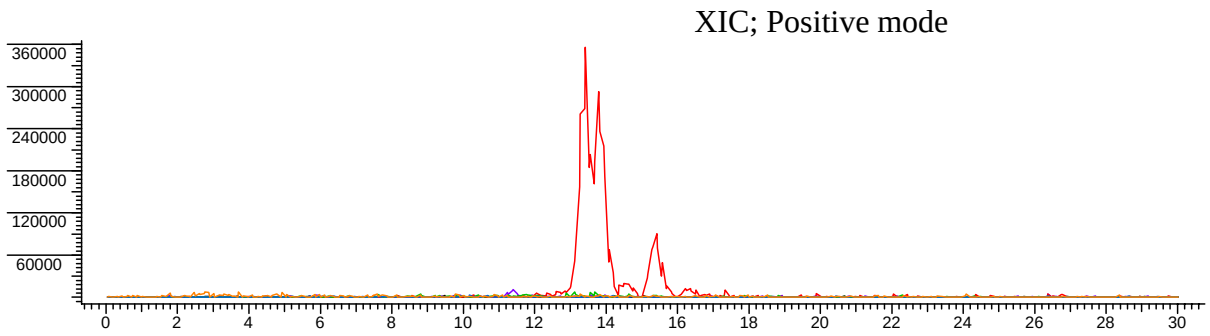


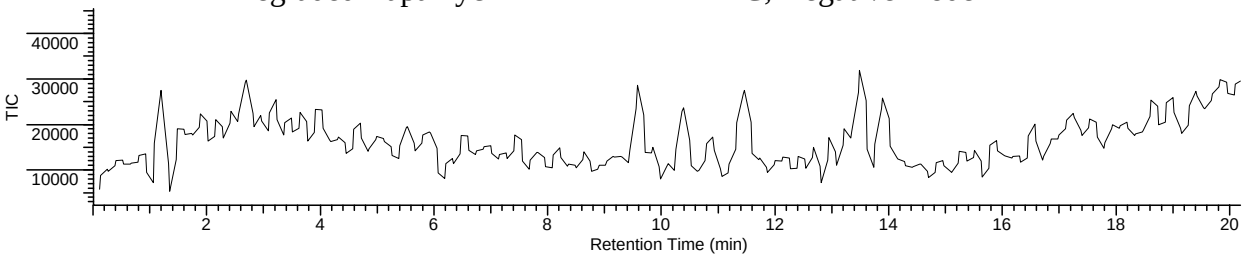
No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	321.20	-	-	-	-	-	-	-	-	-	-		Manual
2	590.50	-	-	-	-	-	-	-	-	-	-		Manual
3	608.50	-	-	-	-	-	-	-	-	-	-		Manual
4	912.50	793791.00	13.466	-	199	172271.016	4.000	89.371	2.416	1	912.590		Manual
5	930.50	-	-	-	-	-	-	-	-	-	-		Manual
6	948.50	-	-	-	-	-	-	-	-	-	-		Manual



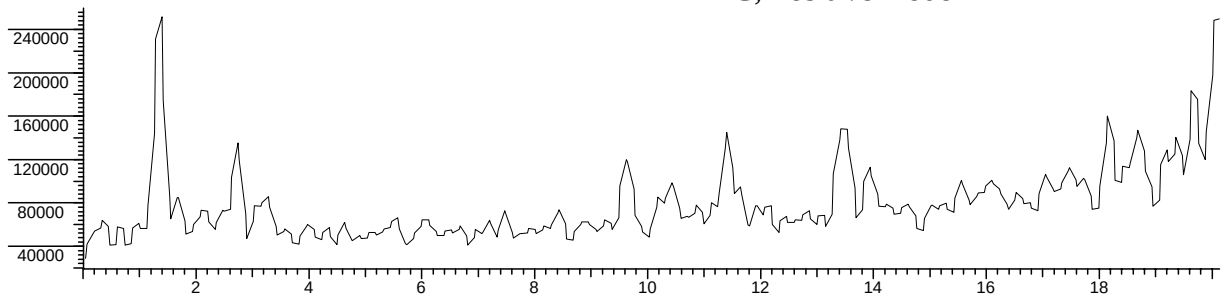
No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	323.20	-	-	-	-	-	-	-	-	-	-		Manual
2	614.50	31754.99	11.396	-	170	11288.996	2.000	7.345	0.887	1	923.458		Manual
3	632.50	-	-	-	-	-	-	-	-	-	-		Manual
4	936.50	1668539.38	13.418	-	200	356406.000	5.000	115.964	0.739	1	936.624		Manual
5	954.50	12768.50	13.128	-	195	7394.000	2.000	1.880	2.937	1	936.684		Manual
6	972.50	-	-	-	-	-	-	-	-	-	-		Manual

Figure S3. RP-HPLC-UV and –ESI-MS traces of secorapamycin solution in 30/70 MeCN-water, no other additives.

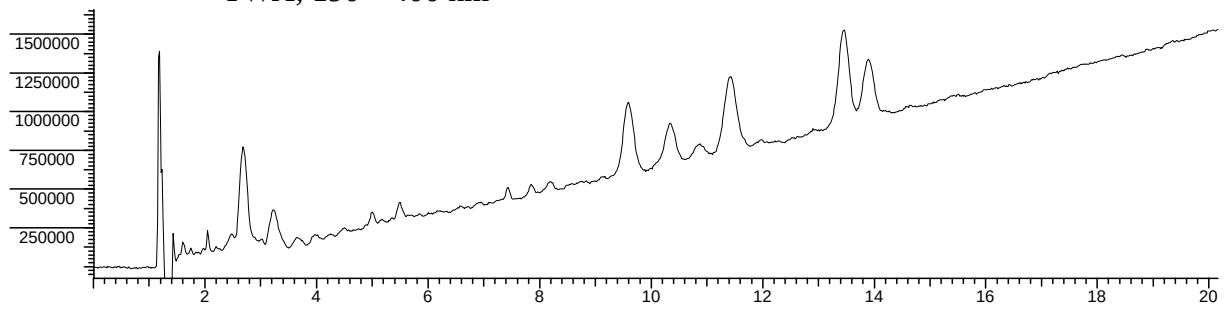
Degraded Rapamycin TIC; Negative mode



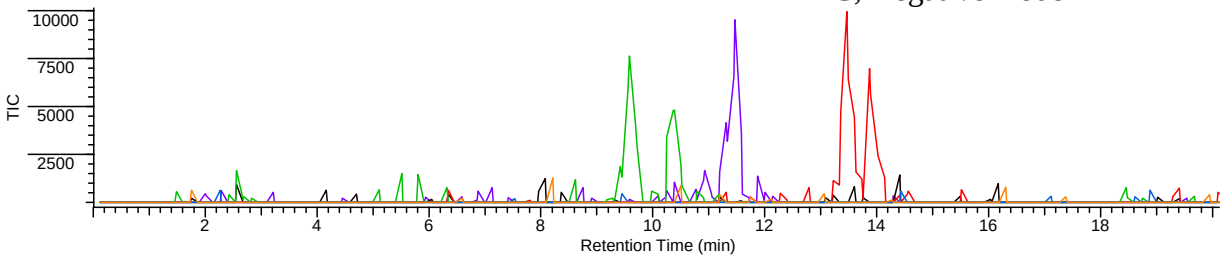
TIC; Positive mode



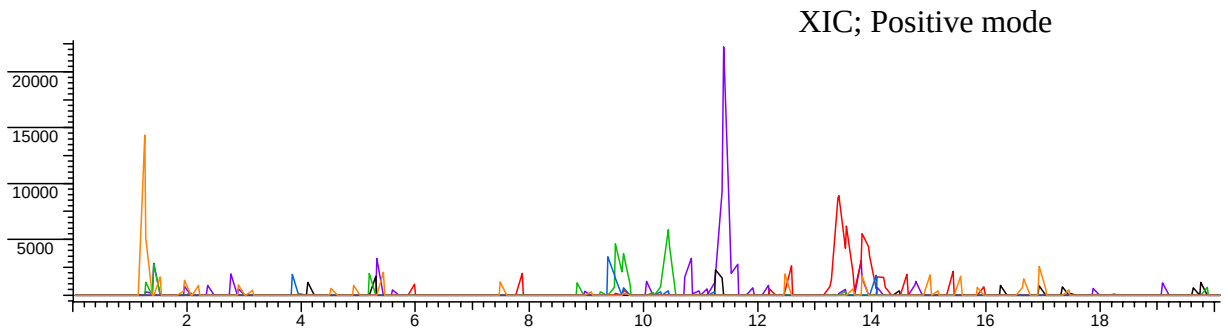
TWA; 190 – 400 nm



XIC; Negative mode



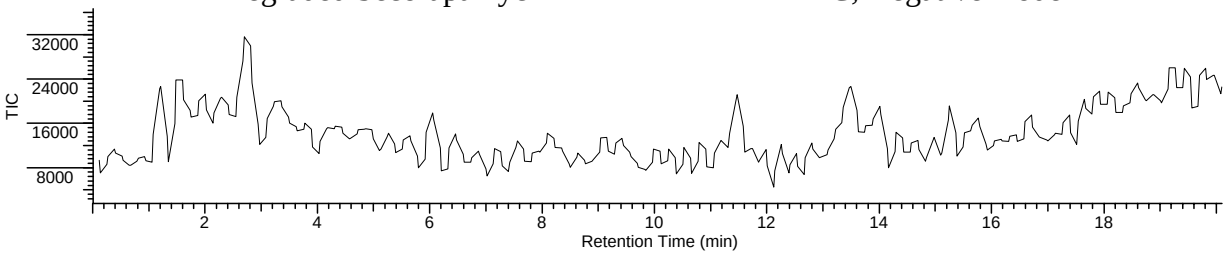
No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	321.20	-	-	-	-	-	-	-	-	-	-		Manual
2	590.50	21850.82	11.467	-	170	9519.725	2.000	100.038	0.879	1	590.285		Manual
3	608.50	-	-	-	-	-	-	-	-	-	-		Manual
4	912.50	30380.87	13.470	-	199	9945.145	2.000	104.508	1.064	1	912.567		Manual
5	930.50	23734.45	9.579	-	142	7638.293	3.000	80.267	0.608	1	930.616		Manual
6	948.50	-	-	-	-	-	-	-	-	-	-		Manual



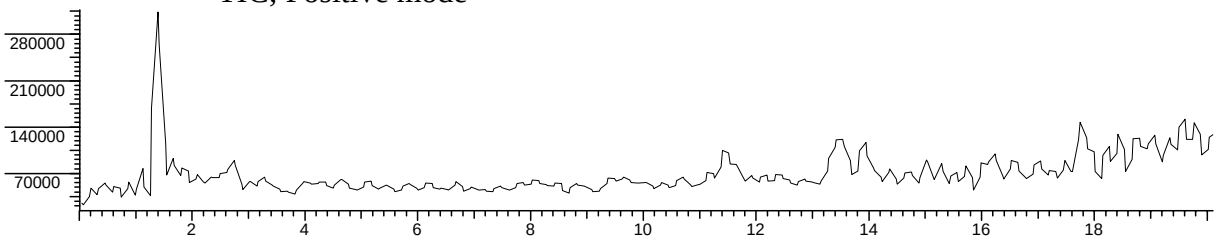
No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	323.20	19443.36	1.262	-	19	14324.150	1.000	6.758	3.624	1	322.077		Manual
2	614.50	45775.73	11.399	-	170	22218.764	1.000	56.432	0.215	1	614.449		Manual
3	632.50	-	-	-	-	-	-	-	-	-	-		Manual
4	936.50	21763.11	13.423	-	200	8904.870	2.000	22.617	0.801	1	352.239		Manual
5	954.50	12758.46	10.435	-	155	5865.164	2.000	5.285	1.292	1	352.055		Manual
6	972.50	-	-	-	-	-	-	-	-	-	-		Manual

Figure S4. RP-HPLC-UV and –ESI-MS traces of rapamycin solution in 30/70 MeCN-water, 237 mM $MeCOONH_4$; Reaction time ~6 months.

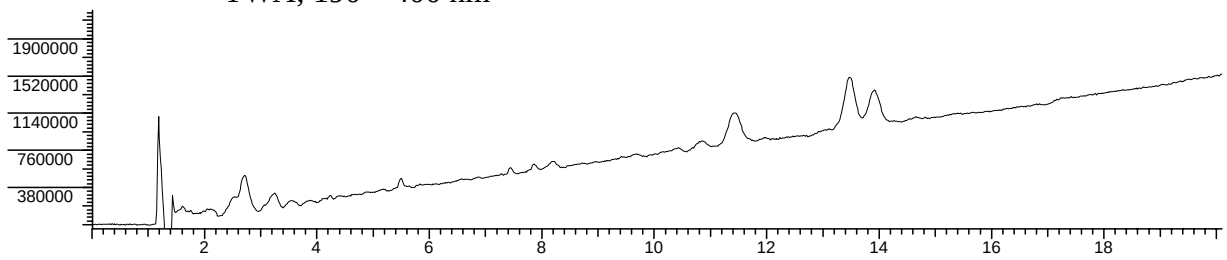
Degraded Secorapamycin TIC; Negative mode



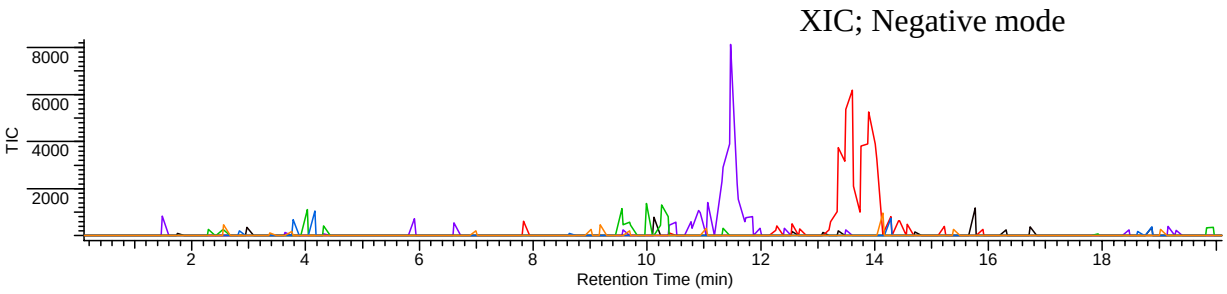
TIC; Positive mode



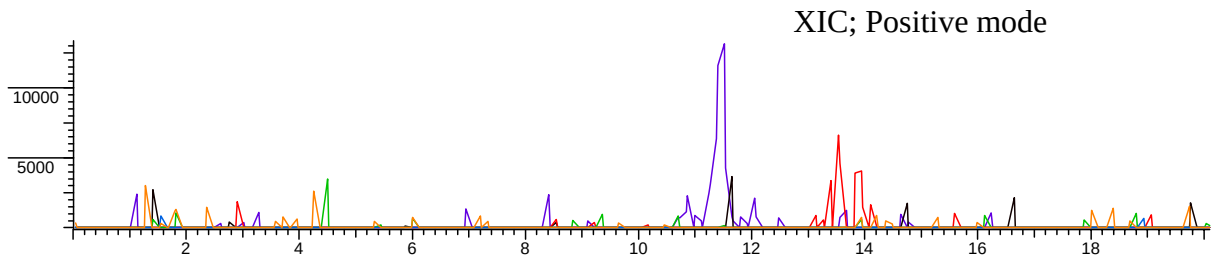
TWA; 190 – 400 nm



XIC; Negative mode



No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	321.20	-	-	-	-	-	-	-	-	-	-		Manual
2	590.50	21572.99	11.469	-	170	8125.200	1.000	91.032	0.243	1	590.243		Manual
3	608.50	-	-	-	-	-	-	-	-	-	-		Manual
4	912.50	22931.47	13.608	-	201	6183.926	4.000	69.283	16.556	1	912.781		Manual
5	930.50	-	-	-	-	-	-	-	-	-	-		Manual
6	948.50	-	-	-	-	-	-	-	-	-	-		Manual

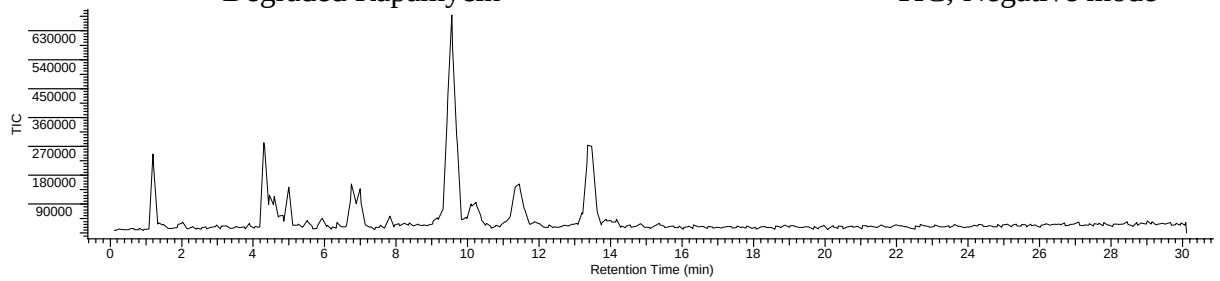


No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	323.20	-	-	-	-	-	-	-	-	-	-		Manual
2	614.50	42297.19	11.517	-	171	13172.168	2.000	37.547	9.056	1	542.388		Manual
3	632.50	-	-	-	-	-	-	-	-	-	-		Manual
4	936.50	11249.70	13.540	-	201	6635.371	2.000	18.914	1.102	1	352.259		Manual
5	954.50	-	-	-	-	-	-	-	-	-	-		Manual
6	972.50	-	-	-	-	-	-	-	-	-	-		Manual

Figure S5. RP-HPLC-UV and –ESI-MS traces of secorapamycin solution in 30/70 MeCN-water, 237 mM $MeCOONH_4$; Reaction time ~6 months.

Degraded Rapamycin

TIC; Negative mode



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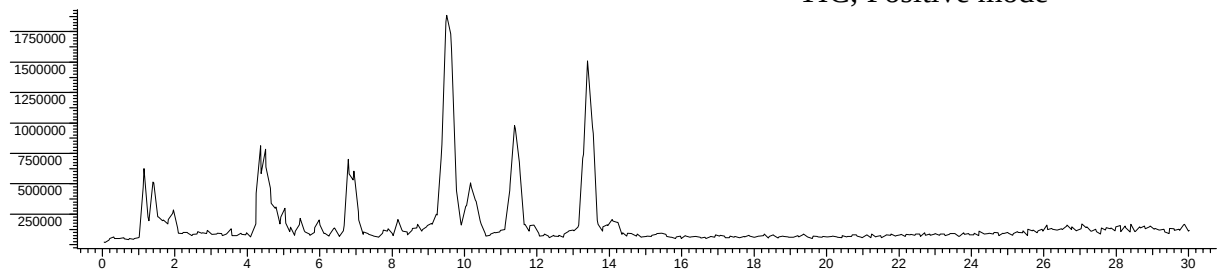
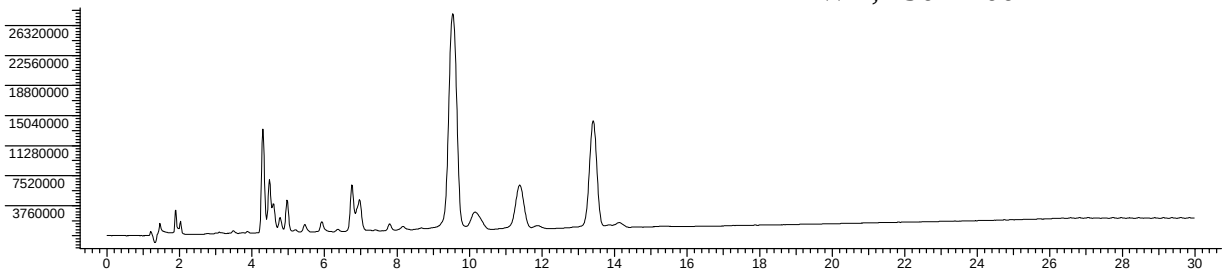


TWA; 190 – 400 nm



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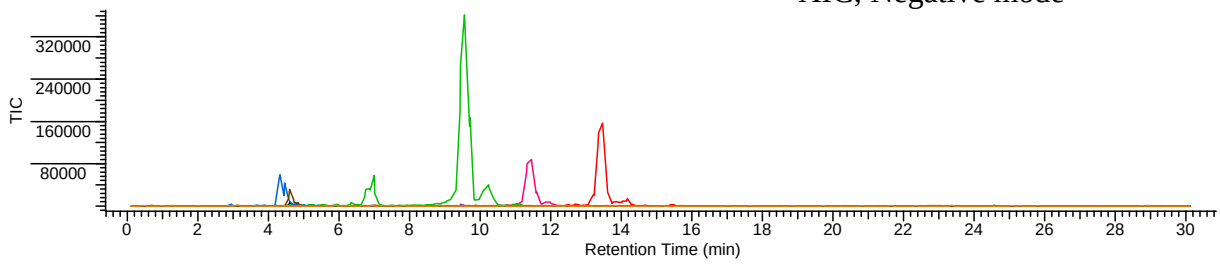
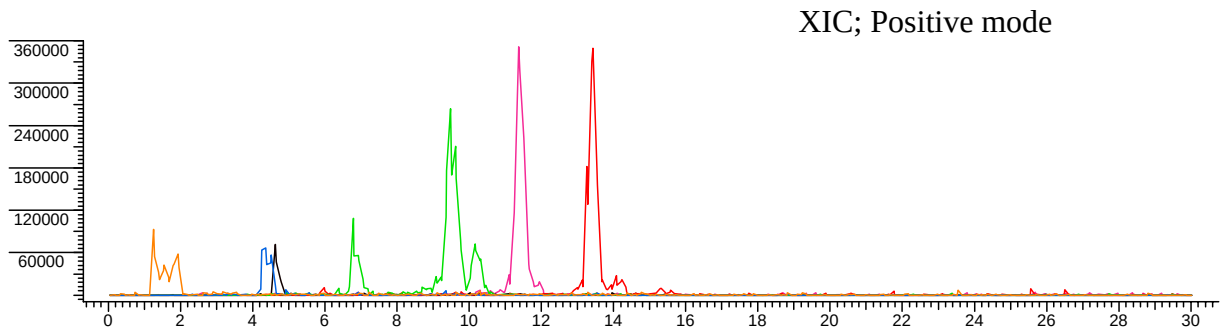


No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	321.20	-	-	-	-	-	-	-	-	-	-		Manual
2	564.50	72688.99	4.588	-	68	31022.994	1.000	45.642	0.203	1	564.241		Manual
3	590.50	394280.50	11.446	-	169	87430.008	4.000	128.631	1.938	1	590.266		Manual
4	608.50	-	-	-	-	-	-	-	-	-	-		Manual
5	912.50	641948.94	13.470	-	199	156680.969	4.000	230.516	2.387	1	912.582		Manual
6	930.50	1754708.00	9.558	-	141	361197.031	3.000	531.408	1.187	1	930.595		Manual
7	948.50	121600.51	4.318	-	64	58677.004	2.000	86.328	0.807	1	904.691		Manual

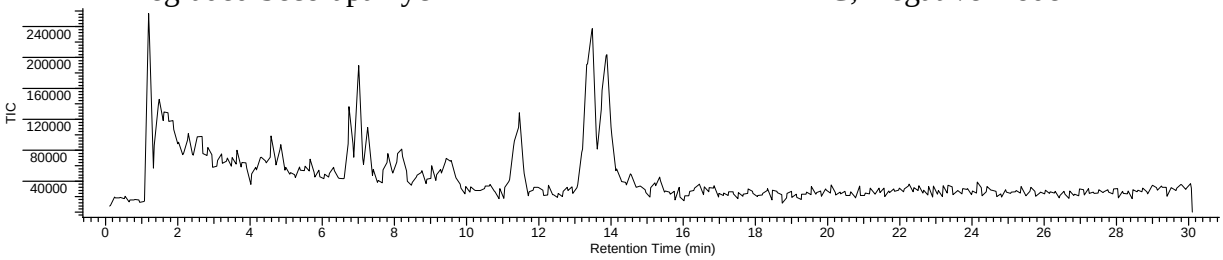


No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	323.20	191168.48	1.262	-	19	93093.992	2.000	30.138	0.929	1	323.170		Manual
2	614.50	1468877.13	11.379	-	169	351300.969	4.000	95.926	0.541	1	614.252		Manual
3	632.50	162047.98	4.635	-	69	71934.992	2.000	38.183	0.786	1	632.237		Manual
4	936.50	1088129.38	13.422	-	200	349448.000	3.000	185.487	0.925	1	936.728		Manual
5	954.50	645546.00	9.491	-	141	263793.969	3.000	140.022	6.367	1	954.644		Manual
6	972.50	161522.48	4.365	-	65	66422.984	3.000	11.831	6.233	1	972.640		Manual

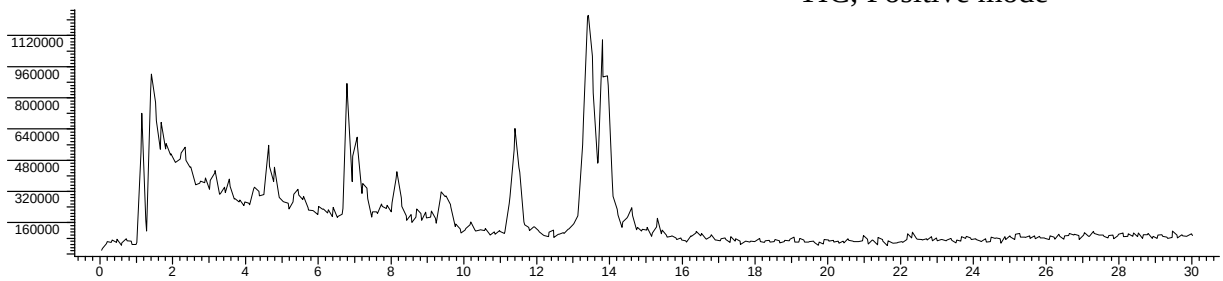
Figure S6. RP-HPLC-UV and -MS traces of rapamycin solution in 30/70 MeCN-water, 3 mM NaOH; Reaction time 1 h.

Degraded Secorapamycin

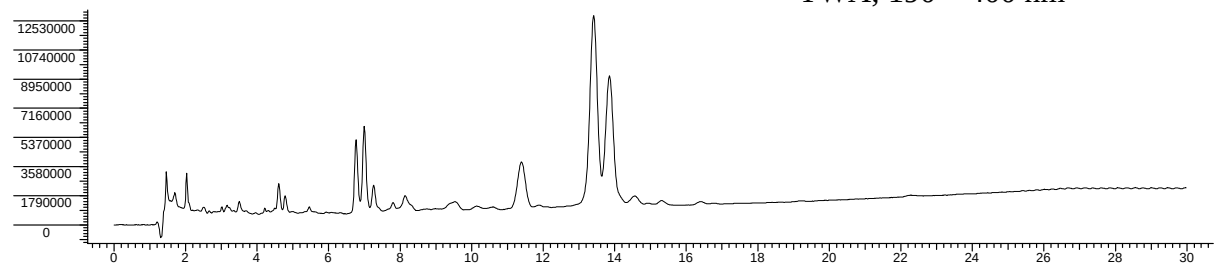
TIC; Negative mode



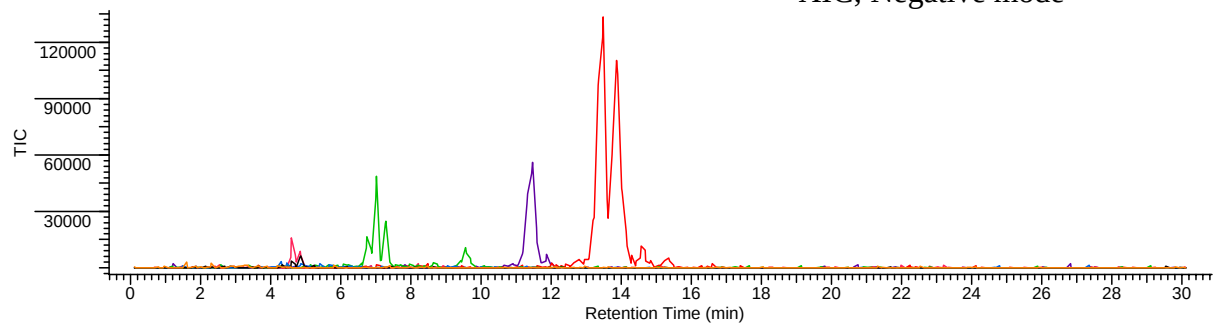
TIC; Positive mode



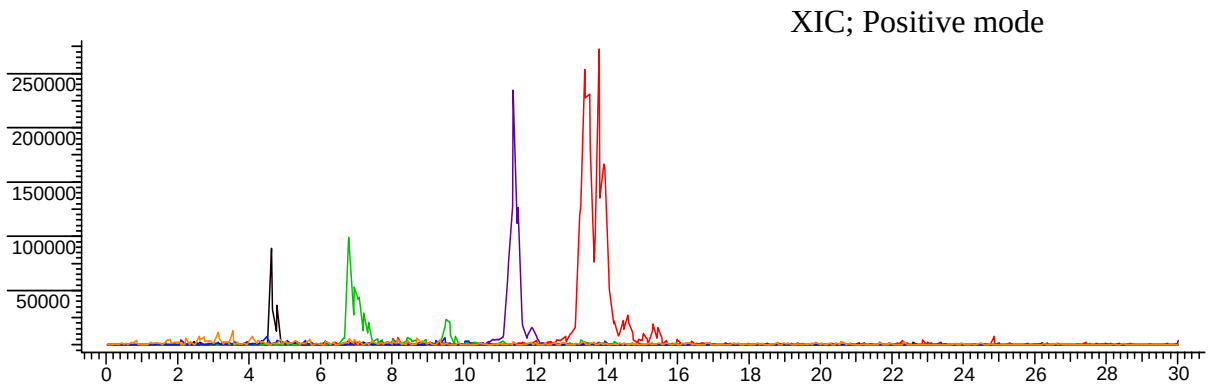
TWA; 190 – 400 nm



XIC; Negative mode



No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	321.20	-	-	-	-	-	-	-	-	-	-		Manual
2	564.50	29771.50	4.587	-	68	15948.001	1.000	13.296	0.155	1	564.257		Manual
3	590.50	250183.00	11.465	-	170	56135.004	4.000	27.920	1.980	1	590.215		Manual
4	608.50	12653.50	4.857	-	72	6240.000	2.000	5.533	1.341	1	564.287		Manual
5	912.50	569987.94	13.488	-	200	133447.000	4.000	58.277	2.591	1	912.597		Manual
6	930.50	91181.49	7.015	-	104	48652.996	2.000	189.384	1.113	1	930.573		Manual
7	948.50	-	-	-	-	-	-	-	-	-	-		Manual



No.	m/z	Area (counts)	tR (min)	RRT	Scan No.	Height (counts)	FWHM (scans)	S / N	Asymmetry	Number of maxima	Base Peak Mass	Color	Origin
1	323.20	13500.00	3.555	-	53	13171.003	1.000	1.598	5.994	1	323.078		Manual
2	614.50	798310.38	11.398	-	170	234859.953	3.000	129.235	0.294	1	614.222		Manual
3	632.50	128965.52	4.634	-	69	88533.023	1.000	27.190	3.692	1	632.286		Manual
4	936.60	478650.94	13.805	-	205	272659.969	1.000	40.828	4.532	1	936.765		Manual
5	954.50	221039.55	6.793	-	101	99021.992	2.000	80.978	0.630	1	954.747		Manual
6	972.50	14741.00	4.499	-	67	7601.000	2.000	1.770	1.530	1	972.642		Manual

Figure S7. RP-HPLC-UV and -MS traces of secorapamycin solution in 30/70 MeCN-water, 3 mM NaOH; Reaction time 1 h.

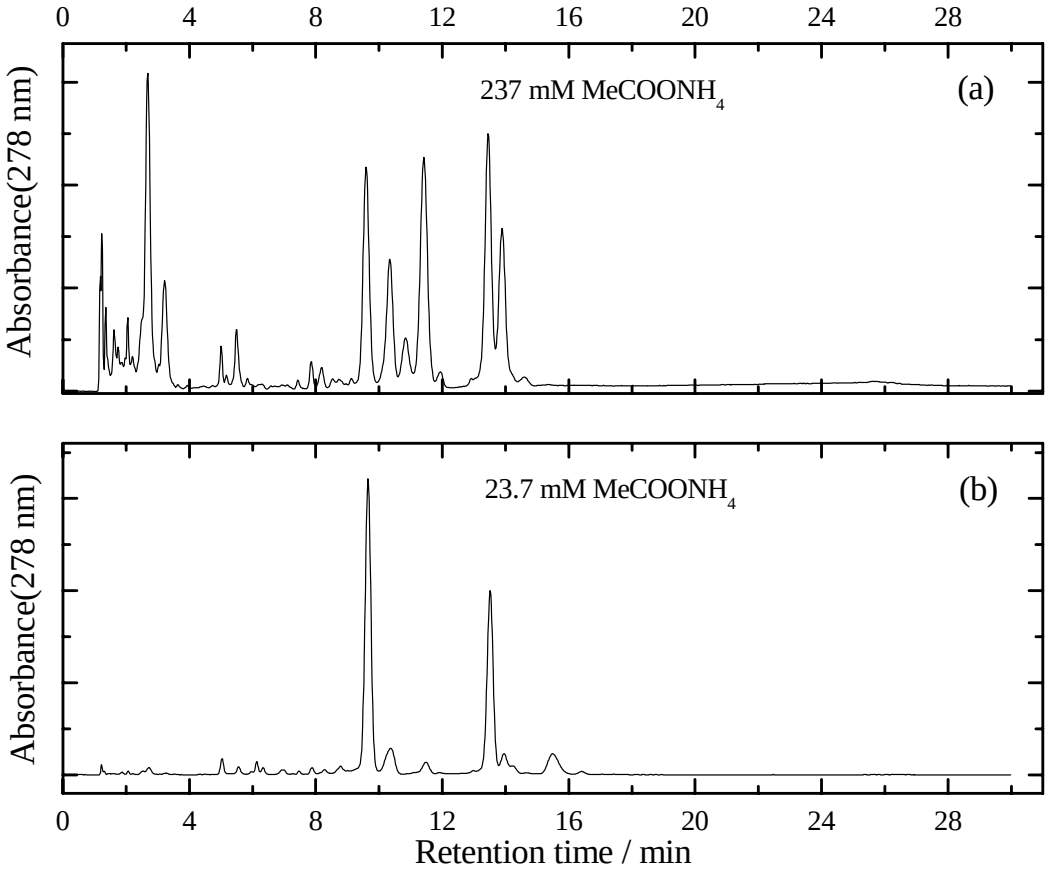


Figure S8. RP-HPLC-UV profiles for degraded rapamycin solutions containing 237 mM $MeCOONH_4$ ${}^{s}_{w}$pH =7.27 (a) or 23.7 mM $MeCOONH_4$, ${}^{s}_{w}$pH =7.24 (b). Reaction time (time elapsed between solution mixing and sample injection) was ~ 6 months. Mobile phase program: 50/50 $MeCN/H_2O$ to 90/10 $MeCN/H_2O$ over 25 min; Flow rate 1 mL/min; Column temperature 40°C. Mobile phase contained 0.1 vol% HCOOH.

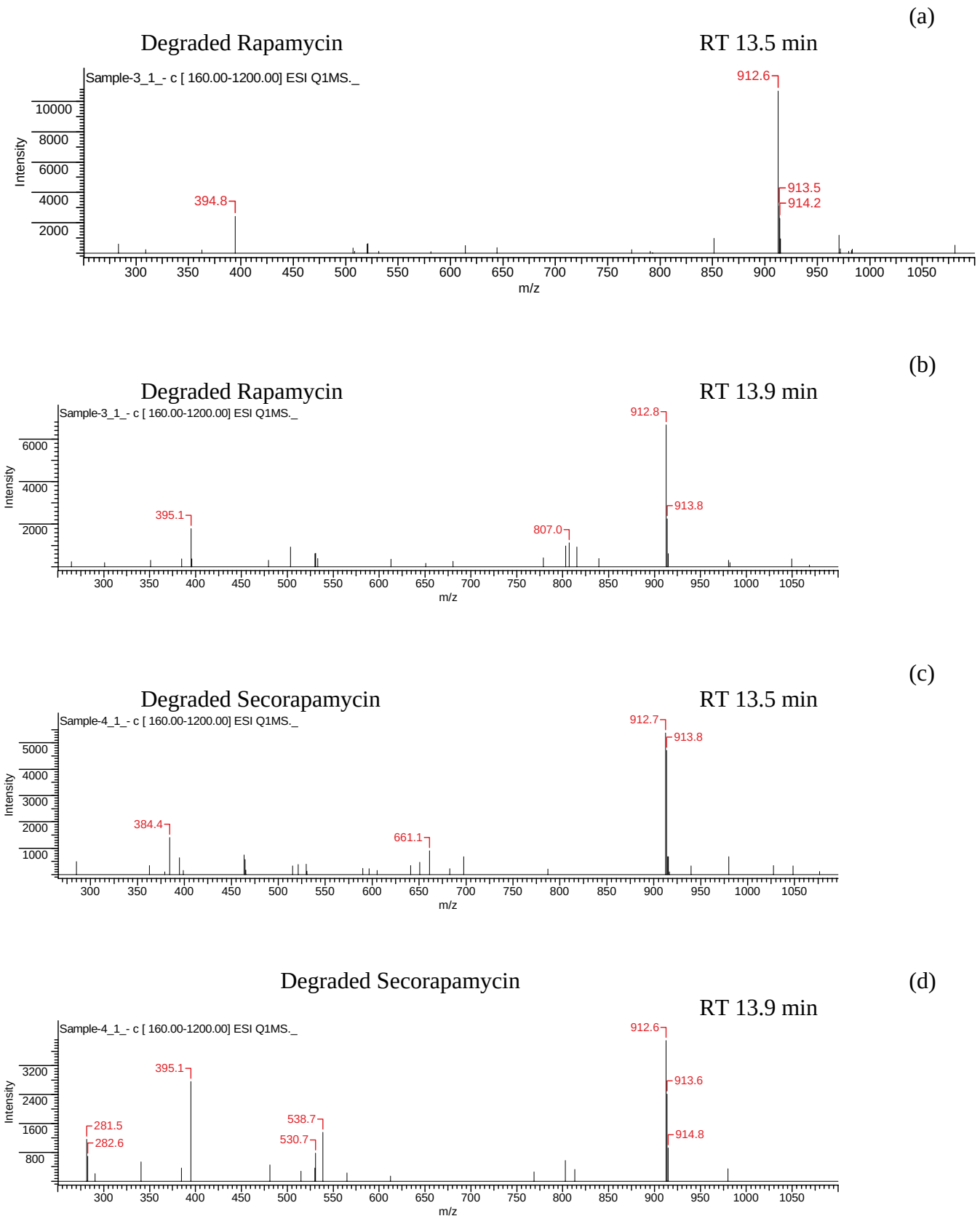


Figure S9. Negative ESI mass spectra acquired at RT 13.5 min (a, d) and 13.9 min (c, d) for rapamycin (a, b) and secorapamycin (c, d) solutions stored for ~ 6 months. HPLC-UV and –MS traces are presented in Figures S3 and S4. Solvent: 30/70 MeCN-water, 237 mM $MeCOONH_4$.

Table S2. ^{1}H and ^{13}C NMR chemical shifts (ppm) of rapamycin and secorapamycin salt with triethylamine (TEA) in 1:1 THF-d_8/D_2O[a]

	Rapamycin		**Secorapamycin**		**Secorapamycin, 0.025% TFA**	
No.	^{13}C chemical shift (ppm) [b]	^{1}H chemical shift (ppm) [c]	^{13}C chemical shift (ppm) [b]	^{1}H chemical shift (ppm) [c]	^{13}C chemical shift (ppm) [b]	^{1}H chemical shift (ppm) [c]
1	170.3	---	175.8	---	172.7, 173.0	---
2	51.7 (t), 57.1 (c)	4.99 (t), 4.18 (c)	54.2 (t), 59.6 (c)	4.86 (t), 4.01 (c)	51.6 (t), 56.7 (c)	5.06 (t), 4.31 (c)
3	27.0	2.12	28.0	2.19, 1.43	26.7; 27.9	2.20, 1.56; 2.10, 1.65
4	20.9	1.64	21.4	1.54, 1.46	21.1	1.66, 1.36
5	25.1	1.52, 1.33	25.0	1.63, 1.21	25.3	1.57, 1.37
6	44.5	3.41, 3.22	44.9 (t); 39.4 (c)	3.44, 3.31 (t); 4.16, 2.83 (c)	44.9 (t); 39.0 (c)	3.43, 3.26 (t); 4.25, 2.89 (c)
8	168.0	---	167.5	---	167.9, 167.3	---
9	198.4	---	180.0	---	175.9	---
10	99.8	---	100.2, 99.1	---	100.0, 99.6	---
11	35.1	2.00	35.2	1.95	34.7	1.96
12	26.9	1.52	27.1	1.45	27.2	1.45
13	30.6	1.72, 1.19	30.7	1.59, 0.83	30.7	1.58, 0.81
14	67.3	3.95	67.5	3.80, 3.69	67.6, 67.4	3.77, 3.69
15	40.5	1.87, 1.28	40.2	1.74, 1.36	40.1	1.72, 1.46
16	83.7	3.58	83.9	3.60	84.0	3.59
17	138.4	---	137.2	---	136.7	---
18	128.1	5.98	126.6	6.08	126.5	6.07
19	127.2	6.31	127.1	6.28	126.9	6.31
20	133.2	6.17	133.3	6.18	133.4	6.15
21	130.9	6.03	130.5	6.03	130.5	6.04
22	139.9	5.43	139.7	5.47	139.9	5.45
23	35.8	2.16	35.1	2.17	35.1	2.18, 2.04
24	40.3	1.36, 1.08	39.7	1.50, 1.07	39.6; 39.3	1.48, 1.07; 1.73, 1.50
25	41.0	2.43	40.9, 41.2	2.75, 2.69	41.2, 40.9	2.67, 2.74
26	215.3, 213.4	---	215.0, 214.4	---	214.5, 215.0	---
27	85.9, 87.3	3.99, 3.61	87.0, 87.4	3.84, 3.79	87.5, 86.9	3.78, 3.82
28	76.4	4.12, 3.98	76.6	4.09	76.6	4.07
29	137.6	---	137.7	---	137.7	---
30	125.7	5.16	126.8, 127.0	5.26, 5.22	127.0, 126.7	5.22, 5.25
31	46.4	3.37, 3.21	44.2	3.50	44.2	3.50
32	209.0	---	201.9	---	202.2, 201.8	---
33	40.7	2.65, 2.38	128.7	6.04	129.1	5.98
34	75.1	4.99	153.3, 153.2	6.67, 6.63	153.3	6.65, 6.63
35	34.1	1.71	34.2	2.34	34.2	2.33
36	39.0	1.05, 0.97	43.2	1.19	43.1	1.18
37	33.3	1.27	33.7	1.26	33.6	1.25
38	35.5	1.94, 0.60	36.1	1.95, 0.70	36.0	1.96, 0.69
39	84.4	3.58, 2.89	84.3	3.70, 2.88	84.4	3.65, 2.88

Table S2.(continued)

	Rapamycin		**Secorapamycin**		**Secorapamycin, 0.025% TFA**	
No.	^{13}C chemical shift (ppm)	^{1}H chemical shift (ppm)	^{13}C chemical shift (ppm)	^{1}H chemical shift (ppm)	^{13}C chemical shift (ppm)	^{1}H chemical shift (ppm)
41	32.5	1.82, 1.21	32.4	1.82, 1.15	32.5	1.81, 1.16
42	31.8	1.54, 0.86	31.1	1.55, 1.23	31.4	1.51, 1.27
43	15.5	0.74	15.4	0.71	15.7, 15.6	0.75, 0.71
44	10.3	1.58	10.5	1.56	10.5	1.56
45	21.2	0.93	21.5	0.92	21.3	0.91
46	13.1	0.82	14.2, 14.7	0.86, 0.88	14.7, 14.3	0.87, 0.86
47	13.7	1.72	12.5, 12.9	1.66, 1.64	12.9, 12.5	1.63, 1.66
48	15.5	0.87	16.4	1.00	16.5, 16.2	0.99, 0.98
49	15.1	0.76	19.4	0.93	19.5	0.93
50	55.4	3.01	55.3	3.03	55.3	3.02
51	57.7, 58.3	3.13, 3.18	58.3, 58.1	3.21, 3.18	58.4, 58.1	3.20, 3.18
52	56.9	3.31	56.6	3.29	56.8	3.29

[a] The chemical shifts were obtained from DEPT-edited gHSQC and gHMBC experiments. The chemical shifts for carbon No. 9 were obtained from a ^{13}C inverse-gated decoupled NMR experiment for rapamycin and from an additional gHMBC experiment with a smaller $^{n}J_{CH}$ value for secorapamycin. Resonances corresponding to *trans*- and *cis*-rotamers were labeled with letters “t” and “c”, respectively. For secorapamycin, the measurements were performed in the absence and in the presence of 0.025% trifluoroacetic acid (TFA);

[b] Relative to the residual proton signal of THF-d8 assigned to 3.58 ppm;

[c] Relative to the signal of THF-d8 assigned to 67.57 ppm;

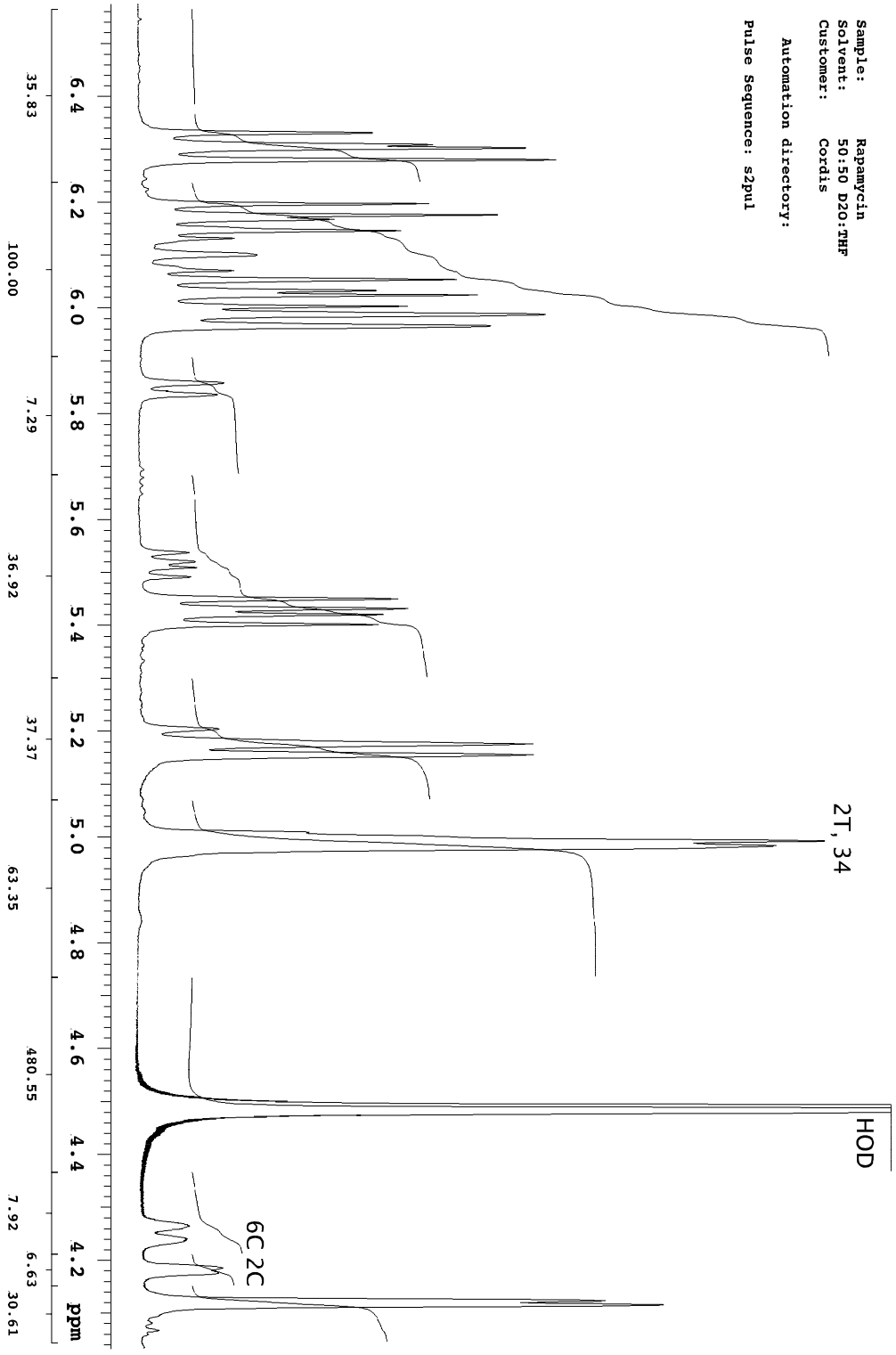


Figure S10. ^{1}H-NMR spectrum of rapamycin in 1:1 THF-d_8/D_2O. Resonances corresponding to *trans*- and *cis*-rotamers were labeled with letters “T” and “C”, respectively.

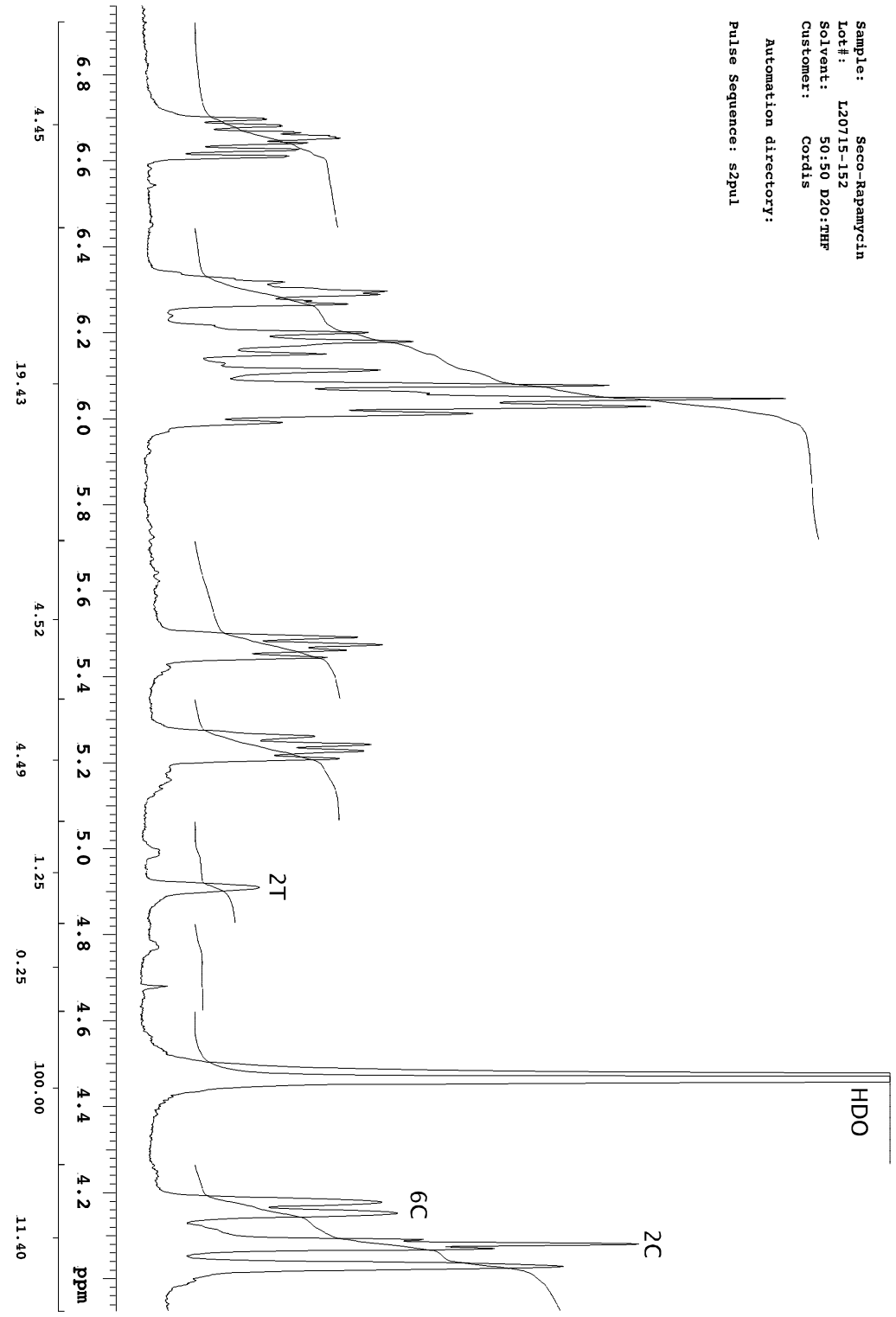


Figure S11. ^{1}H-NMR spectrum of secorapamycin in 1:1 THF-d_8/D_2O. Resonances corresponding to *trans*- and *cis*-rotamers were labeled with letters "T" and "C", respectively.

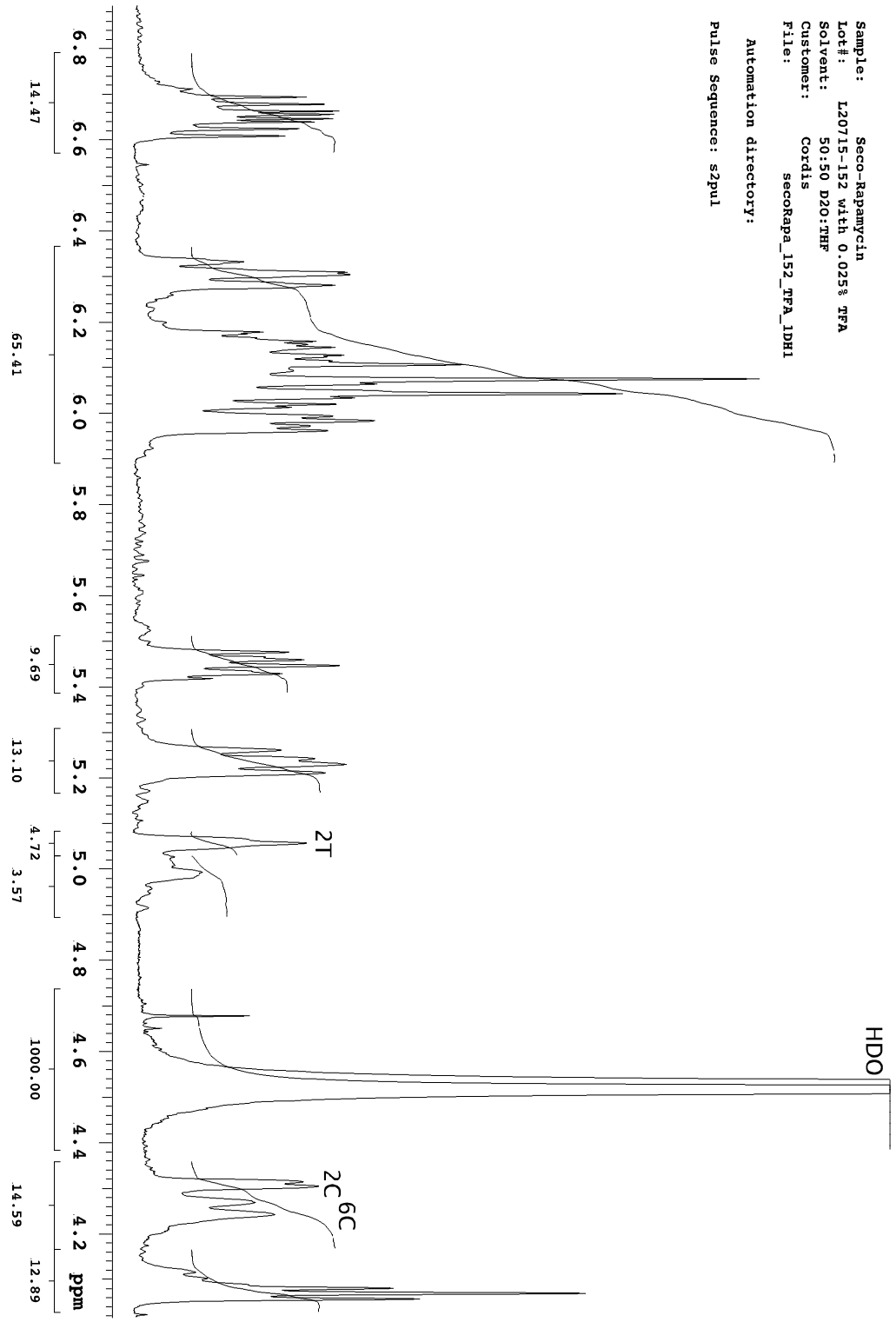


Figure S12. ^{1}H-NMR spectrum of secorapamycin in 1:1 THF-d_8/D_2O containing 0.025% trifluoroacetic acid. Resonances corresponding to *trans*- and *cis*-rotamers were labeled with letters "T" and "C", respectively.