

Supplementary information

Mass spectrometry guided discovery and design of novel Asperphenamate analogues from *Penicilium astrolabium*

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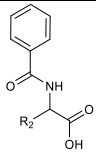
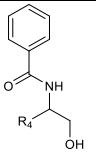
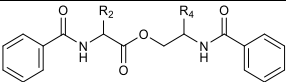
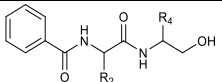
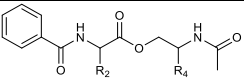
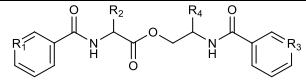
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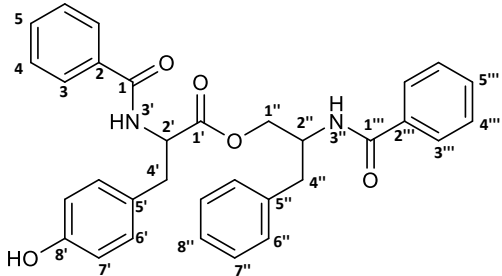
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Supplementary Table 1: m/z values of protonated adduct of potential asperphenamate analogues.

No.	AA										
		(A) R ₂ = AA	(B) R ₄ = AA	(C) R ₂ = AA R ₄ = Phe (D) R ₂ = Phe R ₄ = AA (E) R ₂ = R ₄ = AA	(F) R ₂ = AA R ₄ = Phe (G) R ₂ = Phe R ₄ = AA (H) R ₂ = R ₄ = AA	(I) R ₂ = AA R ₄ = Phe (J) R ₂ = Phe R ₄ = AA (M) R ₂ = R ₄ = AA	(K) R ₂ = AA R ₄ = Phe (L) R ₂ = Phe R ₄ = AA (N) R ₂ = R ₄ = AA	(O) R ₁ = N R ₂ = AA R ₃ = CH R ₄ = Phe (P) R ₁ = N R ₂ = Phe R ₃ = CH R ₄ = AA (Q) R ₁ = CH R ₂ = AA R ₃ = N R ₄ = Phe (R) R ₁ = CH R ₂ = Phe R ₃ = N R ₄ = AA (S) R ₁ = R ₃ = N R ₂ = AA, R ₄ = Phe (T) R ₁ = R ₃ = N R ₂ = Phe, R ₄ = AA			
1	Phe	270.1125	256.1332	507.2278		403.2016		445.2122		508.2231	509.2183
2	Tyr	285.1001	272.1281	523.2227	539.2177	419.1965	435.1914	461.2071	477.2020	524.2180	525.2132
3	Trp	309.1234	295.1441	546.2387	585.2496	442.2125	481.2234	484.2231	523.2340	547.2340	548.2292
4	Leu/Ile	236.1281	222.1489	473.2435	439.2591	369.2173	335.2329	411.2278	377.2435	474.2387	475.2340
5	Ala	194.0812	180.1019	431.1965	355.1652	327.1703	251.1390	369.1809	293.1496	432.1918	433.1870
6	Gly	180.0655	166.0863	417.1809	327.1339	313.1547	223.1077	355.1652	265.1183	–	–
7	His	260.1030	246.1237	497.2183	487.2088	393.1921	383.1826	435.2027	425.1932	498.2136	499.2088
8	Val	222.1125	208.1332	459.2278	411.2278	355.2016	307.2016	397.2122	349.2122	460.2231	461.2183
9	Met	254.0845	240.1053	491.1999	475.1720	387.1737	371.1458	429.1843	413.1563	492.1952	493.1904
11	Cys	226.0532	212.0740	463.1686	419.1094	359.1424	315.0832	401.1530	357.0937	–	–
12	Ser	210.0761	196.0968	447.1914	387.1551	343.1652	283.1288	385.1758	325.1394	–	–
13	Thr	224.0917	210.1125	461.2071	415.1864	357.1809	311.1601	399.0914	353.1707	–	–
14	Asn	237.0870	223.1077	474.2023	411.1769	370.1761	337.1506	412.1867	379.1612	–	–
15	Gln	251.1026	237.1234	488.2180	469.2082	384.1918	365.1819	426.2023	407.1925	–	–
16	Lys	251.1390	237.1598	488.2544	469.2809	384.2282	365.2547	426.2387	407.2653	–	–
17	Arg	279.1452	265.1659	516.2605	525.2932	412.2343	421.2670	454.2449	463.2776	–	–
18	Pro	220.0968	206.0968	457.2122	407.1965	353.1860	277.1547	395.1965	345.1809	–	–
19	Glu	252.0866	238.1074	489.2020	471.1762	385.1758	337.1500	427.1864	409.1605	–	–
20	Asp	238.0710	224.0917	475.1864	443.1449	371.1601	339.1187	413.1707	381.1292	–	–
21	p-Cl-Phe	304.0735	290.0942	541.1889	575.1499	437.1626	471.1237	479.1732	513.1542	542.1841	543.1794
22	p-Br-Phe	348.0230	334.0437	585.1383	663.0489	481.1121	559.0226	523.1227	601.0332	586.1336	587.1288
23	p-NH ₂ -Phe	285.1234	271.1441	522.2387	537.2496	418.2125	433.2234	460.2231	494.1841	523.2340	524.2292
24	p-NO ₂ -Phe	315.0978	301.1183	552.2129	597.1980	448.1867	463.1976	490.1973	535.1823	553.2082	554.2034

Supplementary Table S2: NMR data for Asperphenamate Y (**4**) in chloroform (CDCl₃).



No.	δ_H , mult. (J in Hz)	δ_C	COSY	H2BC	HMBC
1		167.7			
2		133.4			
3	7.65, m	127.2	4	C-4	C-1, C-3, C-5,
4	7.39, m	128.8	3, 5	C-3, C-5	C-2, C-4
5	7.5, tt (7.4, 1.2)	132.2	4	C-4	C-3
1'		172.2			
2'	4.87, d (6.7)	54.8	3', 4'	C-4'	C-1, C-1', C-4', C-5'
3'	6.59, d (6.6)		2'	C-2'	C-1, <u>C-1'</u> , <u>C-4'</u>
4'	3.20, dd (14.0, 6.5) 3.14, dd (14.0, 6.9)	36.9	2'	C-2'	C-1', C-2', C-5', C-6'
5'		127.6			
6'	7.05, m	130.5	7'	C-7'	C-4', C-6', <u>C-7'</u> , C-8'
7'	6.76, m	116.0	6'	C-6'	C-5', C-7', C-8'
8'		155.3			
1''	4.50, dd (11.4, 3.6) 4.04, dd (11.4, 4.5)	65.5	2''	C-2''	C-1', C-4'', C-2''
2''	4.60, m	50.6	1'', 3'', 4''	<u>C-1''</u> , C-4''	
3''	6.71, d (8.4)		2''	C-2''	C-1''
4''	3.01, dd(13.9, 6.5) 2.91, dd (13.9, 8.2)	37.4	2''	C-2''	C-1'' , C-2'', C-5'', C-6''
5''		137.1			
6''	7.22, m	129.5	7''	C-7''	C-4'',C- 8''
7''	7.31, m	128.9	6'', 8''	C-6'', C-8''	C-5'', C-7'', C-6''
8''	7.24, m	127.0	7''	C-7''	C-6''
1'''		167.8			
2'''		134.1			
3'''	7.68, m	127.3	4'''	C-4'''	C-1''', C-3''', C-5'''
4'''	7.31, m	128.6	3''', 5'''	C-3''', C-5'''	C-2''', C-4'''

Weak correlations are underlined.

Supplementary Table S3: NMR data for Asperphenamate W (**12**) in chloroform (CDCl₃).

No.	δ_H , mult. (J in Hz)	δ_C	COSY	H2BC	HMBC
1		167.6			
2		133.5			
3	7.63, m	127.3	4	C-4	C-1, C-3, C-5
4	7.29, m	128.6	3, 5	C-3, C-5	C-2, <u>C-3</u> , C-4
5	7.48, t (7.4)	132.1	4	C-4	C-3, <u>C-4</u>
1'		172.5			
2'	5.04, q (6.5)	54.3	3', 4'	C-4'	C-1, C-1', C-4', C-5'
3'	6.69, d (6.4)		2'		C-1, <u>C-1'</u> , C-2', C-4'
4'	3.43, d (6.0)	27.7	2'	C-2'	C-1', C-2', C-5', C-6', C-13'
5'		110.1			
6'		127.5			
7'	7.64, m	118.7	8'	C-8'	<u>C-5'</u> , C-9', C-11'
8'	7.12, t (7.4)	120.1	7', 9'	C-7', C-9'	C-6', C-10'
9'	7.20, m	122.7	8', 10'	C-8', C-10'	C-7', C-11'
10'	7.33, d (7.9)	111.6	9'	C-9'	C-6', C-8'
11'		136.4			
12'	8.06, s		13'		<u>C-5'</u> , <u>C-6'</u> , <u>C-13'</u>
13'	7.06, d (2.2)	123.1	12'		<u>C-4</u> , C-5', C-6', C-11'
1''	4.46, dd (11.6, 3.6) 4.06, dd (11.6, 4.6)	65.4	1'', 2''	C-2'''*	C-1', <u>C-2''</u> , <u>C-4''</u>
2''	4.55, m	50.6	1'', 3'', 4''	C-1'', C-4''	<u>C-1''</u>
3''	6.59 d (8.4)		2''		<u>C-1''</u> , <u>C-2''</u> , C-1'''
4''	2.94, dd (13.6, 6.7) 2.81, dd (13.6, 8.4)	37.3	2'', 4''	C-2''	C-1'', C-2'', C-5'', C-6''
5''		137.4			
6''	7.18, s (7.6)	129.5	7''	C-7''	C-4'', C-6'', <u>C-7''</u> , C-8''
7''	7.36, t (7.8)	128.7	6'', 8''	C-6'', C-8''	5'', 6'', 7''
8''	7.23, m	126.9	7''	C-7''	C-6'', C-7''
1'''		167.4			
2'''		134.4			
3'''	7.63, m	127.2	4'''	C-4'''	C-1''', C-3''', C-5'''
4'''	7.29, m	128.8	3''', 5'''	C-3''', C-5'''	C-2''', C-4'''
5'''	7.43, t (7.4)	131.5	4'''	C-4'''	C-3''', <u>C-4'''</u>

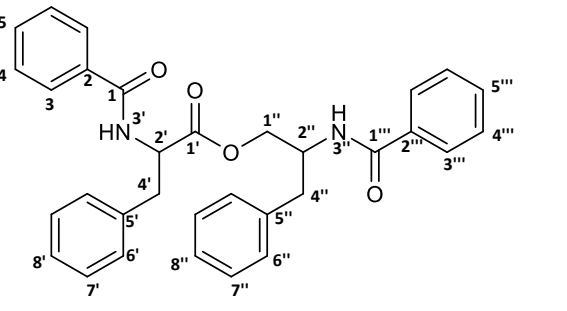
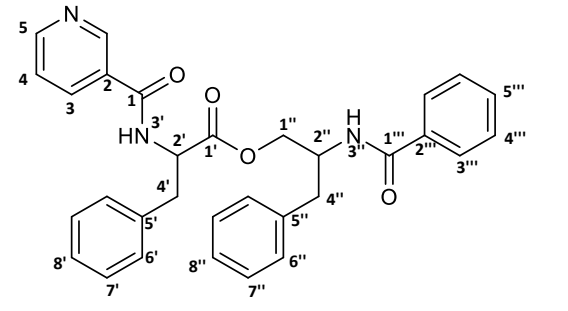
Weak correlations are underlined.

Supplementary Table S4: NMR data for Asperphenamate L (**13**) in chloroform (CDCl₃).

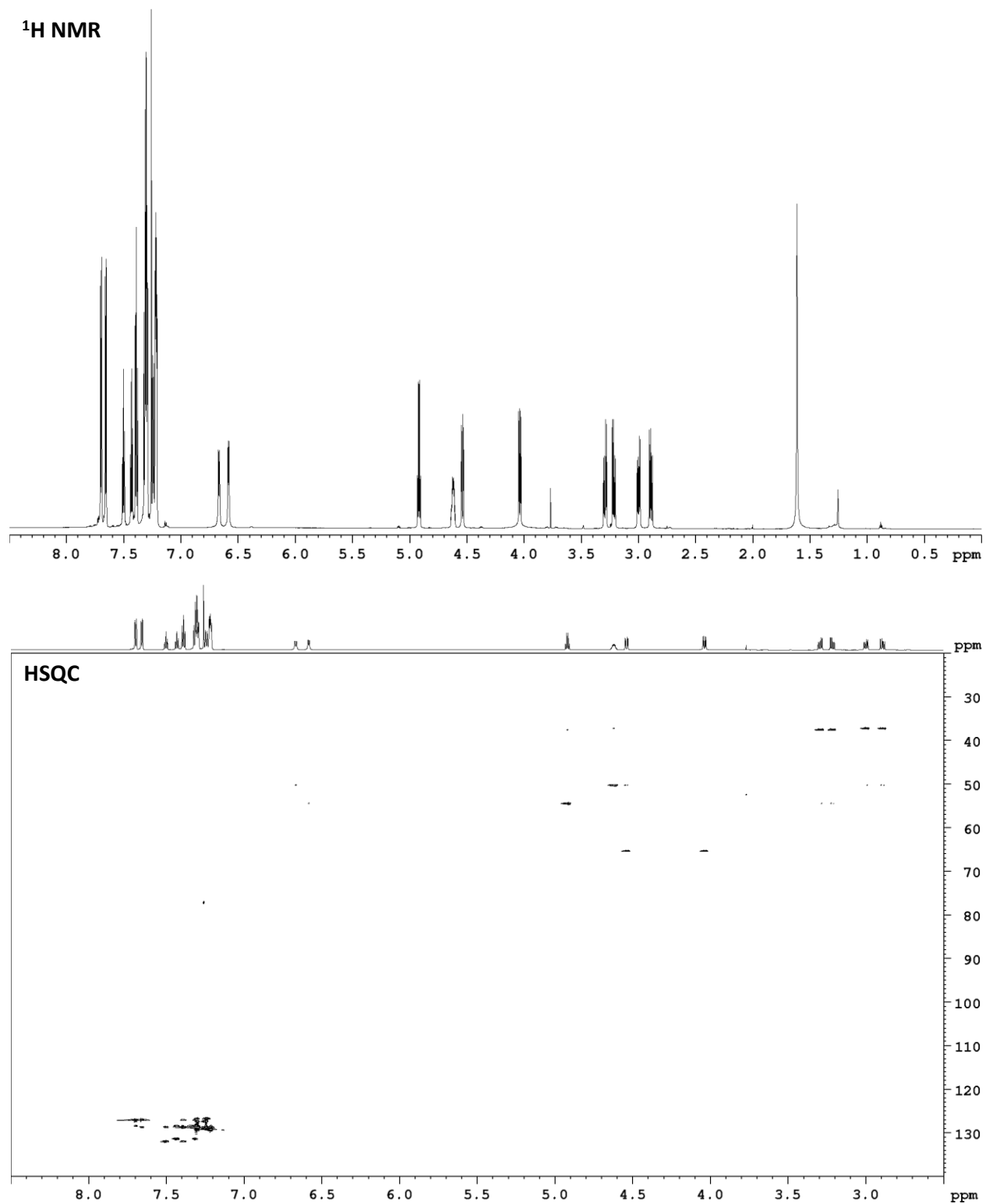
No.	δ_H , mult. (<i>J</i> in Hz)	δ_C	COSY	HMBC
1		167.8		
2		133.3		
3	7.72, m	127.1	C-4	C-1, C-3, C-5
4	7.41, m	128.6	C-3, C-5	C-2, C-4
5	7.52, m	132.2	C-4	C-3, <u>C-4</u>
1'		173.2		
2'	4.71, m	52.1	C-3', C-4'	C-1', C-4'
3'	6.46, d (6.8)		C-2'	<u>C-1</u>
4'	1.79, m 1.69, m	40.8	C-2', C-4', C-5'	C-1', C-2', C-5', C-6', C-7'
5'	1.75, m	25.1	C-4', C-6', C-7'	C-4', C-6'
6'	0.99, d (6.5)	22.2	C-5'	C-4', C-5', C-7'
7'	1.02, d(6.5)	22.8	C-5'	C-4', C-5', C-6'
1''	4.59, dd (11.5, 3.3) 4.08, dd (11.5, 4.6)	65.1	C-1'', C-2''	C-1'
2''	4.69, m	50.5	C-1'', C-3'', C-4''	
3''	6.73, d (8.2)		C-2''	<u>C-1'''</u>
4''	3.10, dd (13.6, 6.5) 3.01, dd (13.8, 8.2)	37.3	C-2'', C-4''	C-1'', C-2'', C-5'', C-6''
5''		137.3		
6''	7.29, m	129.3	C-7''	C-4'', C-6'', C-8''
7''	7.32, m	128.7	C-6'', C-8''	C-5'', C-7''
8''	7.25, m	126.8	C-7''	
1'''		167.3		
2'''		134.3		
3'''	7.70, m	127.1	C-4'''	C-1''', C-3''', C-5'''
4'''	7.28, m	128.4	C-3''', C-5'''	C-2''', C-4'''
5'''	7.42, m	131.3	C-4'''	C-3'''

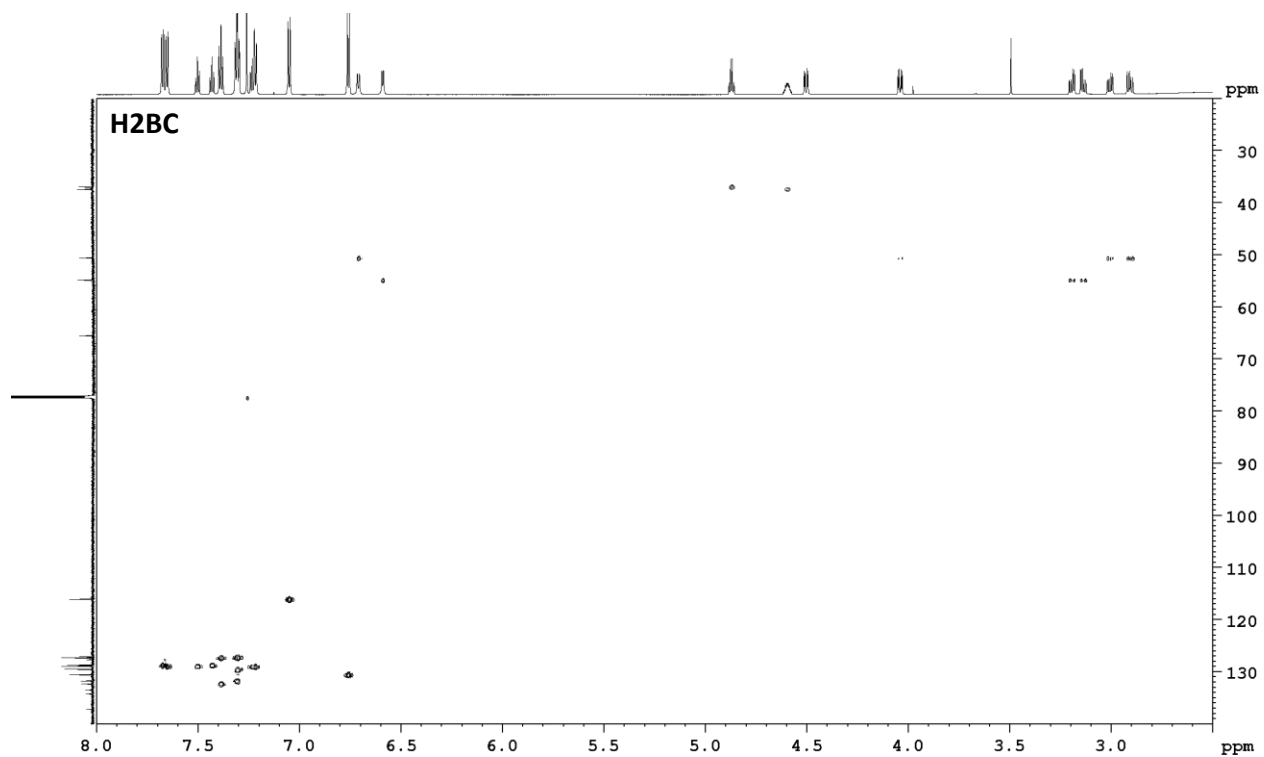
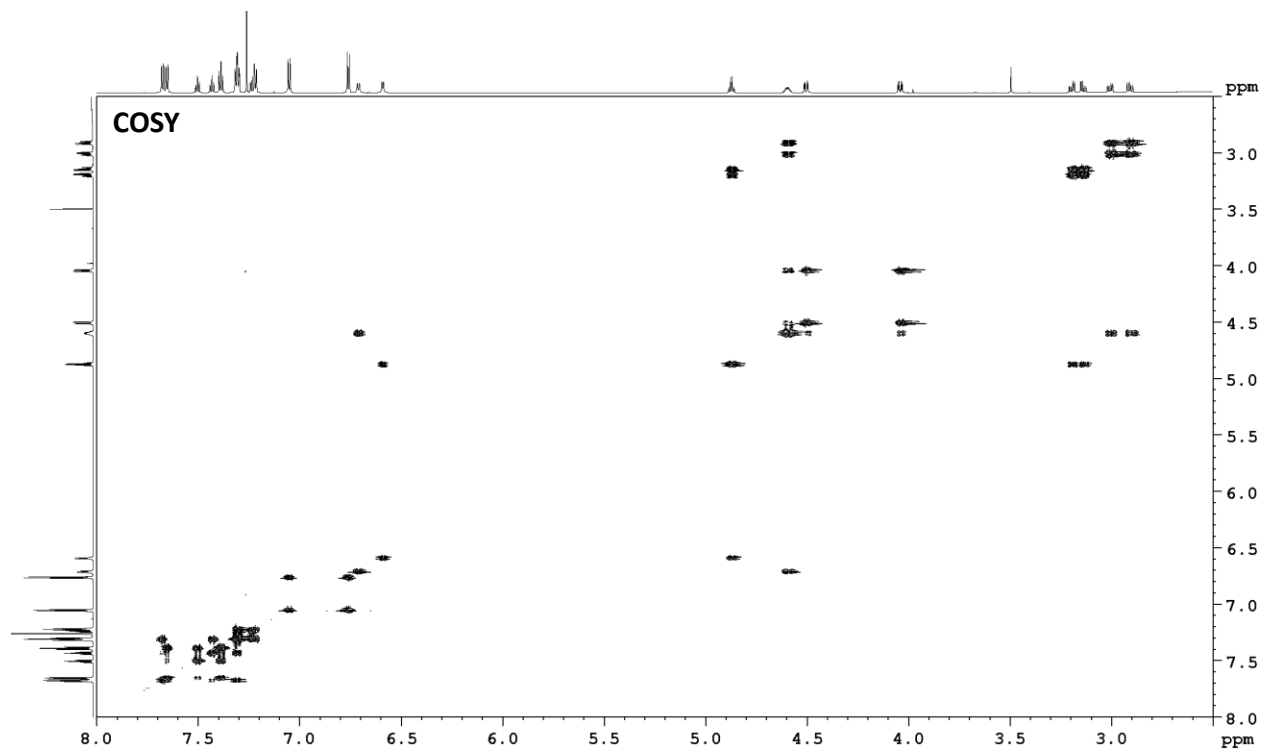
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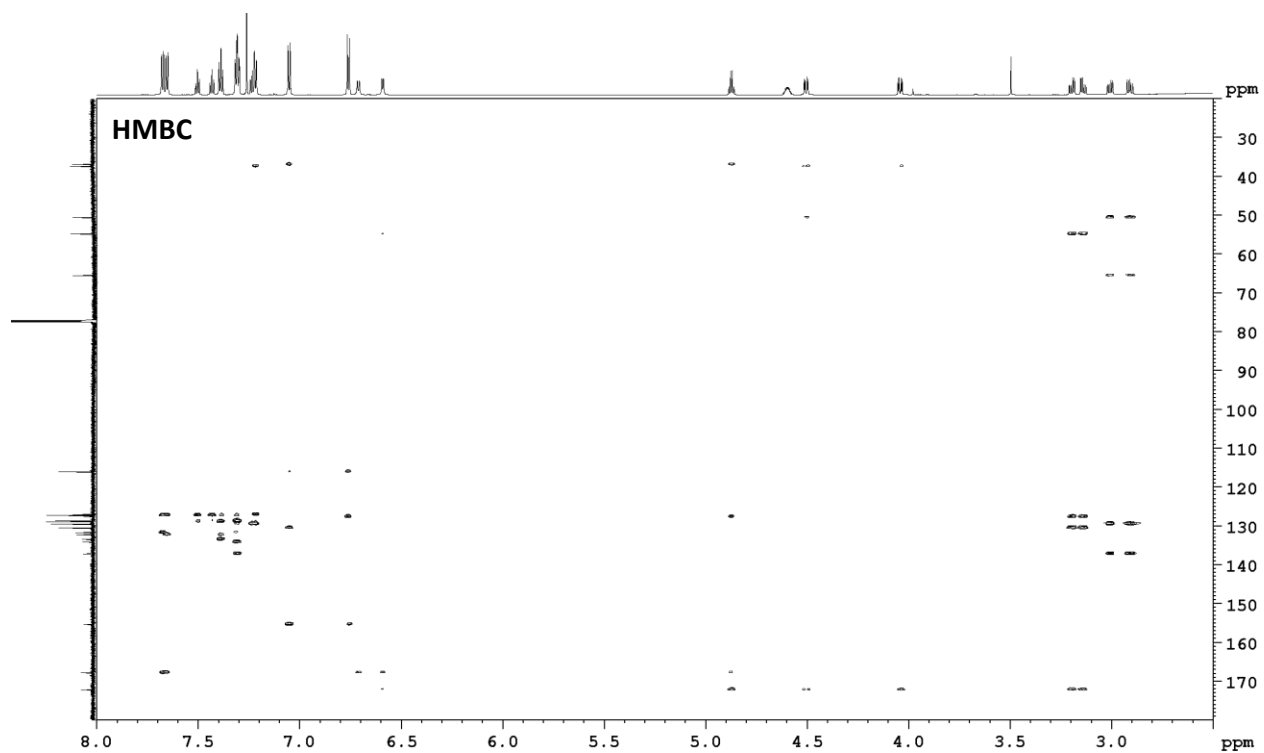
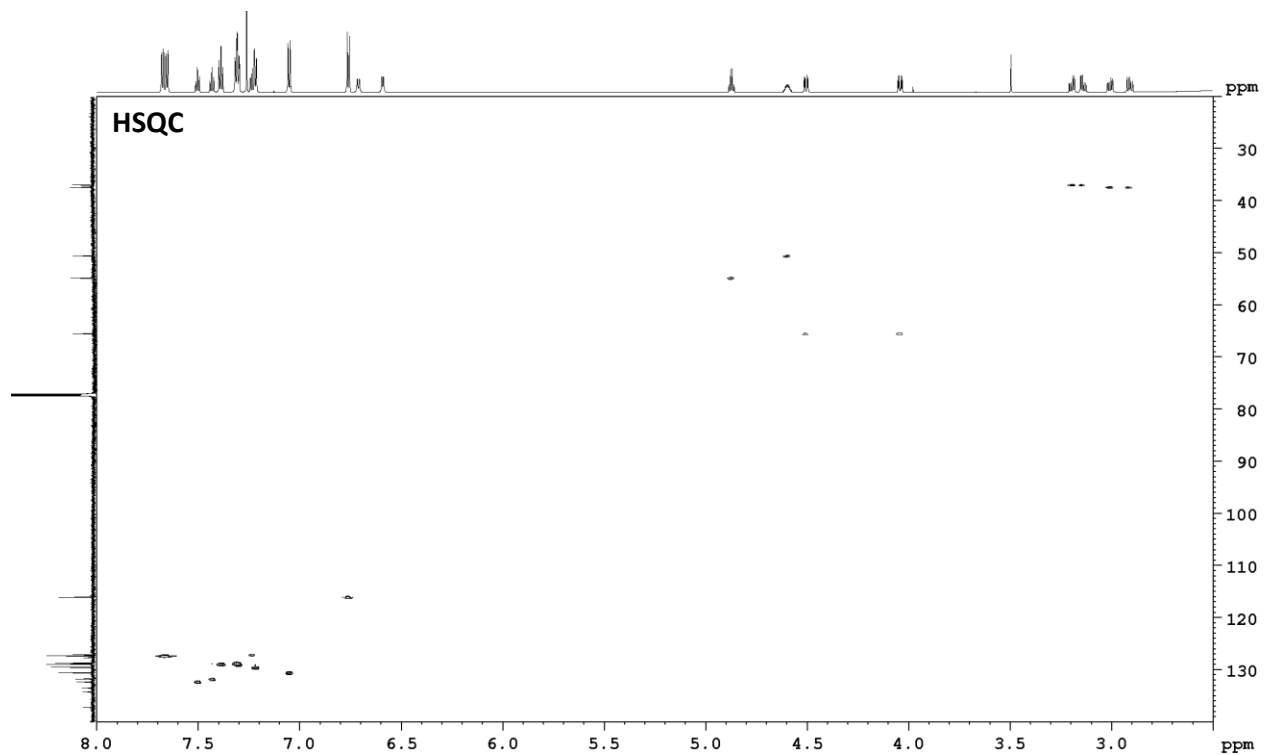
Supplementary Table S5: NMR data for Asperphenamate F (**3**) and Asperphenidine F1 (**3a**) in chloroform (CDCl₃).

					
No.	δ_H , mult. (<i>J</i> in Hz)	δ_C	No.	δ_H , mult. (<i>J</i> in Hz)	
1			1		
2			2		
3	7.7, dd (8.3, 1.1)	126.9	3	7.94, m	
4	7.39, m	128.6	4	7.18-7.35, m	
5	7.50, tt (7.5, 1.1)	131.9	5	8.72, dd (4.8, 1.5)	
7				8.87, d (2.0)	
1'			1'		
2'	4.92, q (6.6)	54.4	2'	4.96, q (6.7)	
3'	6.58, d (6.6)		3'	6.48, d	
4'	3.29, dd (14.0, 6.6) 3.21, dd (14.0, 7.0)	37.5	4'	3.30, dd (14.1, 6.5) 3.21, dd (14.0, 7.0)	
5'			5'		
6'	7.21, m	129.1	6'	7.18-7.35, m	
7'	7.29, m	128.8	7'	7.18-7.35, m	
8'	7.24, m	126.7	8'	7.18-7.35, m	
1''	4.54, dd (11.4, 3.4) 4.04, dd (11.4, 4.4)	65.3	1''	4.51, dd (11.4, 3.5) 4.09, dd (11.4, 4.1)	
2''	4.62, m	50.2	2''	4.63, m	
3''	6.67, d (8.4)		3''	6.59, d	
4''	3.00, dd (13.7, 6.4) 2.89, dd (13.8, 8.5)	37.2	4''	3.00, dd (13.8, 6.5) 2.90, dd (13.8, 8.1)	
5''			5''		
6''	7.23, m	129.2	6''	7.18-7.35, m	
7''	7.32, m	128.3	7''	7.18-7.35, m	
8''	7.25, m	127.3	8''	7.18-7.35, m	
1'''			1'''		
2'''			2'''		
3'''	7.65, dd (8.3, 1.1)	127.0	3'''	7.66, dd (8.3, 1.0)	
4'''	7.31, m	128.6	4'''	7.18-7.35, m	
5'''	7.43, tt (7.5, 1.1)	131.3	5'''	7.45, m	

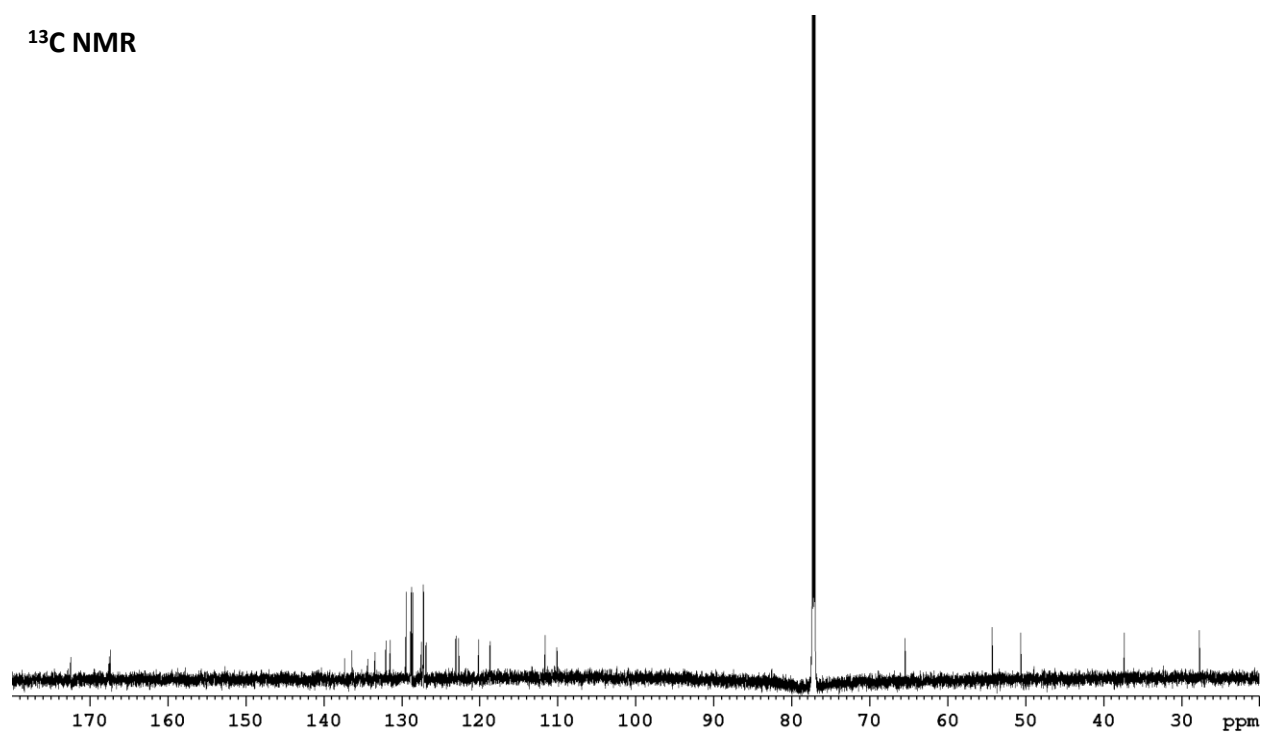
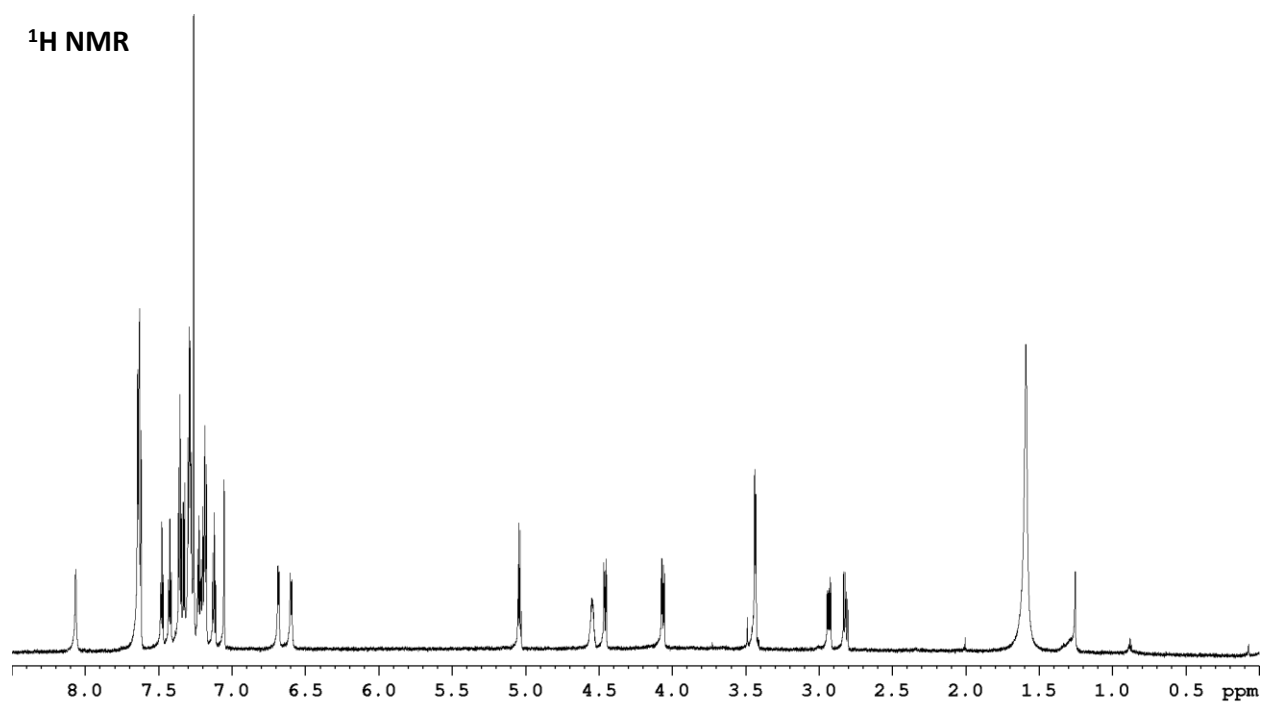
Supplementary Figure S1: NMR spectra for Asperphenamate F (3).

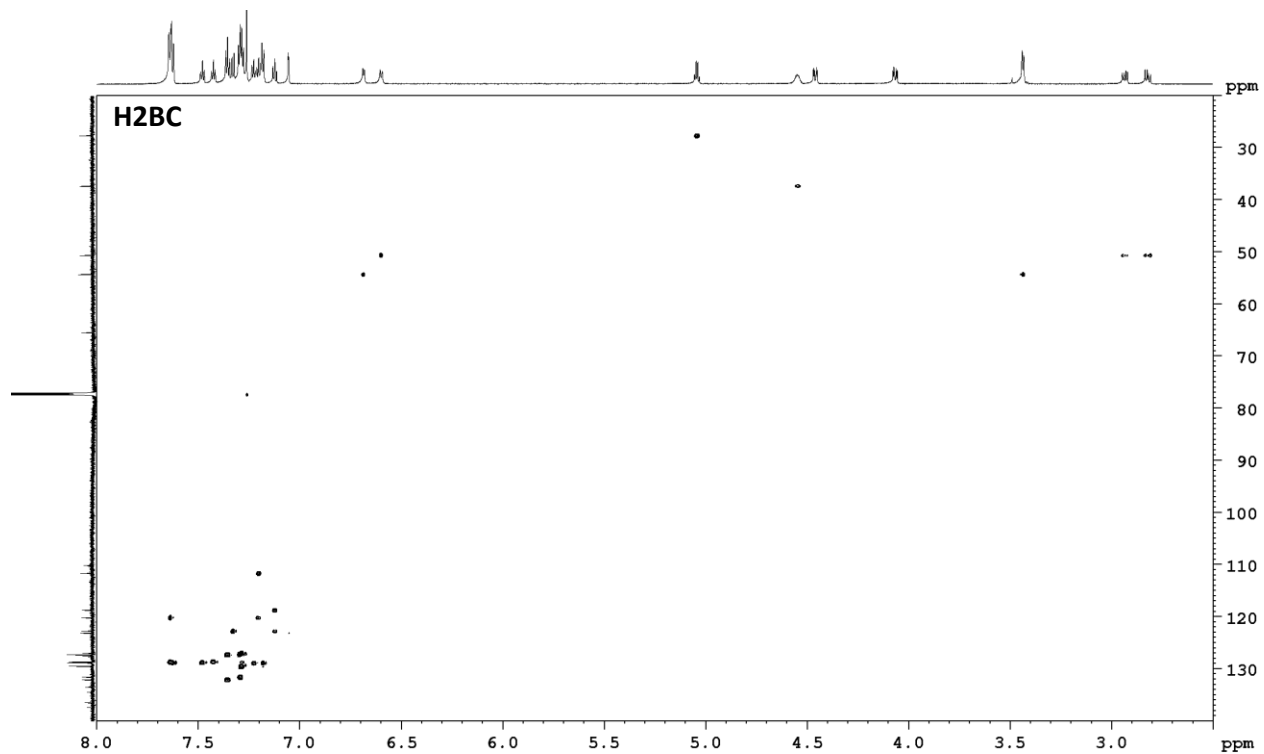
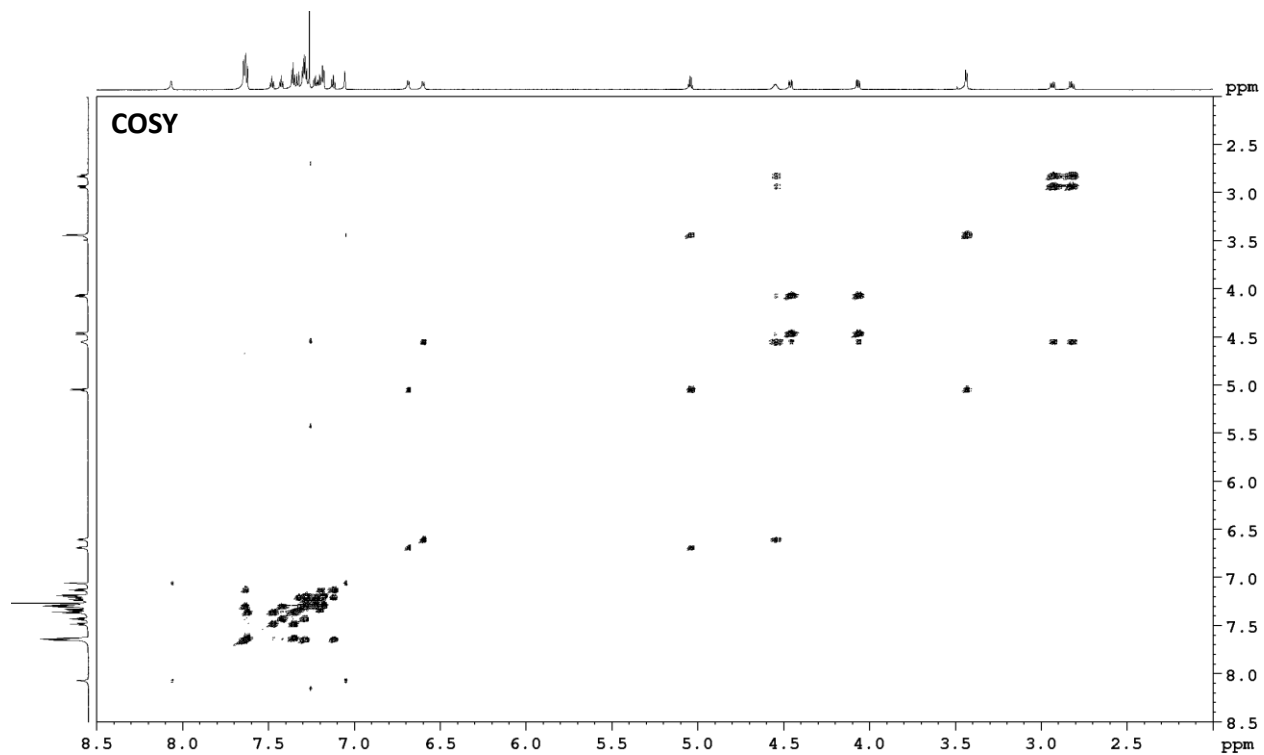


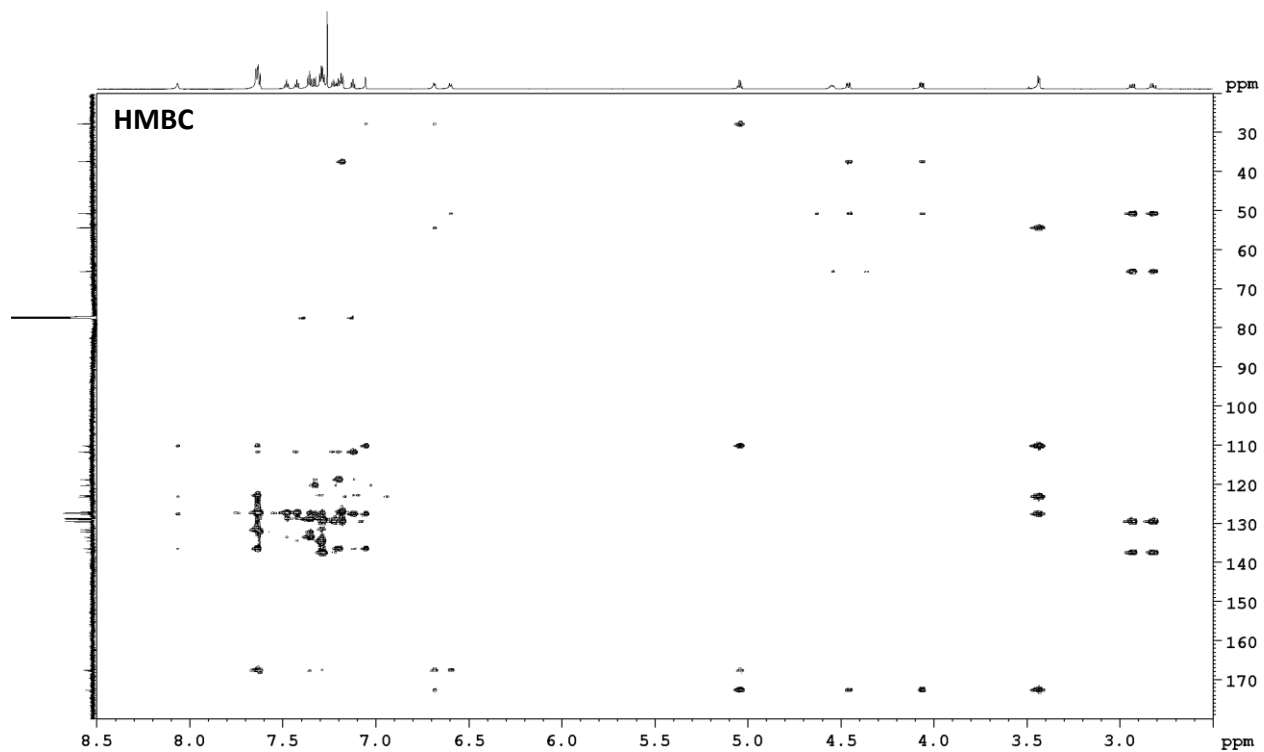
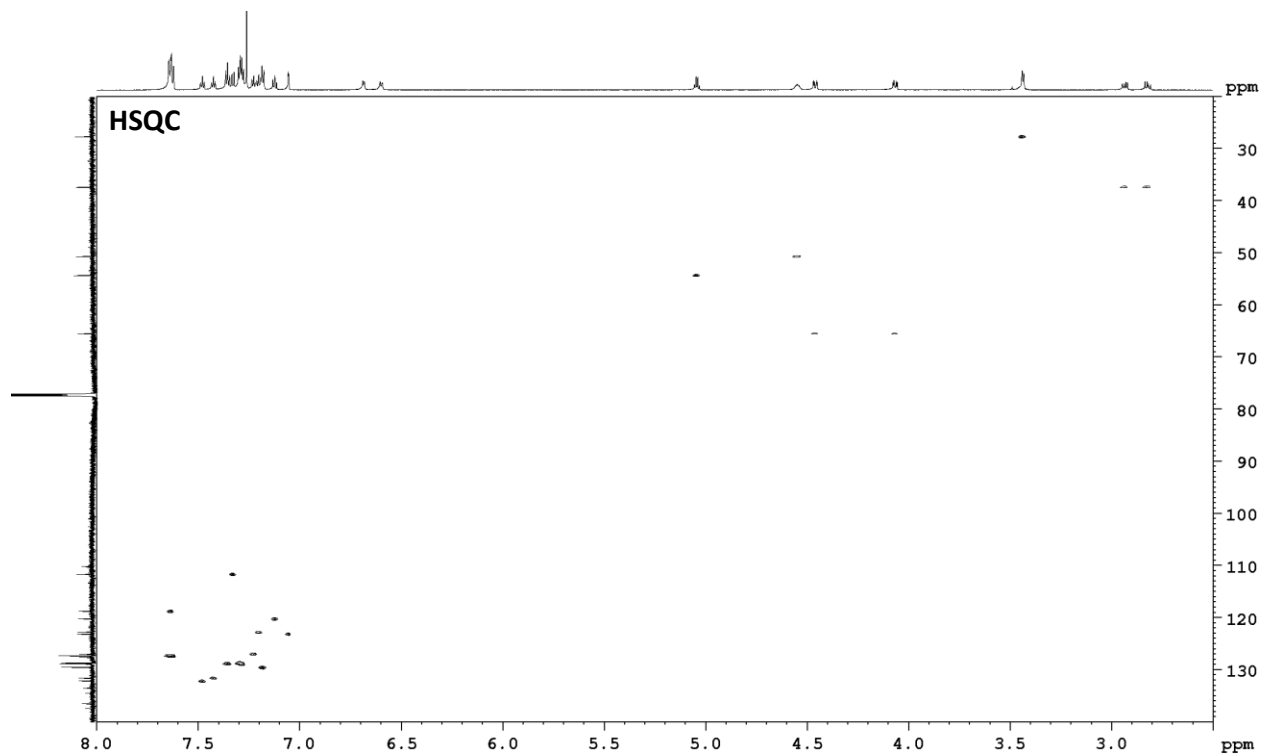


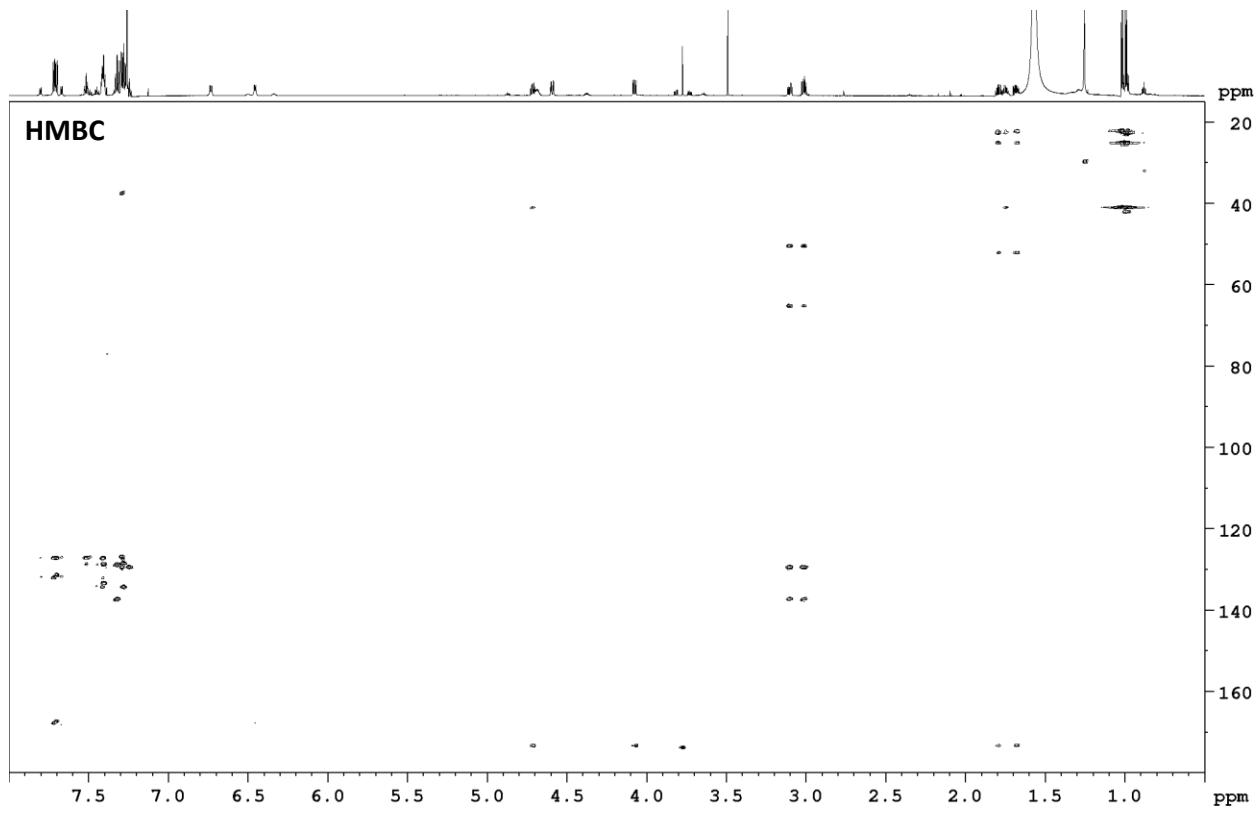
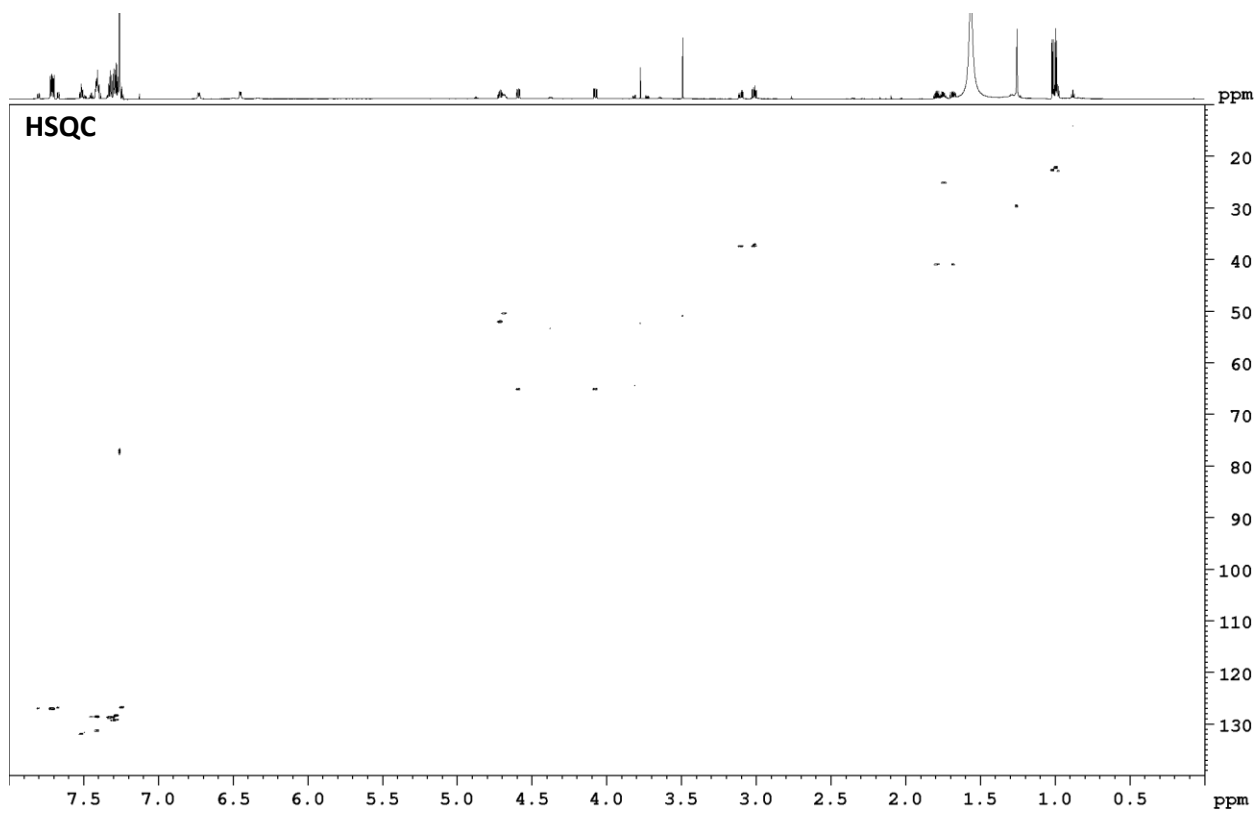


Supplementary Figure S3: NMR spectra for Asperphenamate W (12).

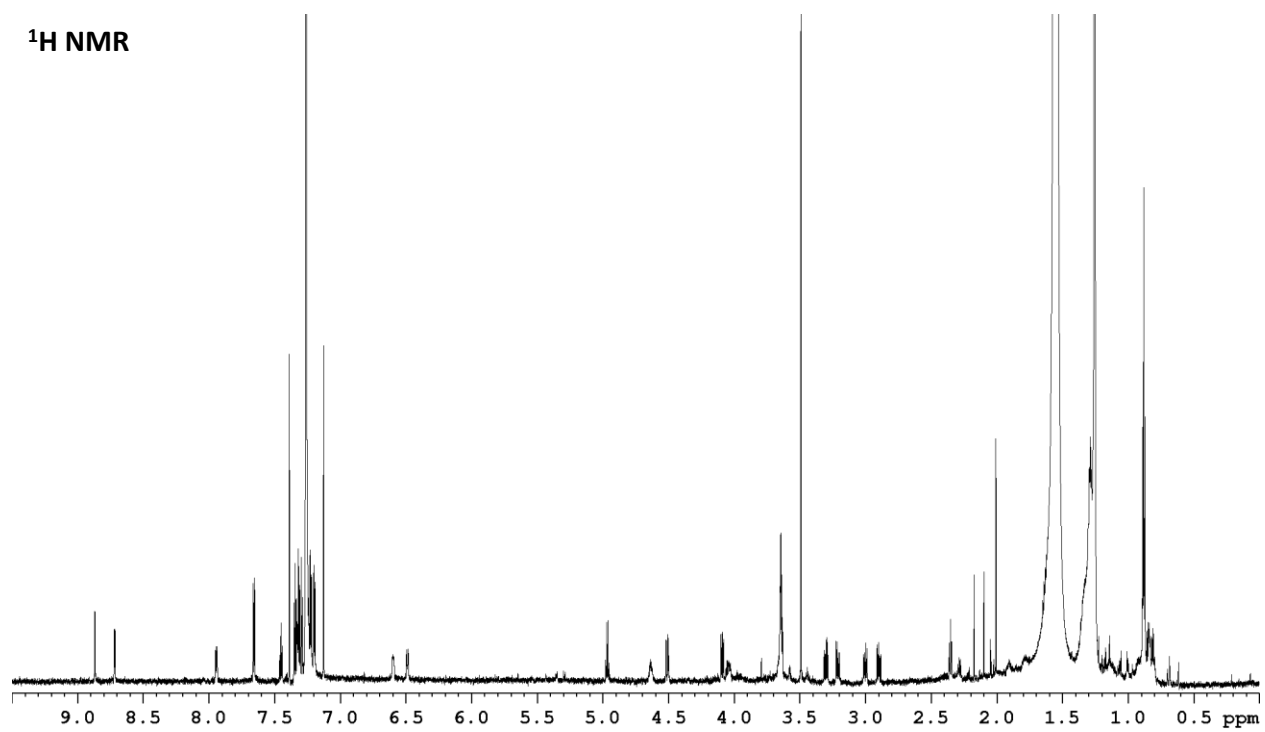




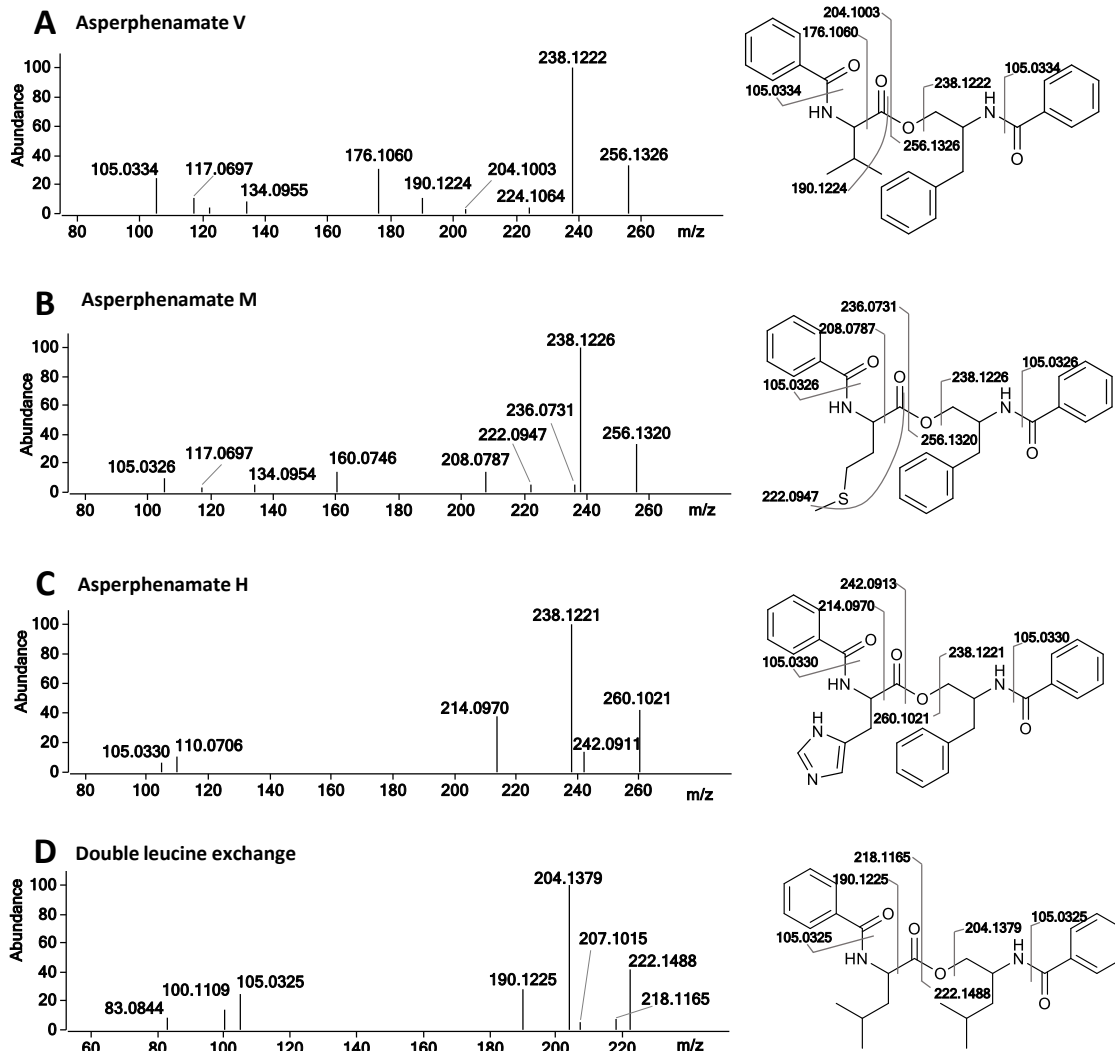




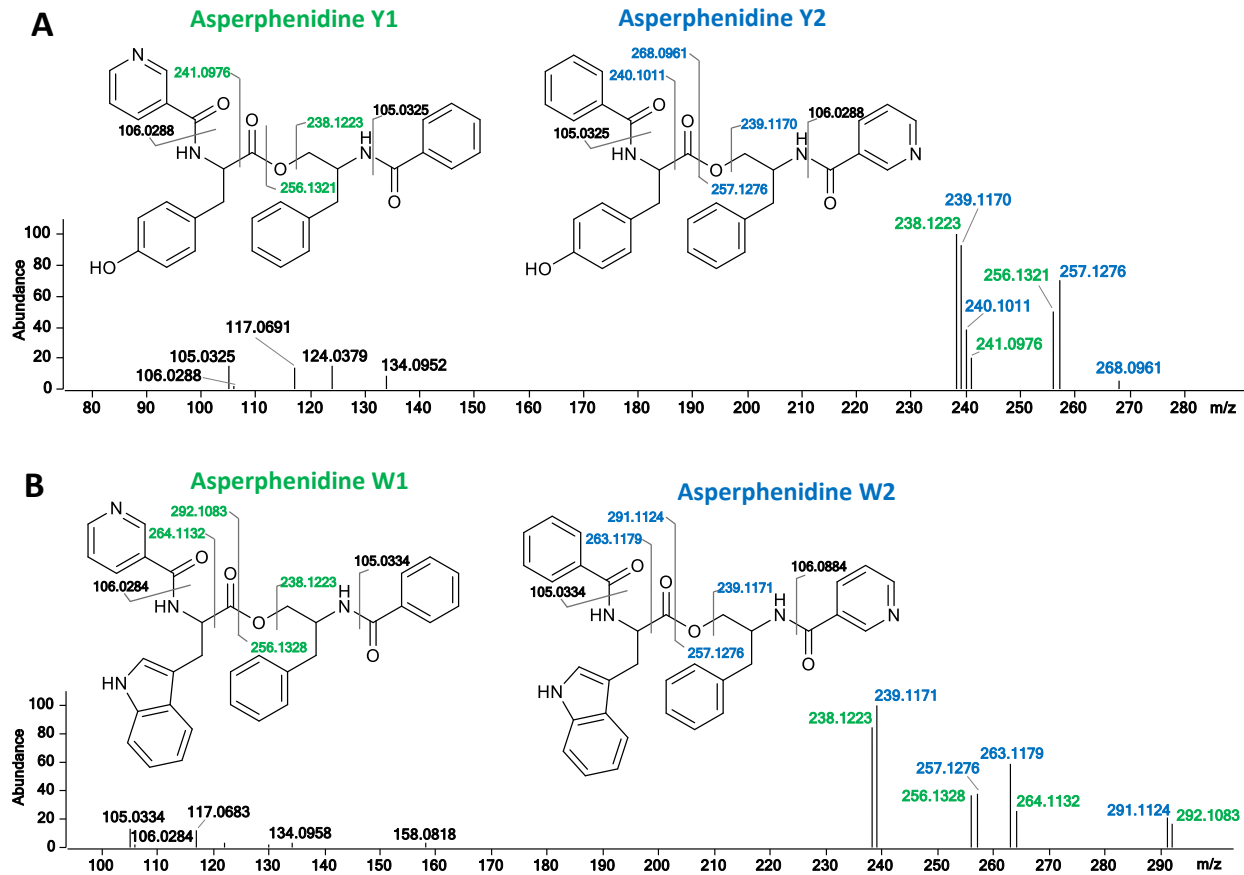
Supplementary Figure S5: ^1H NMR spectrum for Asperphenidine F1 (**3a**).



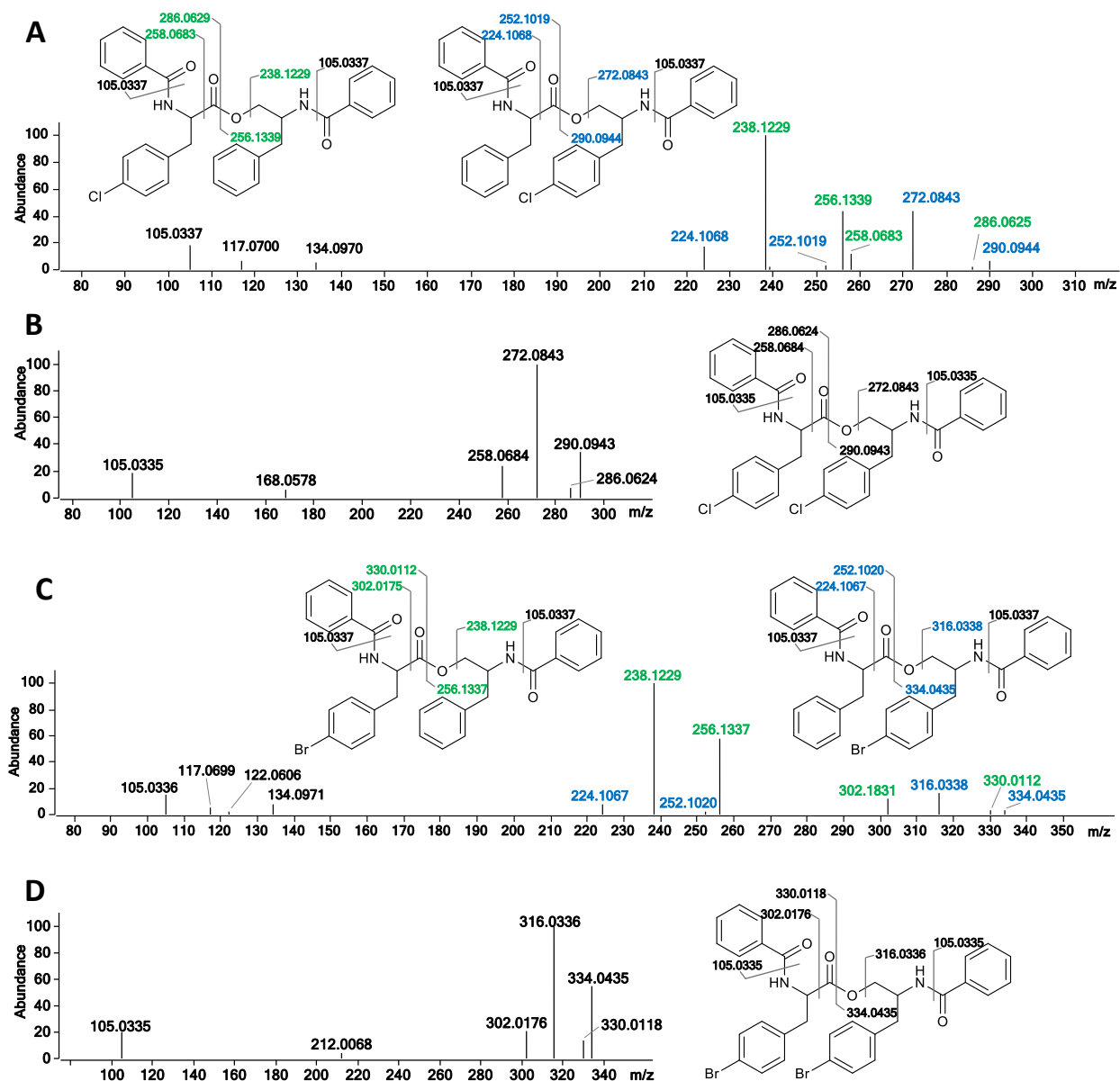
Supplementary Figure S6: MS/MS spectra and assignment of natural Asperphenamate analogues. (A) valine, (B) methionine, (C) histidine, and (D) double leucine exchange analogues.



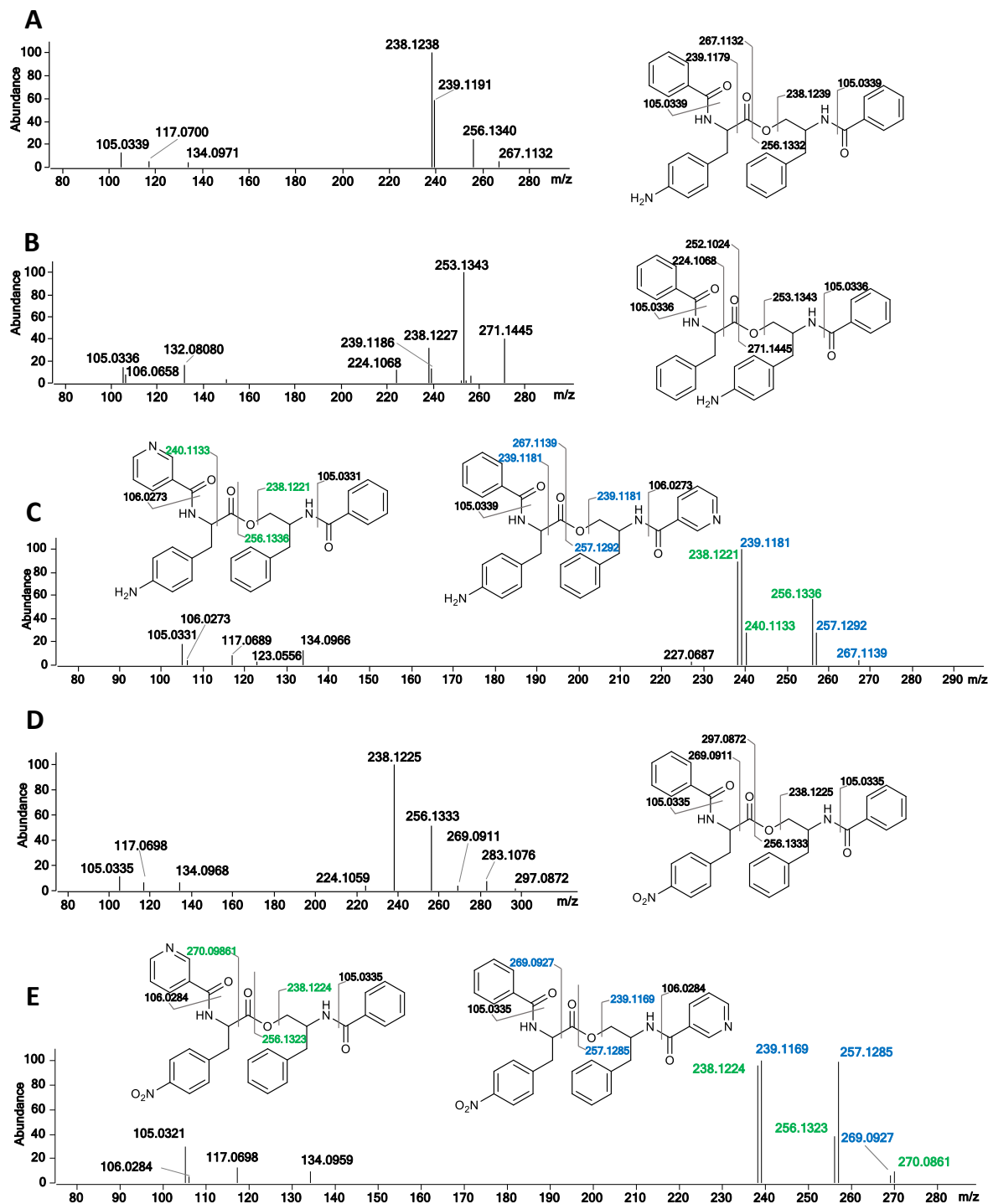
Supplementary Figure S7: MS/MS spectra and assignment of Asperphenidines Y1-Y2 (A) and W1-W2 (B). Fragment values highlighted in green correspond to nicotinic acid incorporation in the non-reduced amino acid moiety, whereas fragment values highlighted in blue correspond to nicotinic acid incorporation in the reduced amino acid moiety.



Supplementary Figure S8: MS/MS spectra and assignment of chloro- and bromo- phenylalanine asperphenamate derivatives, with single (A) and double (B) p-chloro-phenylalanine, and single (C) and double (D) p-bromo-phenylalanine incorporation. Fragment values highlighted in green correspond to substituted amino acid incorporation in the non-reduced amino acid moiety, whereas fragment values highlighted in blue correspond to substituted amino acid incorporation in the reduced amino acid moiety.



Supplementary Figure S9: MS/MS spectra and assignment of amino- and nitro- phenylalanine asperphenamate derivatives, with single substituted amino acid incorporation as well as corresponding asperphenidine analogues for p-amino-phenylalanine (AB/C) and p-nitro-phenylalanine (D/E). Fragment values highlighted in green correspond to nicotinic acid incorporation in the non-reduced amino acid moiety, whereas fragment values highlighted in blue correspond to nicotinic acid incorporation in the reduced amino acid moiety.



Supplementary Figure S10: BPCs of selected fungi from *Brevicompacta* section grown on YES media. Shaded peaks are EIC of $[M+H]^+$ adducts of most abundant asperphenamate analogues: m/z 508.2231 (red), m/z 523.2227 (green), m/z 546.2387 (yellow), m/z 473.2435 (blue) and m/z 507.2278 (grey). The numbers correspond to characterized asperphenamate analogues.

