Supporting data

Identification and characterization of degradation products of Remdesivir using liquid chromatography/mass spectrometry

Table S1. Stress degradation conditions of the RDV

Degradation study	Exposure conditions	Degradation products formed
Acid hydrolysis	1 N HCl reflux at 70 °C for 6 h	DP1, DP2,DP3,DP4 and DP5
Alkaline hydrolysis	0.1 N NaOH at RT for 10 min	DP5, DP6, DP7 and DP8
Neutral hydrolysis	Reflux at 70 °C for 24 h	DP5
Oxidative degradation	3% H_2O_2at room temperature for 48 h	DP9
UV light	200 $Whrm^{-2}$	No degradation
Fluorescent light	1.2 million lux hours	No degradation
Thermal	100 °C for 5 d	No degradation

h-hours, d-days, min-minutes

Table S2. Elemental composition of precursor and product ions of RDV and its degradation products.

RDV and its DPS (Retention time in min)	***Elemental composition***	***Theoretical m/z***	***Experimental m/z***	***Mass error (Δ ppm)***
RDV(12.55)	$C_{27}H_{36}N_6O_8P^+$	603.2327	603.2333	-0.96
	$C_{21}H_{24}N_6O_8P^+$	519.1388	519.1393	-1.04
	$C_{21}H_{22}N_6O_7P^+$	501.1282	501.1287	-0.94
	$C_{20}H_{22}N_6O_6P^+$	473.1333	473.1322	2.26
	$C_{18}H_{17}N_5O_6P^+$	430.0911	430.0917	-1.38
	$C_{18}H_{29}NO_7P^+$	402.1676	402.1677	-0.1

	$C_{18}H_{27}NO_6P^+$	384.1571	384.1575	-1.26
	$C_{15}H_{25}NO_5P^+$	330.1465	330.1468	-0.83
	$C_{12}H_{17}NO_7P^+$	318.0737	318.0744	-2
	$C_{12}H_{15}NO_6P^+$	300.0632	300.0635	-1.3
	$C_{12}H_{13}NO_5P^+$	282.0523	282.0526	0.89
	$C_{12}H_{12}N_5O_3^+$	274.0935	274.0936	-0.38
	$C_{11}H_{15}NO_5P^+$	272.0682	272.0686	-1.25
	$C_{12}H_{10}N_5O_2^+$	256.0829	256.0831	-0.71
	$C_{11}H_{13}NO_4P^+$	254.0577	254.0578	-0.56
	$C_9H_{13}NO_5P^+$	246.0526	246.0529	-1.12
	$C_{11}H_9N_4O_2^+$	229.0720	229.0724	-1.71
	$C_9H_{11}NO_4P^+$	228.0420	228.0422	-0.73
	$C_9H_8NO_4P^+$	211.0155	211.0151	1.89
	$C_9H_8N_5O^+$	202.0723	202.0726	-1.13
	$C_8H_{11}NOP^+$	200.1471	200.1473	-1.16
	$C_8H_7N4O^+$	175.0614	175.0618	-1.93
	$C_9H_{20}NO_2^+$	174.1489	174.1490	-0.67
	$C_7H_7N_4^+$	147.0665	147.0668	-1.71
	$C_3H_8NO_2^+$	90.0550	90.0550	-0.52
	$C_2H_6N^+$	44.0495	44.0493	3.99
DP1(3.21)	$C_{12}H_{15}N_5O_7P^+$	372.0704	372.0720	-4.35
	$C_{12}H_{13}N_5O_6P^+$	354.0598	354.0597	0.4
	$C_{11}H_{14}N_4O_7P^+$	345.0595	345.0610	-4.42
	$C_{12}H_{12}N_5O_3^+$	274.0935	274.0934	0.09
	$C_{12}H_{10}N_5O_2^+$	256.0829	256.0839	-4.04

	$C_{11}H_9N_4O_2^+$	229.0720	229.0723	-1.12
	$C_9H_8N_5O^+$	202.0723	202.0727	-1.91
	$C_8H_7N_4O^+$	175.0614	175.0619	-2.75
	$C_3H_8O_6P^+$	171.0053	171.0053	-0.03
	$C_{17}H_7N_4^+$	147.0665	147.0670	-3
	$H_4O_4P^+$	98.9842	98.9844	-2.23
DP2(6.52)	$C_{12}H_{14}N_5O_4^+$	292.1040	292.1048	-2.59
	$C_{12}H_{12}N_5O_3^+$	274.0935	274.0926	3.07
	$C_{11}H_{13}N_4O_4^+$	265.0931	265.0928	1.1
	$C_{11}H_{11}N_4O_3^+$	247.0826	247.0817	3.35
	$C_9H_8N_5O^+$	202.0723	202.0722	0.69
	$C_8H_7N_4O^+$	175.0614	175.0615	-0.46
	$C_7H_7N_4^+$	147.0665	147.0667	-1.26
	$C_6H_6N_4^+$	134.0587	134.0588	-0.85
	$C_5HN_2^+$	92.0369	92.0368	0.8
DP3(7.85)	$C_{18}H_{19}N_5O_7P^+$	448.1017	448.1030	-2.96
	$C_{18}H_{17}N_5O_6P^+$	430.0911	430.0927	-3.62
	$C_{17}H_{18}N_4O_7P^+$	421.0908	421.0911	-0.84
	$C_{18}H_{15}N_5O_5P^+$	412.0805	412.0828	-5.55
	$C_{17}H_{16}N_4OP^+$	403.0802	403.0818	-3.91
	$C_{12}H_{12}N_5O_3^+$	274.0935	274.0934	0.18
	$C_{12}H_{10}N_5O_2^+$	256.0829	256.0833	-1.53
	$C_9H_{12}O_6P^+$	247.0366	247.0370	-1.71
	$C_{11}H_9N_4O_2^+$	229.0720	229.0723	-1.23
	$C_9H_8N_5O^+$	202.0723	202.0727	-1.7

	$C_8H_7N_4O^+$	175.0614	175.0617	-1.66
	$C_3H_6O_5P^+$	152.9947	152.9937	6.76
	$C_7H_7N_4^+$	147.0665	147.0666	-0.22
DP4(9.37)	$C_9H_{20}NO_2^+$	174.1489	174.1495	-3.47
	$C_3H_8NO_2^+$	90.0550	90.0551	-1.49
	$C_6H_{13}^+$	85.1012	85.1012	-0.67
	$C_4H_9^+$	57.0699	57.0698	1.22
	$C_2H_6N^+$	44.0495	44.0493	5.01
	$C_3H_7^+$	43.0542	43.0542	1.55
	$C_3H_5^+$	41.0386	41.0385	1.03
DP5(10.47)	$C_{27}H_{38}N_6O_9P^+$	621.2432	621.2439	-1.13
	$C_{18}H_{29}NO_7P^+$	402.1676	402.1664	2.91
	$C_{18}H_{27}NO_6P^+$	384.1571	384.1565	1.45
	$C_{15}H_{25}NO_5P^+$	330.1465	330.1447	5.36
	$C_{12}H_{17}NO_7P^+$	318.0737	318.0734	0.68
	$C_{12}H_{15}NO_6P^+$	300.0632	300.0644	-4.05
	$C_{12}H_{14}N_5O_4P^+$	292.1040	292.1040	-0.03
	$C_{12}H_2N_5O_3^+$	274.0935	274.0938	-1.24
	$C_{11}H_{15}NO_5P^+$	272.0682	272.0684	-0.74
	$C_{11}H_{13}NO_4P^+$	254.0577	254.0587	-3.93
	$C_{11}H_{11}N_4O_3^+$	247.0826	247.0819	2.78
	$C_9H_{13}NO_5P^+$	246.0526	246.0527	-0.39
	$C_{11}H_9N_4O_2^+$	229.0720	229.0717	1.14
	$C_9H_{11}NO_4P^+$	228.0420	228.0416	1.81
	$C_9H_{10}N_5O_2^+$	220.0829	220.0827	0.83

	$C_9H_7N_4O_2^+$	203.0564	203.0563	0.25
	$C_8H_{11}NO_3P^+$	200.0471	200.0467	1.98
	$C_8H_7N_4O^+$	175.0614	175.0616	-1.01
	$C_9H_{20}NO_2^+$	174.1489	174.1492	-2.06
	$C_7H_7N_4^+$	147.0665	147.0672	-4.81
	$C_3H_8NO_2^+$	90.0550	90.0551	-1.5
	$C_2H_6N^+$	44.0495	44.0495	0.78
DP6(5.31)	$C_{15}H_{20}N_6O_8P^+$	443.1075	443.1084	-2
	$C_{15}H_{18}N_6O_7P^+$	425.0969	425.0975	-1.42
	$C_{12}H_{13}N_5O_6P^+$	354.0598	354.0613	-4.17
	$C_{12}H_{12}N_5O_3^+$	274.0935	274.0932	1.51
	$C_{12}H_{10}N_5O_2^+$	256.0829	256.0841	-4.79
	$C_6H_{13}NO_7P^+$	242.0426	242.0425	-0.52
	$C_{11}H_9N_4O_2^+$	229.0720	229.0720	0.01
	$C_6H_{11}NO_6P^+$	224.0319	224.0320	-0.68
	$C_9H_8N_5O^+$	202.0723	202.0727	-1.63
	$C_5H_{11}NO_5P^+$	196.0369	196.0374	-2.5
	$C_5H_9NO_4P^+$	178.0264	178.0258	2.98
	$C_8H_7N_4O^+$	175.0614	175.0608	3.81
	$C_3H_9NO_5P^+$	170.0213	170.0212	0.24
	$C_7H_7N_4^+$	147.0665	147.0667	-1.22
	$C_2H_7NO_3P^+$	124.0158	124.0159	-0.98
	$C_3H_8NO_2^+$	90.0550	90.0551	-1.29
	C_2H_6N	44.0495	44.0494	1.52
DP7(8.87)	$C_{21}H_{24}N_6O_8P^+$	519.1388	519.1381	1.3

	$C_{21}H_{22}N_6O_7P^+$	501.1282	501.1272	0.93
	$C_{20}H_{22}N_6O_6P^+$	473.1333	473.1329	0.89
	$C_{18}H_{17}N_5O_6P^+$	430.0911	430.0899	2.7
	$C_{12}H_{17}NO_7P^+$	318.0737	318.0745	-2.46
	$C_{12}H_{15}NO_6P^+$	300.0632	300.0634	-0.91
	$C_{12}H_{13}NO_5P^+$	282.0526	282.0532	-2.28
	$C_{12}H_{12}N_5O_3^+$	274.0935	274.0936	-0.61
	$C_{11}H_{15}NO_5P^+$	272.0682	272.0688	-2.04
	$C_{12}H_{10}N_5O_2^+$	256.0829	256.0831	-0.84
	$C_{11}H_{13}NO_4P^+$	254.0577	254.0582	-2.04
	$C_9H_{13}NO_5P^+$	246.0526	246.0530	-1.87
	$C_{11}H_9N_4O_2^+$	229.0720	229.0723	-1.47
	$C_9H_{11}NO_4P^+$	228.0420	228.0410	4.69
	$C_9H_8N_5O^+$	202.0723	202.0728	-2.13
	$C_8H_{11}NO_3P^+$	200.0471	200.0473	-1.2
	$C_8H_7N4O^+$	175.0614	175.0624	-5.52
	$C_7H_7N_4^+$	147.0665	147.0670	-3.03
	$C_3H_8NO_2^+$	90.0550	90.1547	3.04
	$C_2H_6N^+$	44.0495	44.0493	3.65
DP8(9.86)	$C_{21}H_{32}N_6O_8P^+$	527.2014	527.2000	2.52
	$C_{15}H_{20}N_6O_8P^+$	443.1075	443.1090	-3.49
	$C_{15}H_{18}N_6O_7P^+$	425.0969	425.0971	-0.54
	$C_{12}H_{13}N_5O_6P^+$	354.0598	354.0603	-1.38
	$C_{12}H_{25}NO_7P^+$	326.1363	326.1373	-2.97
	$C_{12}H_{23}NO_6P^+$	308.1258	308.1262	-1.57

	$C_{12}H_{12}N_5O_3^+$	274.0935	274.0941	-2.22
	$C_{12}H_{10}N_5O_2^+$	256.0829	256.0837	-3.29
	$C_9H_{21}NO_5P^+$	254.1152	254.1162	-4.15
	$C_6H_{13}NO_7P^+$	242.0424	242.0430	-2.59
	$C_{11}H_9N_4O_2^+$	229.0720	229.0726	-2.63
	$C_6H_{11}NO_4P^+$	224.0319	224.0324	-2.32
	$C_9H_8N_5O^+$	202.0723	202.0729	-2.84
	$C_5H_{11}NO_5P^+$	196.0369	196.0374	-2.42
	$C_5H_9NO_4P^+$	178.0264	178.0268	-2.6
	$C_8H_7NO_4P^+$	175.0614	175.0618	-2.14
	$C_9H_{20}NO_4^+$	174.1489	174.1493	-2.63
	$C_3H_9NO_5P^+$	170.0213	170.0218	-3.03
	$C_3H_7NOP^+$	152.0107	152.0114	-4.3
	$C_7H_7N_4^+$	147.0665	147.0668	-1.69
	$C_2H_7NO_3P^+$	124.0158	124.0160	-1.87
	$C_3H_8NO_2^+$	90.0550	90.0551	-1.47
	$C_2H_6N^+$	44.0495	44.0493	4.02
DP9(11.96)	$C_{27}H_{36}N_6O_9P^+$	619.2276	619.2293	-2.31
	$C_{21}H_{24}N_6O_9P^+$	535.1337	535.1306	5.71
	$C_{20}H_{22}N_6O_7P^+$	489.1282	489.1274	1.57
	$C_{18}H_{17}N_5O_7P^+$	446.086	446.0881	-4.6
	$C_{18}H_{29}NO_7P^+$	402.1676	402.1675	0.3
	$C_{18}H_{27}NO_6P^+$	384.1571	384.1581	-2.76
	$C_{15}H_{25}NO_5P^+$	330.1465	330.1474	-2.78
	$C_{12}H_{17}NO_7P^+$	318.0737	318.0741	-1.3

	$C_{12}H_{15}NO_6P^+$	300.0632	300.0635	-1.02
	$C_{12}H_{12}N_5O_4^+$	290.0884	290.0889	-1.93
	$C_{11}H_{15}NO_5P^+$	272.0682	272.0682	0.3
	$C_{11}H_{13}NO_4P^+$	254.0577	254.0574	1.2
	$C_9H_{13}NO_5P^+$	246.0526	246.0525	0.32
	$C_{11}H_9N_4O_3^+$	245.0669	245.0676	-2.68
	$C_9H_{11}NO_4P^+$	228.0420	228.0414	2.56
	$C_9H_8N_5O_2^+$	218.0673	218.0673	-0.21
	$C_8H_{11}NO_3P^+$	200.0471	200.0476	-2.62
	$C_8H_7N_4O_2^+$	191.0564	191.0565	-0.82
	$C_9H_{20}NO_2^+$	174.1489	174.1483	2.91
	$C_3H_8NO_2^+$	90.0550	90.0553	-3.93
	$C_2H_6N^+$	44.0495	44.0494	0.72

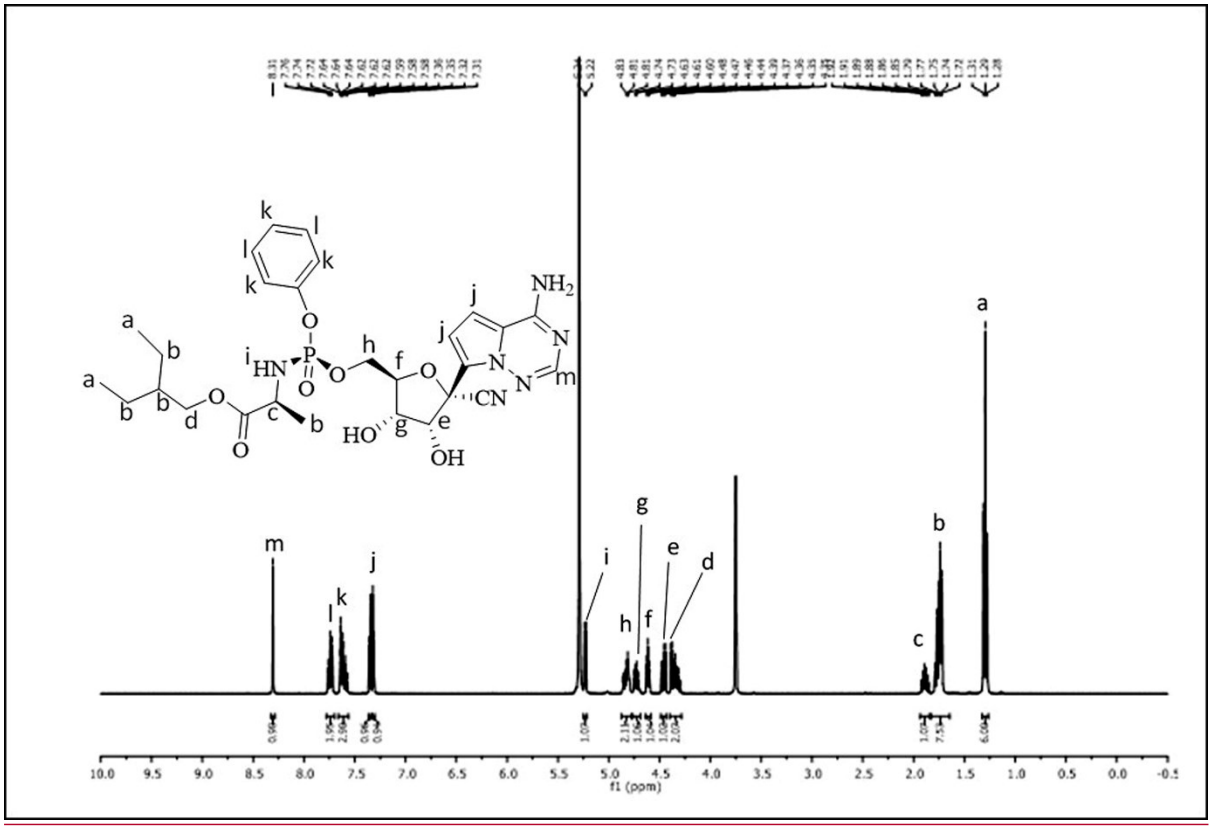


Figure S1. ^{1}H - NMR spectrum of RDV (400 MHz, MeOD): δ 8.31 (s, 1H), 7.78 – 7.70 (m, 2H), 7.66 – 7.56 (m, 3H), 7.35 (d, 1H), 7.32 (d, 1H), 5.23 (d, 1H), 4.87 – 4.78 (m, 2H), 4.73 (ddd, 1H), 4.61 (t, 1H), 4.46 (dd, 5.8 Hz, 1H), 4.40 – 4.28 (m, 2H), 1.89 (m, 1H), 1.83 – 1.65 (m, 8H), 1.29 (t, 6H).

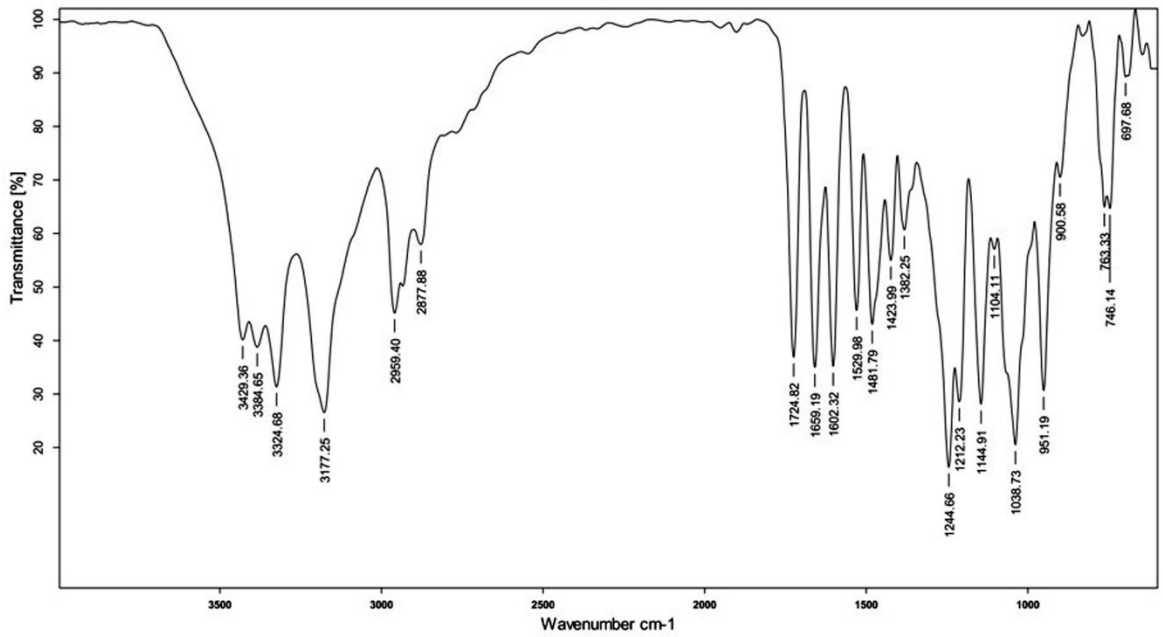


Figure S2.FTIR spectrum of RDV (KBr)

3429.36 cm^{-1}observed due to N-H stretching of primary amine

3324.68 cm^{-1}observed due to N-H stretching of secondary amine

3177.26 cm^{-1}observed due to O-H (intermolecular hydrogen bonding) stretching of alcohol

1724.82 cm^{-1}observed due to C=O stretching of ester

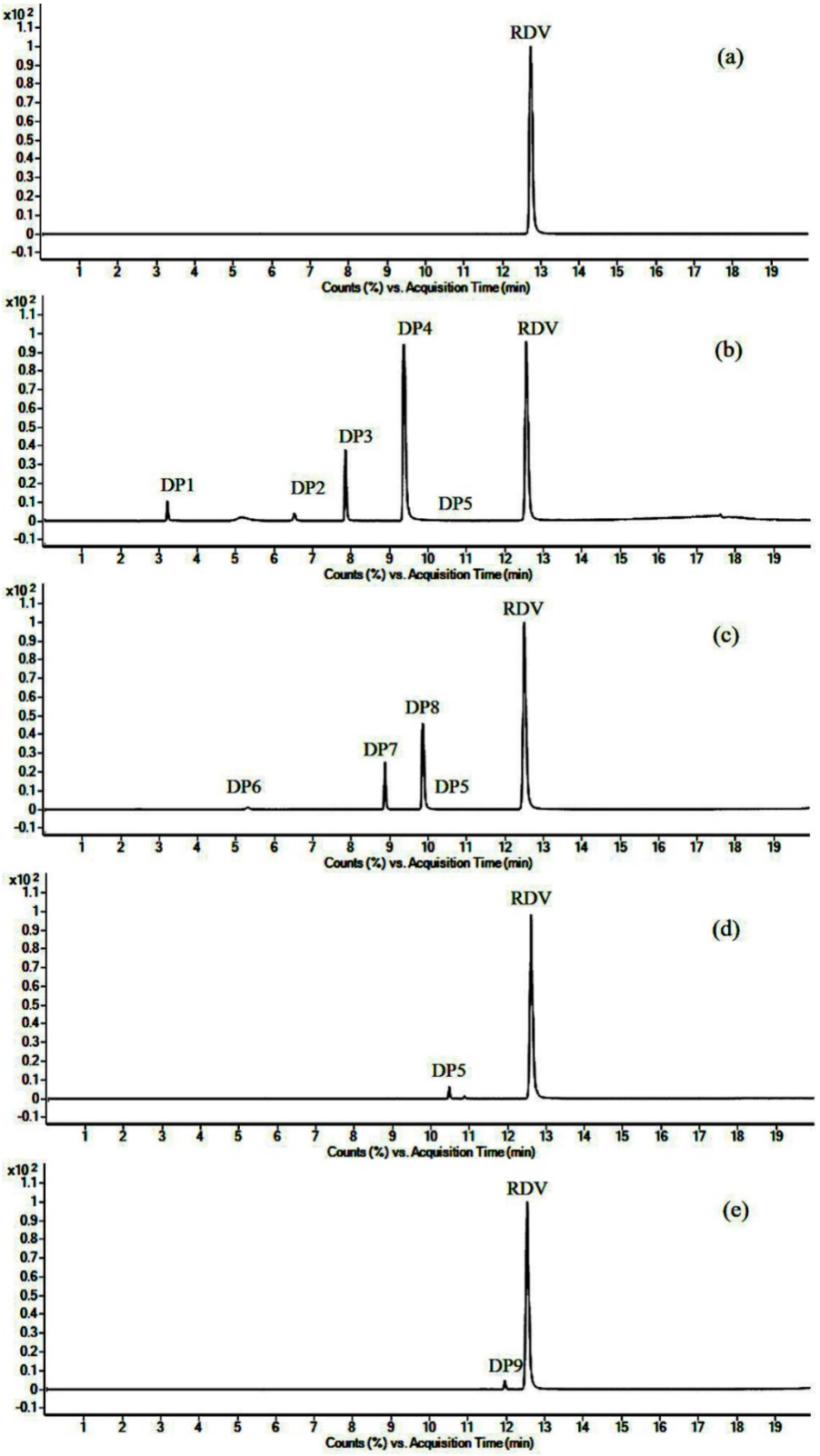


Figure S3. Extracted ion chromatograms (EICs) of (a) RDV(603.2333), (b) RDV (603.2333), DP1 (372.0720), DP2(292.1048), DP3(448.1030), DP4(174.1495), andDP5(621.2439),(c) RDV(603.2333), DP5(621.2439), DP6(443.1084), DP7(519.1381), andDP8 (527.2000),(d) RDV (603.2333), and DP5 (621.2439), (e) RDV (603.2333), and DP9 (619.2293).

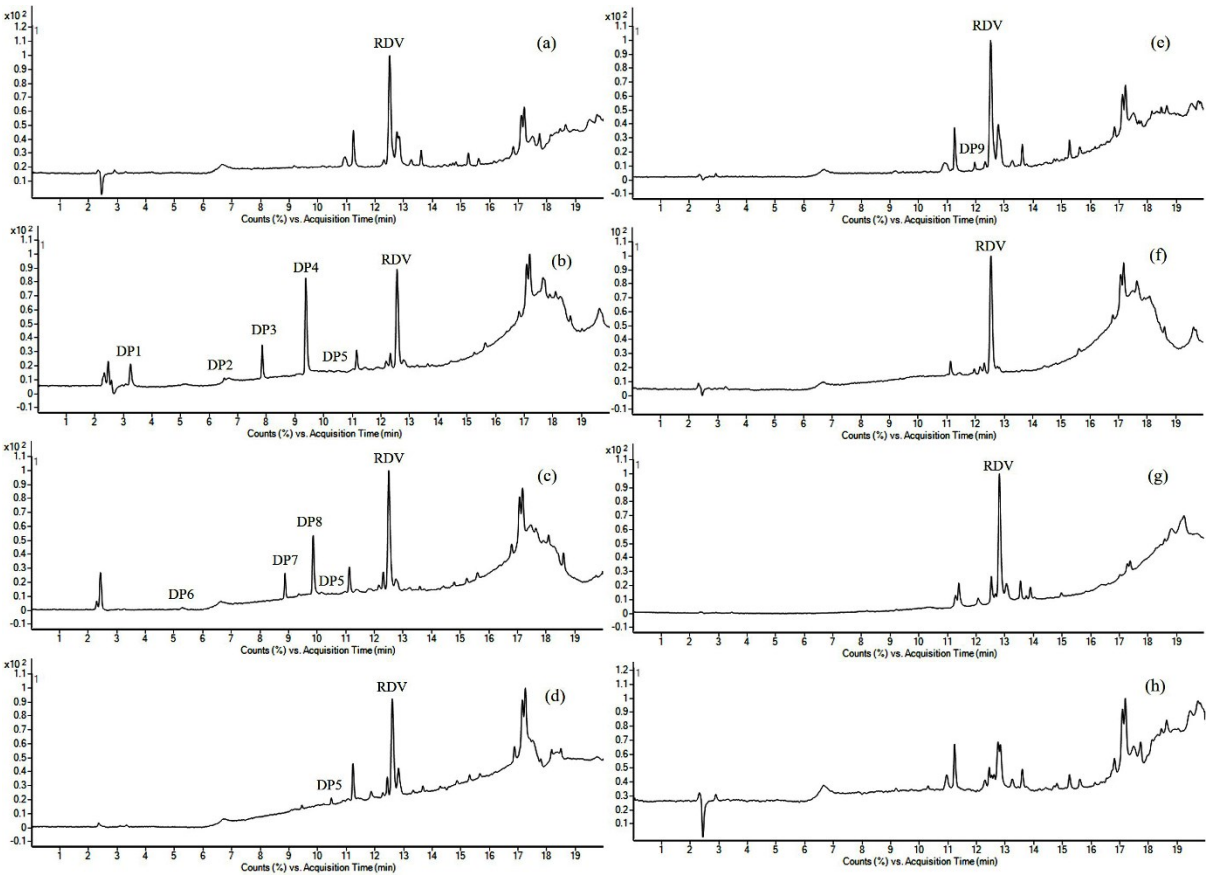
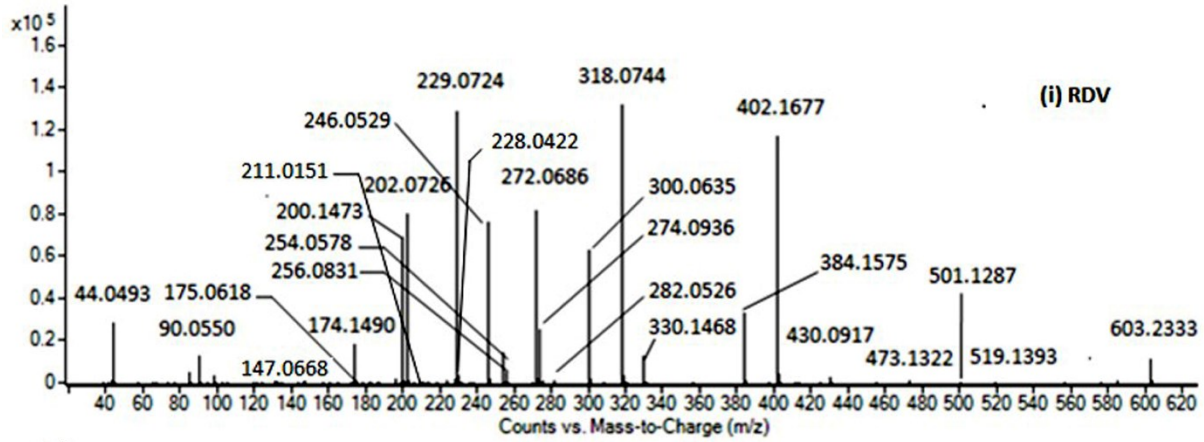
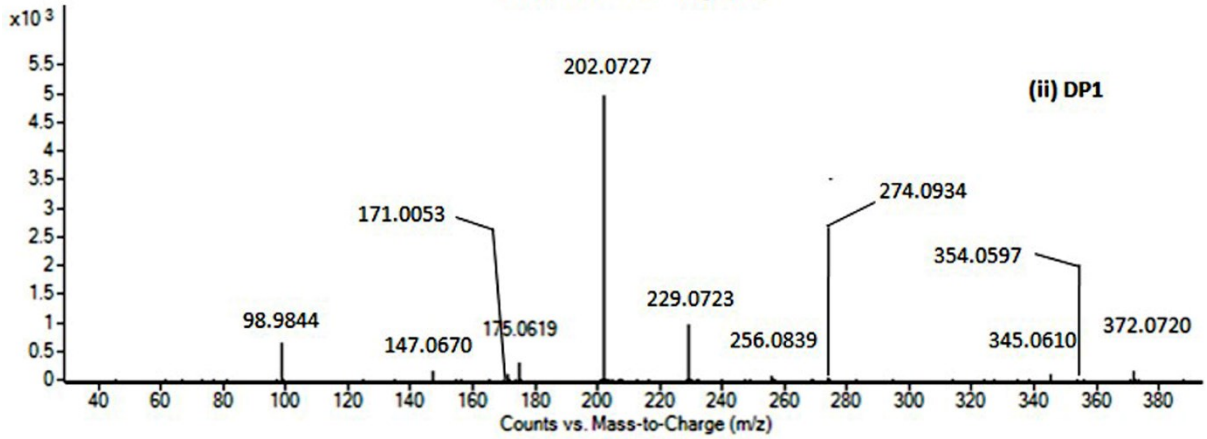
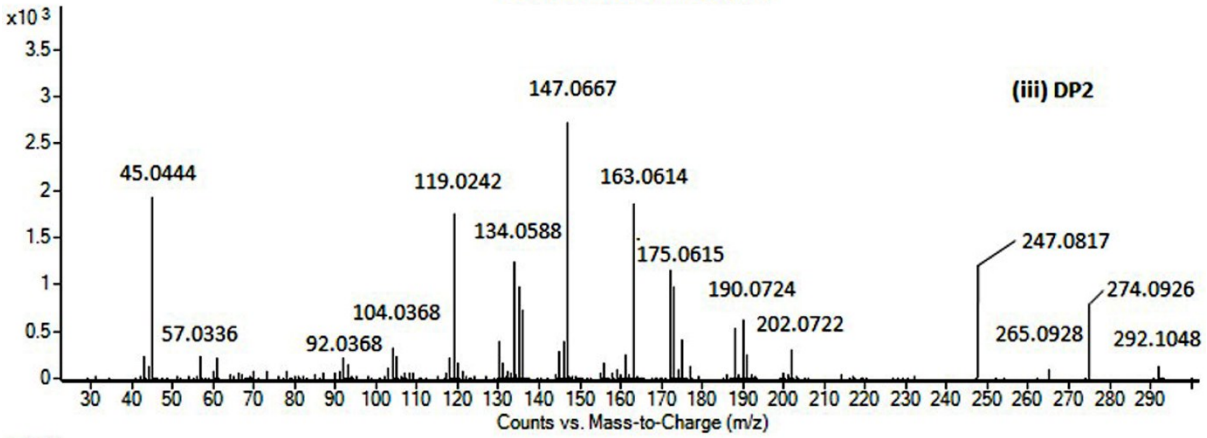
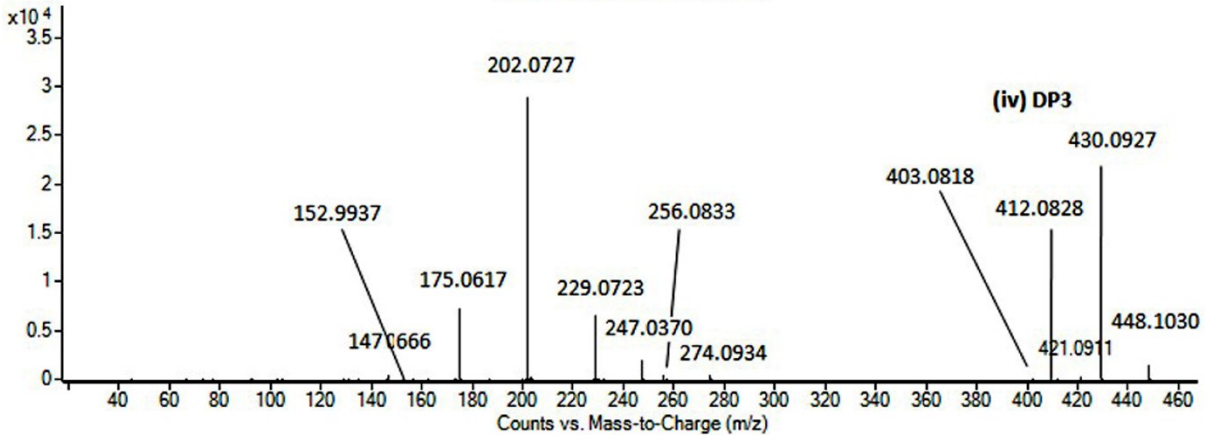


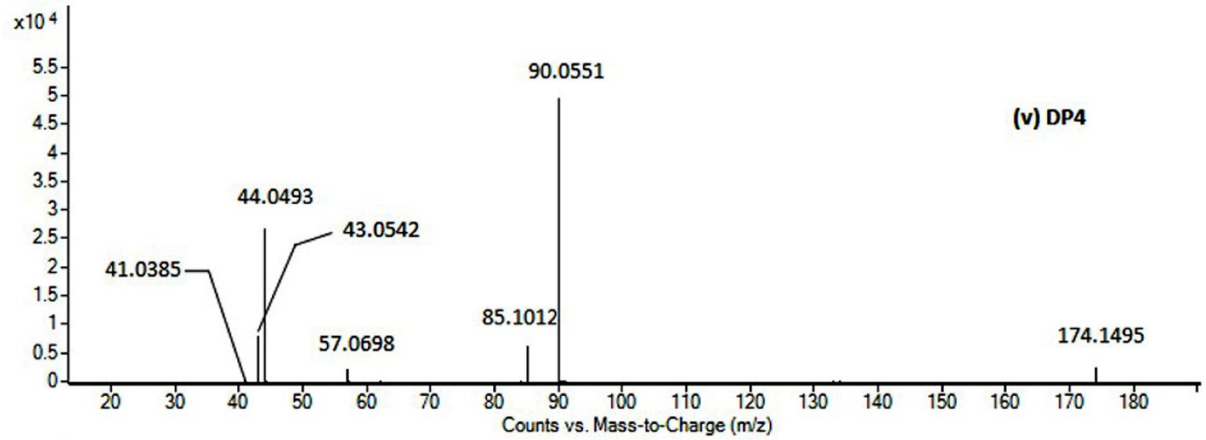
Figure S4. Total ion chromatograms (TICs) of (a) RDV, (b) acid degradation, (c) base degradation, (d) neutral degradation, (e) oxidative degradation, (f) thermal degradation, (g) photo degradation, and (h) blank

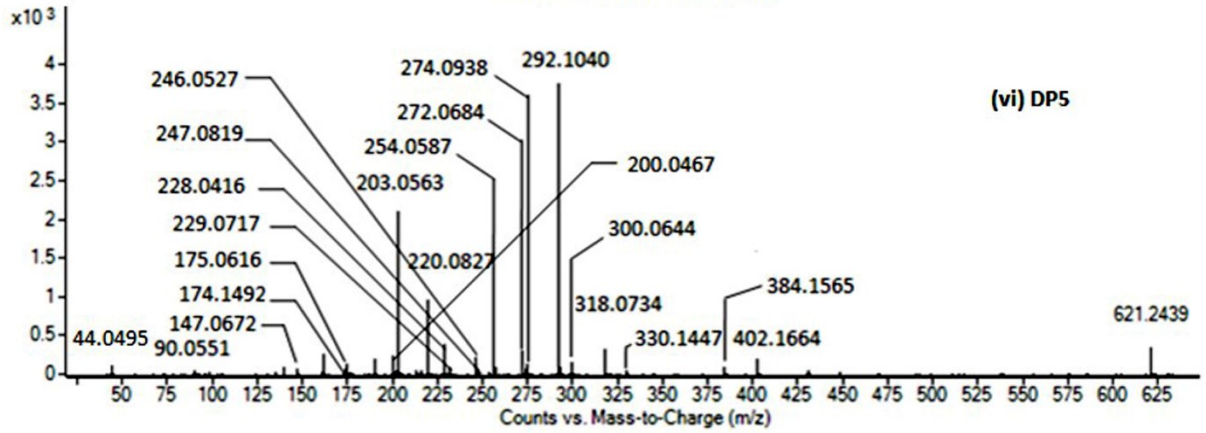

(i) RDV
229.0724
318.0744
402.1677
246.0529
228.0422
211.0151
202.0726
272.0686
300.0635
200.1473
274.0936
254.0578
256.0831
384.1575
501.1287
44.0493
175.0618
282.0526
90.0550
174.1490
330.1468
430.0917
603.2333
473.1322
519.1393
147.0668
Counts vs. Mass-to-Charge (m/z)

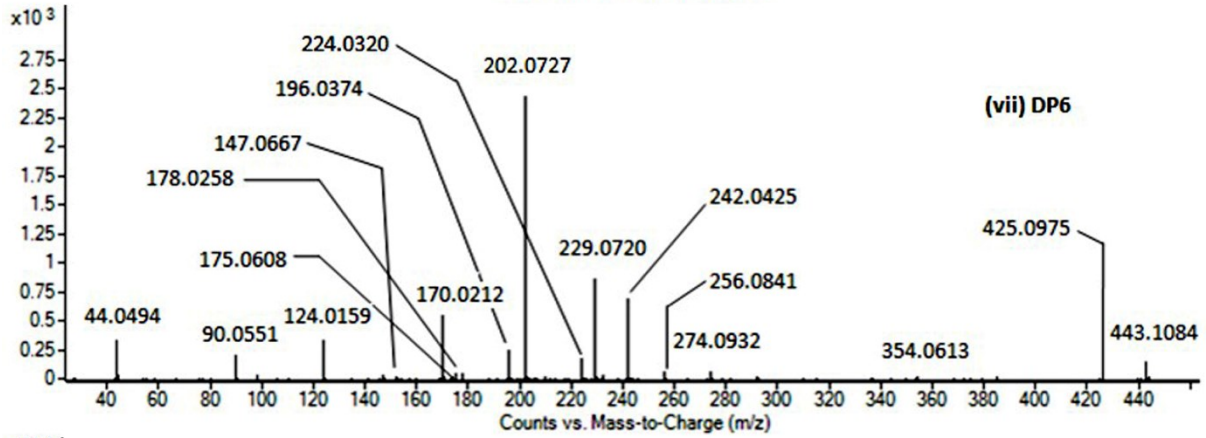

(ii) DP1
202.0727
274.0934
171.0053
354.0597
229.0723
98.9844
175.0619
147.0670
256.0839
345.0610
372.0720
Counts vs. Mass-to-Charge (m/z)

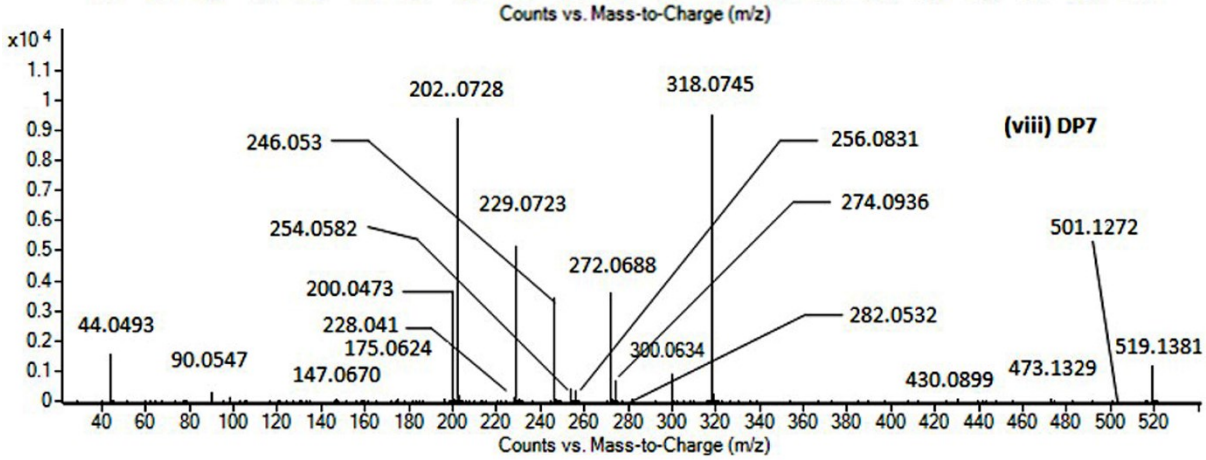

(iii) DP2
147.0667
45.0444
119.0242
163.0614
134.0588
247.0817
175.0615
274.0926
190.0724
104.0368
202.0722
57.0336
92.0368
265.0928
292.1048
Counts vs. Mass-to-Charge (m/z)


(iv) DP3
202.0727
430.0927
403.0818
412.0828
152.9937
256.0833
175.0617
229.0723
448.1030
247.0370
147.0666
274.0934
421.0911
Counts vs. Mass-to-Charge (m/z)


x10 4
90.0551
(v) DP4
44.0493
43.0542
41.0385
85.1012
57.0698
174.1495
Counts vs. Mass-to-Charge (m/z)


x10 3
246.0527
274.0938
292.1040
(vi) DP5
272.0684
247.0819
254.0587
200.0467
228.0416
203.0563
229.0717
300.0644
175.0616
220.0827
174.1492
384.1565
318.0734
147.0672
621.2439
44.0495
90.0551
330.1447
402.1664
Counts vs. Mass-to-Charge (m/z)


x10 3
224.0320
202.0727
196.0374
(vii) DP6
147.0667
178.0258
242.0425
425.0975
229.0720
175.0608
256.0841
170.0212
44.0494
124.0159
90.0551
274.0932
354.0613
443.1084
Counts vs. Mass-to-Charge (m/z)


x10 4
202..0728
318.0745
(viii) DP7
246.053
256.0831
229.0723
274.0936
254.0582
501.1272
272.0688
200.0473
282.0532
44.0493
228.041
519.1381
175.0624
300.0634
90.0547
147.0670
430.0899
473.1329
Counts vs. Mass-to-Charge (m/z)

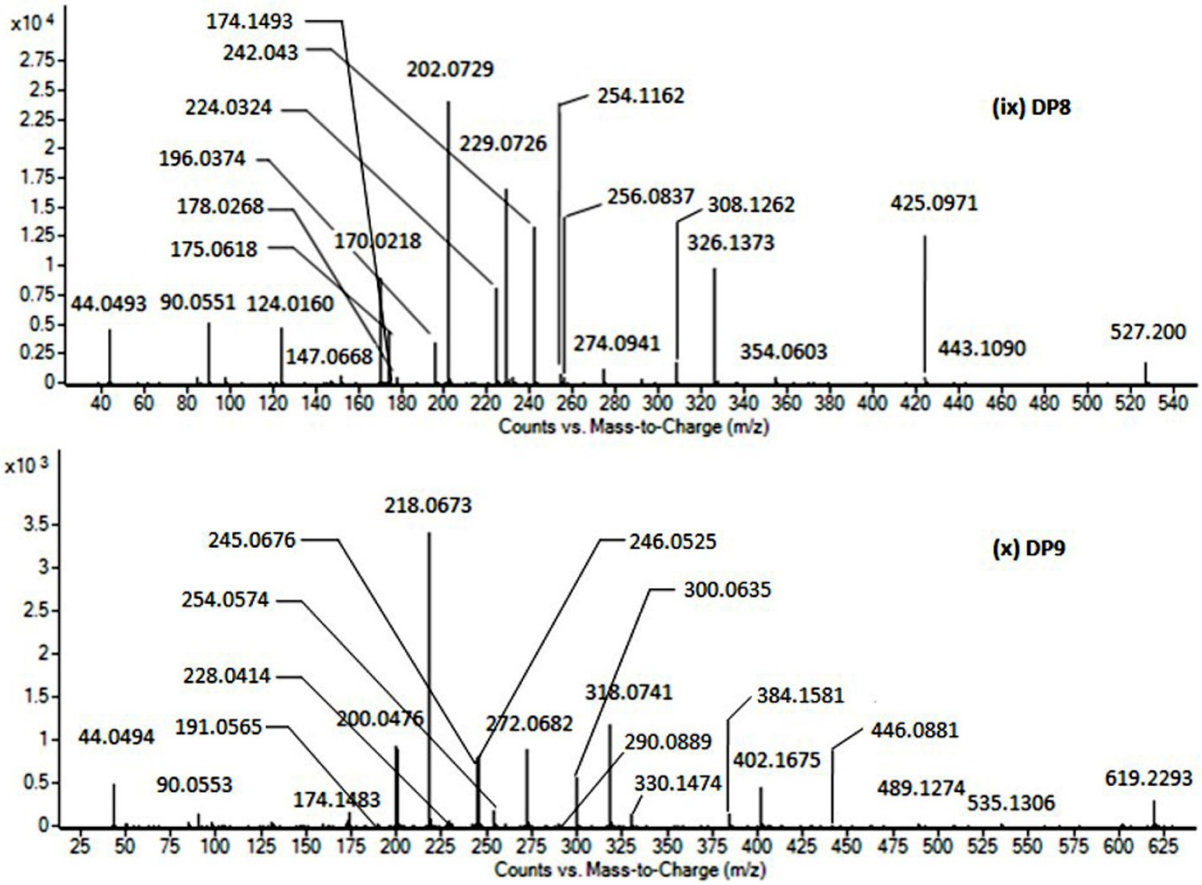


Figure S5. UHPLC-ESI-MS/MS spectra of $[M+H]^+$of (i) RDV (*m/z* 603), (ii) DP1 (*m/z*372), (iii) DP2 (*m/z*292), (iv) DP3 (*m/z*448), (v) DP4 (*m/z*174), (vi) DP5 (*m/z*621), (vii) DP6 (*m/z*443), (viii) DP7 (*m/z*519), (ix) DP8 (*m/z*527), and (x) DP9 (*m/z*619)

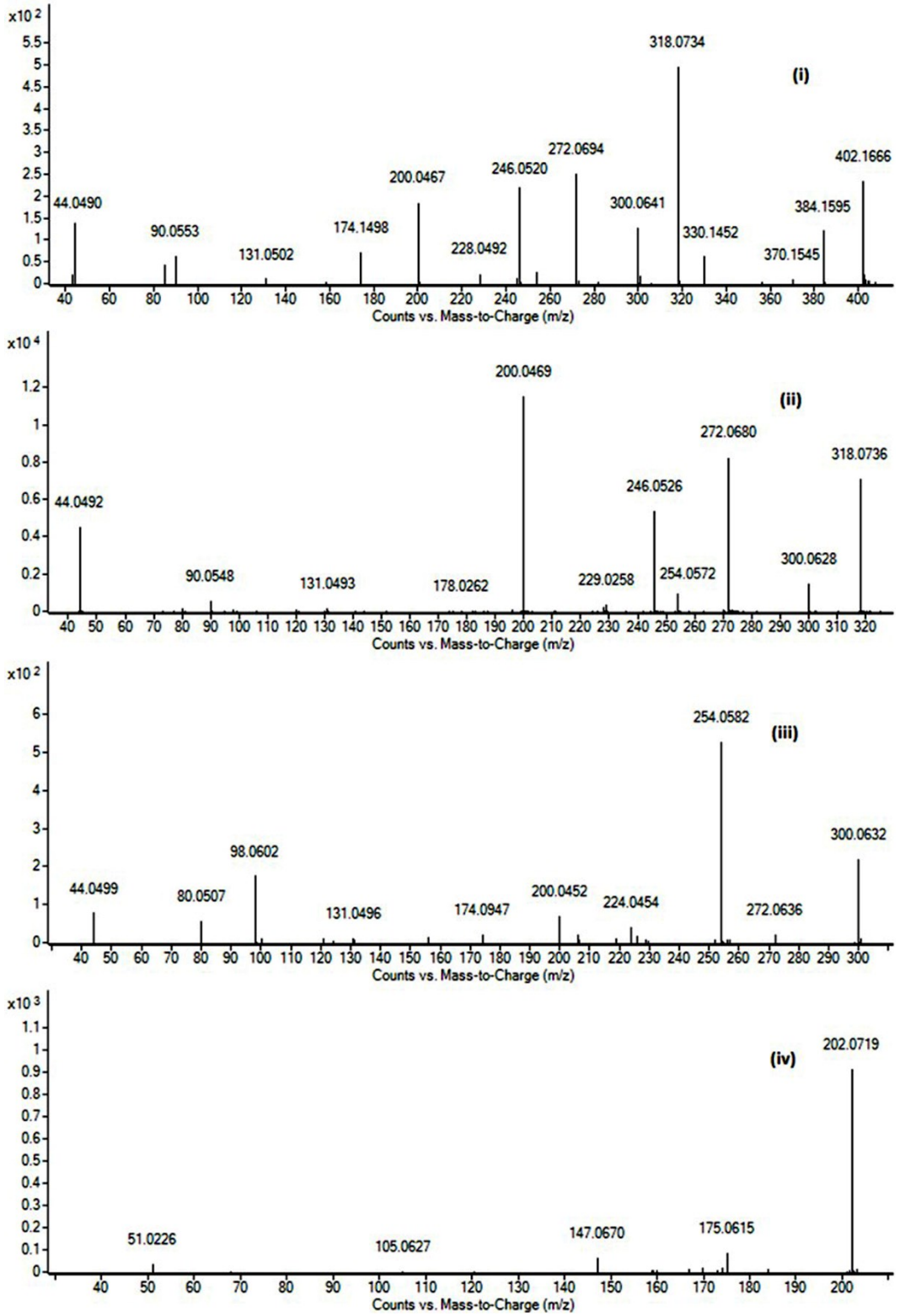
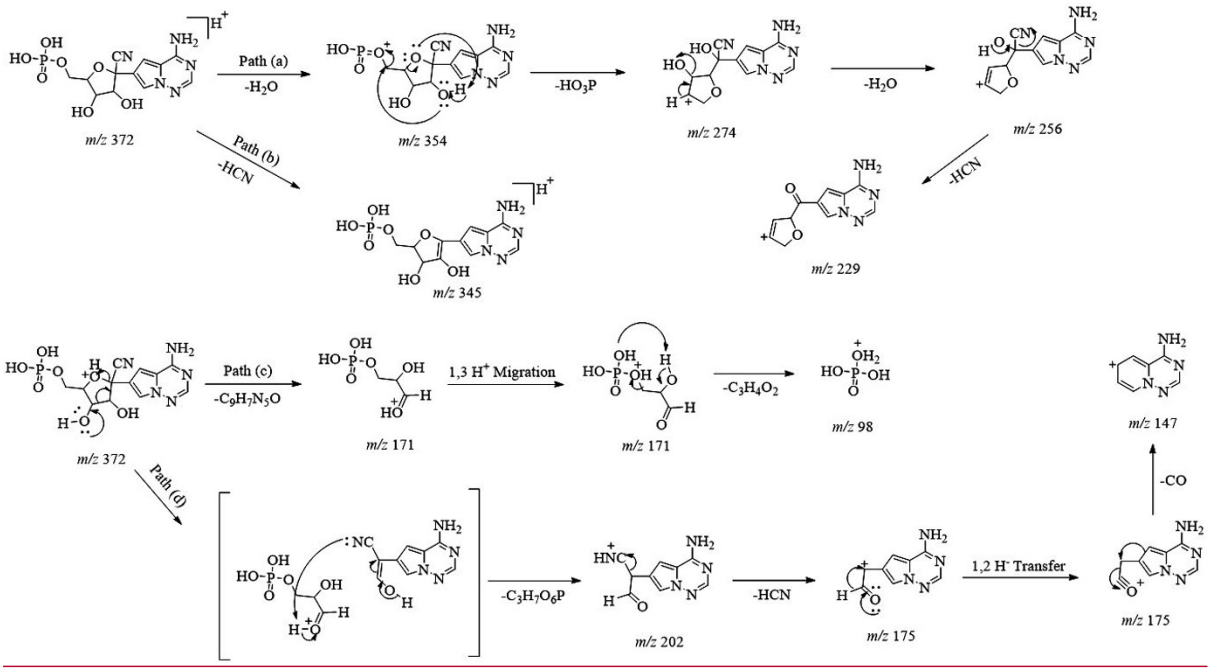
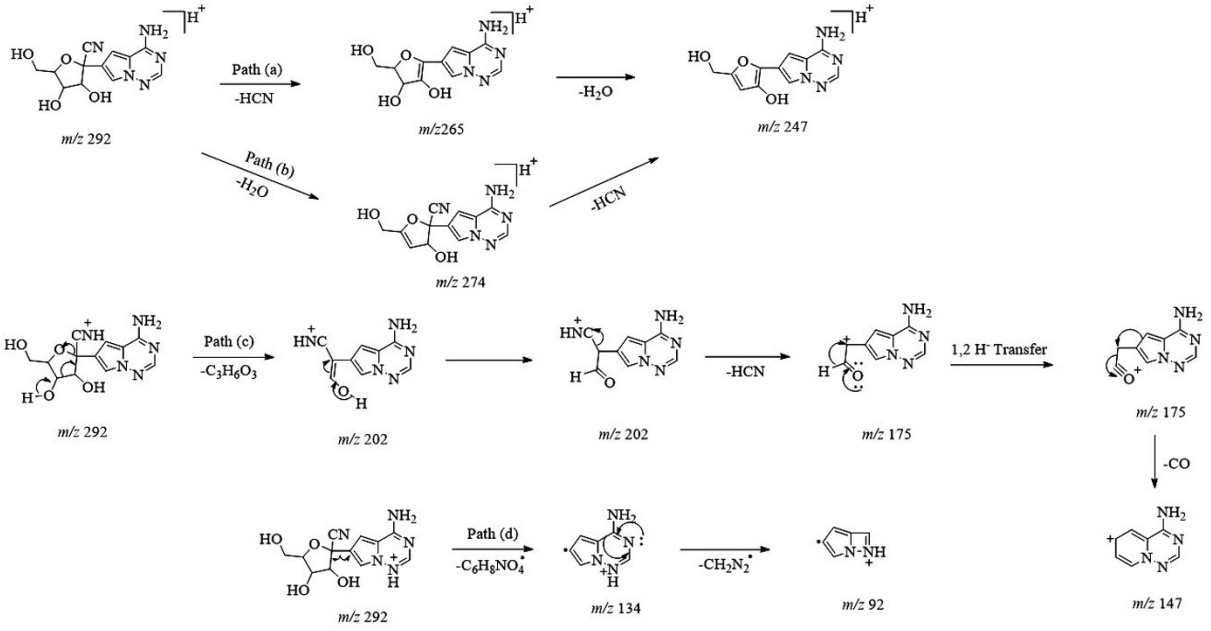


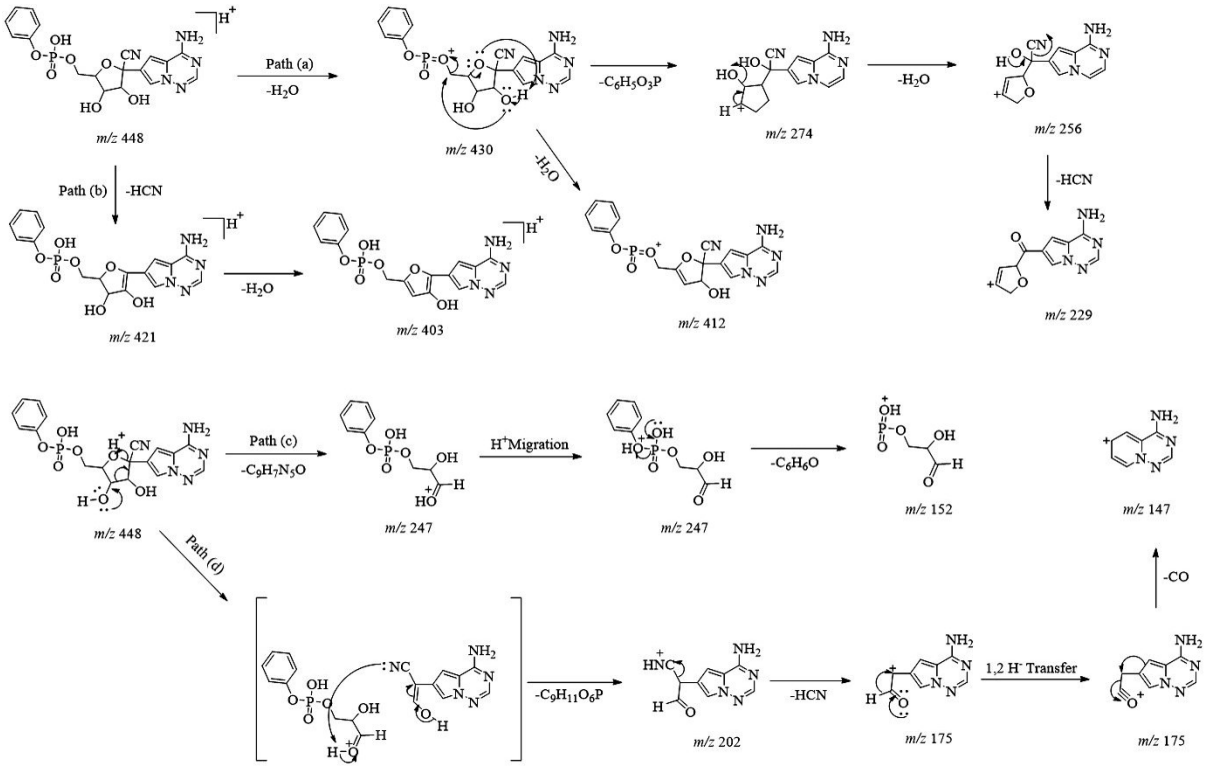
Figure S6. ESI-Pseudo- MS^3spectra ofions at i) *m/z*402, ii) *m/z* 318, iii) *m/z* 300, and iv) *m/z*202.



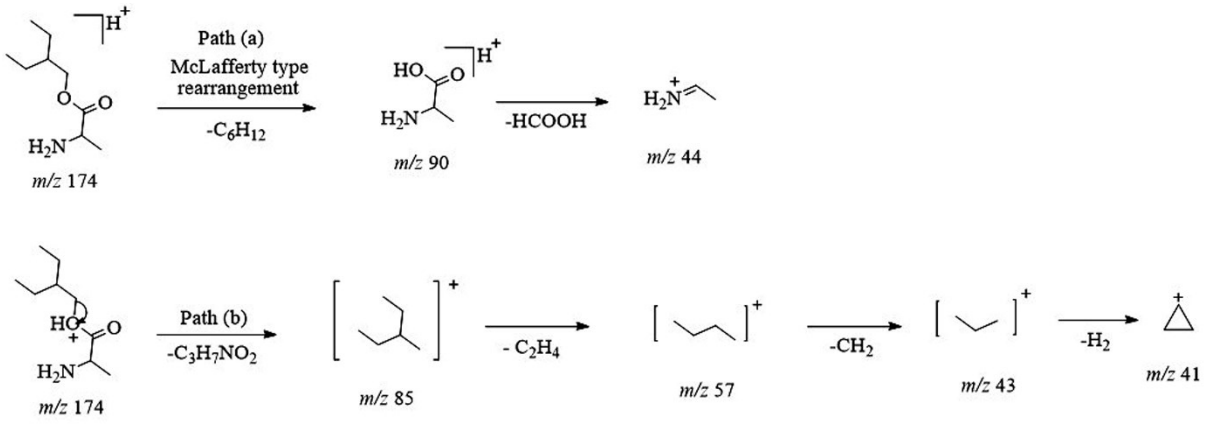
Scheme S1. Proposed fragmentation pathway for $[M+H]^+$ of DP1.



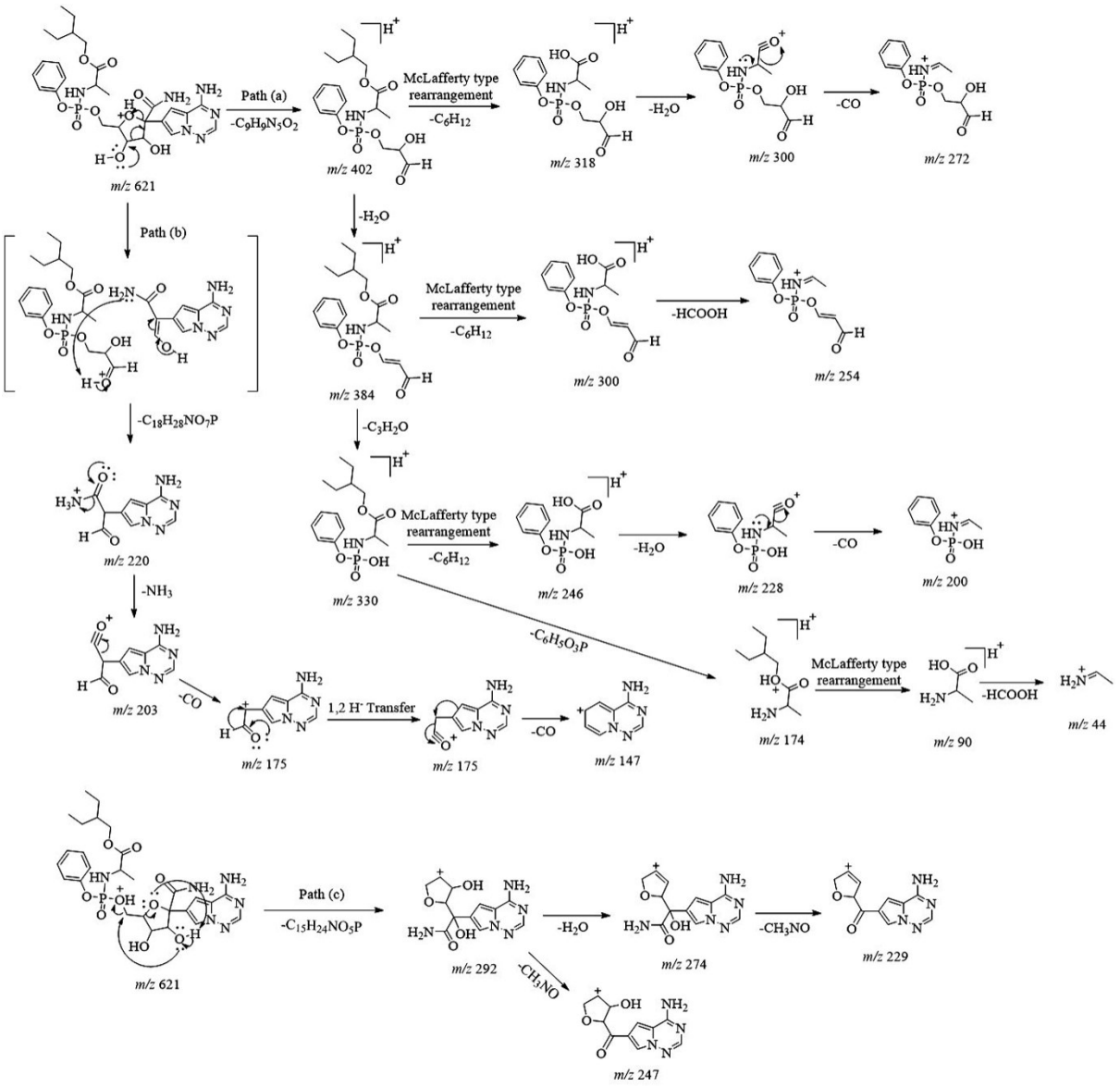
Scheme S2.Proposed fragmentation pathway for $[M+H]^+$ of DP2.



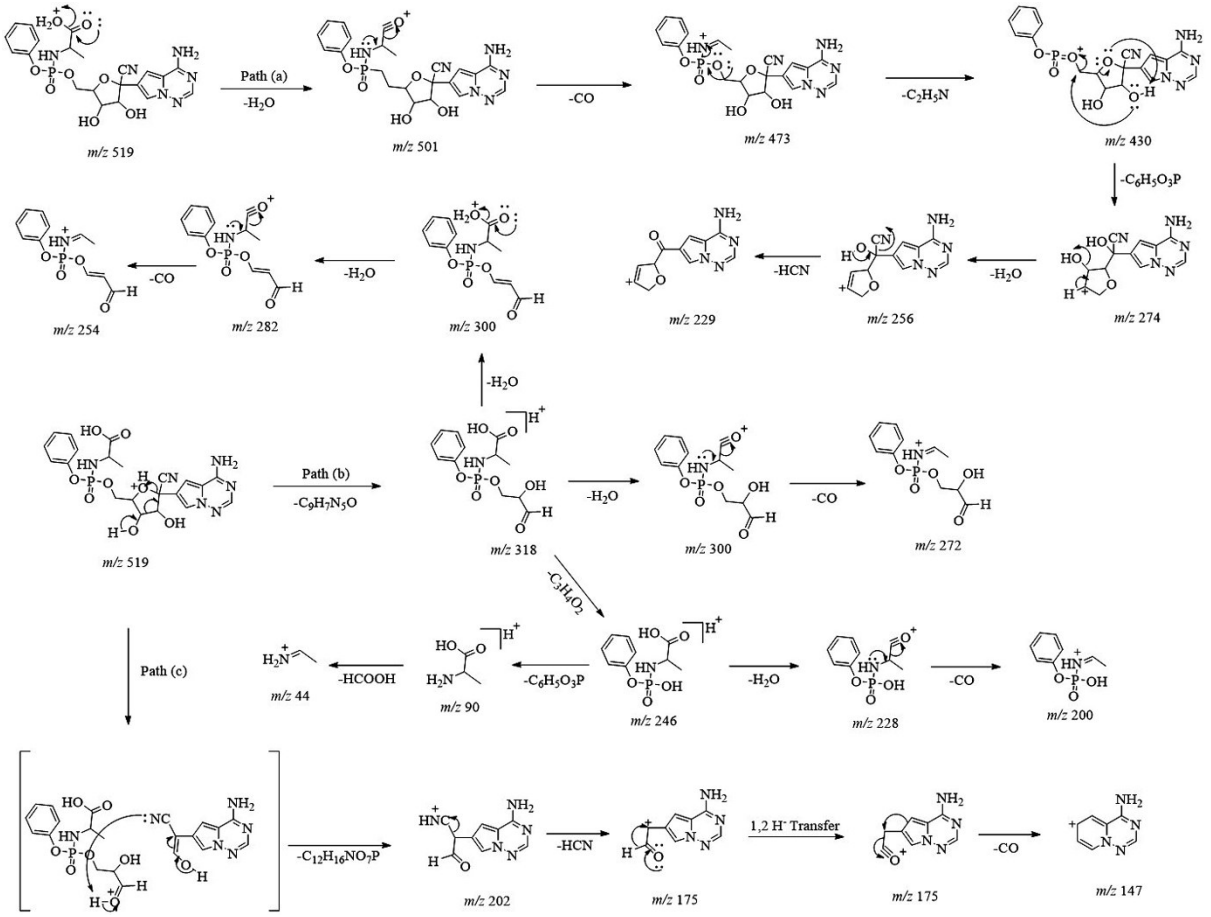
Scheme S3. Proposed fragmentation pathway for $[M+H]^+$ of DP3.



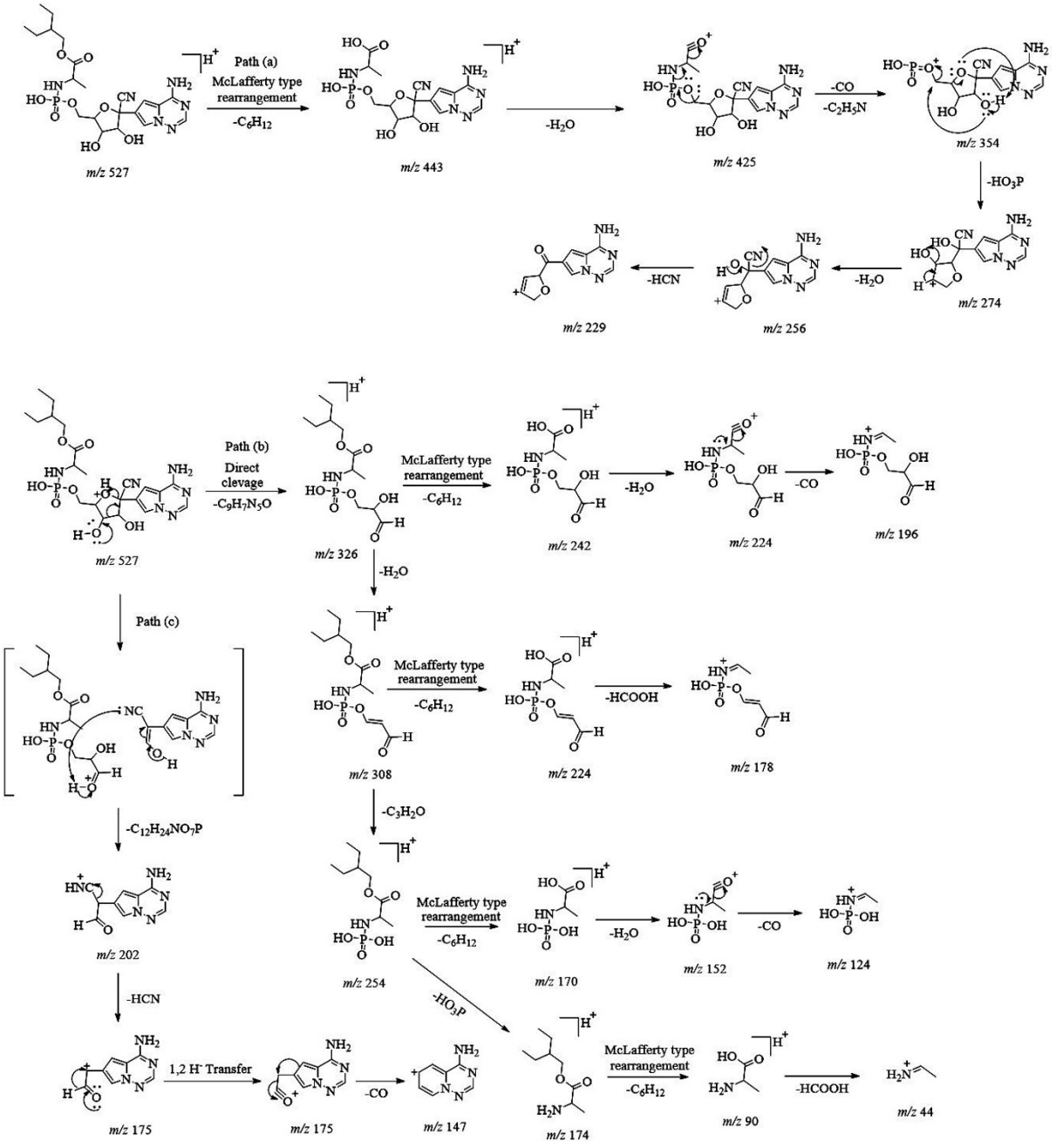
Scheme S4.Proposed fragmentation pathway for $[M+H]^+$ of DP4.



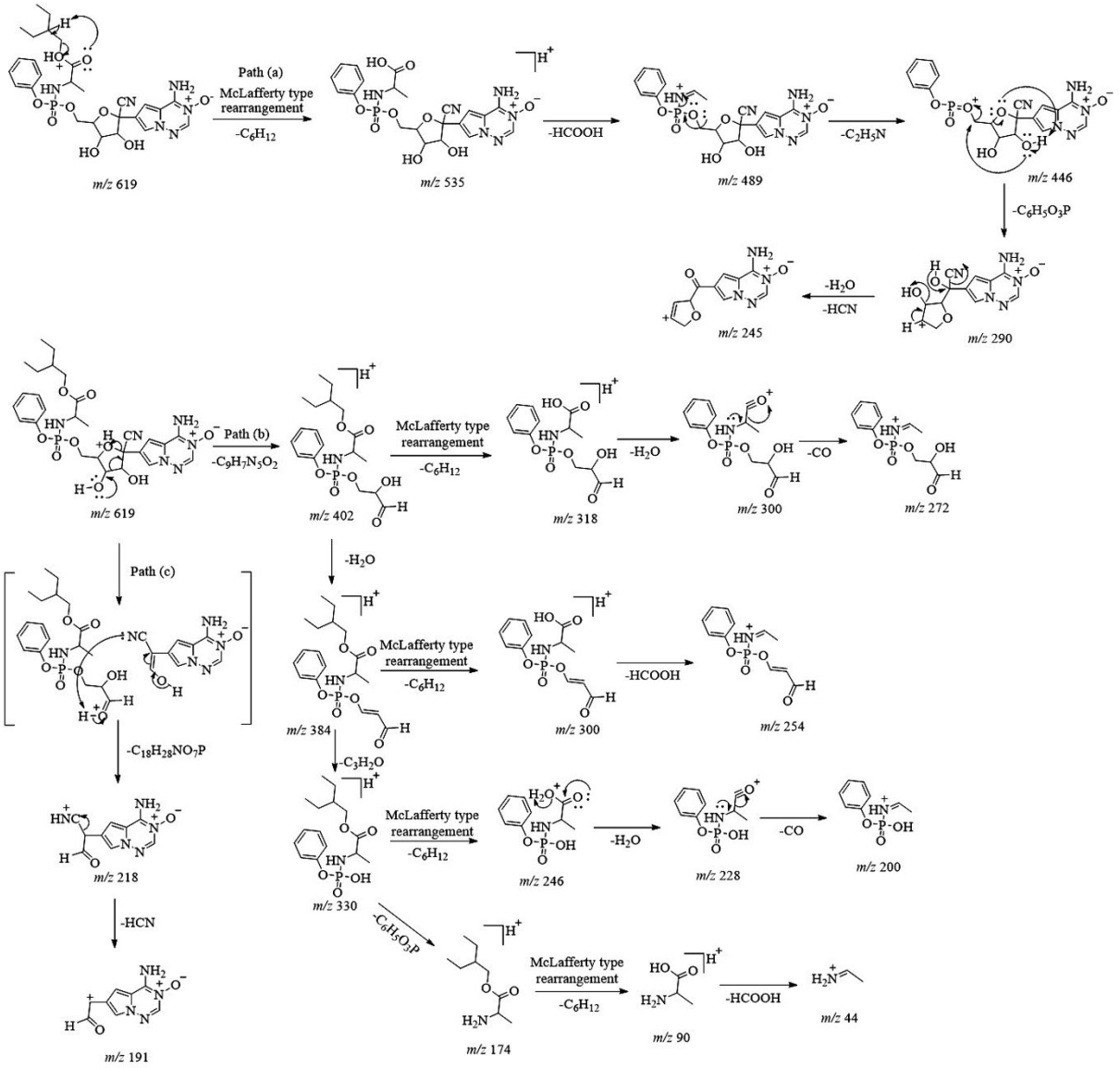
Scheme S5. Proposed fragmentation pathway for $[M+H]^+$ of DP5.



Scheme S6.Proposed fragmentation pathway for $[M+H]^+$ of DP7.



Scheme S7. Proposed fragmentation pathway for $[M+H]^+$ ofDP8.



Scheme S8.Proposed fragmentation pathway for $[M+H]^+$ of DP9.