Supporting Information

Orthogonal Method for Double Bond Placement via Ozone-Induced Dissociation Mass Spectrometry (OzID-MS)

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Structure Elucidation by Traditional NMR and Mass Spectrometry Techniques. For Compound 1

Ent-sartorypyrone E (**1**) was obtained as a white solid with a molecular formula of $C_{26}H_{40}O_5$ as determined via HRESIMS along with ^{1}H, ^{13}C, and edited-HSQC NMR data (Table 1), demonstrating an index of hydrogen deficiency of 7. Inspection of the MS and NMR data suggested **1** as an analogue of sartorypyrone A (**2**).[2] For example, **1** showed a trisubstituted unsaturated *δ*-lactone moiety, as noted by two conjugated double bonds (δ_C 100.6, 165.5, 100.5, and 160.3 for C-3, C-4, C-5, and C-6 respectfully) containing one olefinic proton (δ_H 5.75 for H-5), both of which were conjugated with an ester (δ_C 165.6 for C-2). Additional similarities included NMR signals characteristic of two sequential isoprene units, which were connected to the α-position (δ_C 100.6 for C-3) of the *δ*-lactone moiety. Key differences between compound **2**[2] and **1** were in the terminal part of the terpenoid side chain. Specifically, compound **1** lacked the terminal cyclohexane moiety along with the acetyl group seen in **2**, which were replaced by a dihydroxy unsaturated isoprene unit, as indicated by NMR data characteristic of two methyls (δ_H/δ_C 1.2/26.4; 1.2/23.2 for CH_3-22 and CH_3-23, respectively), two methylenes (δ_H/δ_C 2.22/2.06/26.8; 1.40/1.59/29.5 for CH_2-18 and CH_2-19, respectively), one oxymethine (δ_H/δ_C 3.36/78.3, for CH-20), and a quaternary oxygenated carbon (δ_C 73.1 for C-21). These data, along with further analysis of the 2D-NMR data, including COSY and HMBC experiments (Figure 2 and discussed in the manuscript under section "Structure Elucidation by Traditional NMR and Mass Spectrometry Techniques"), yielded the structure of compound **1**.

Table S1. NMR table of ent-sartorypyrone E (**1**)

Position	δ_H	δ_C	Mult (*J* in Hz)
2		165.6	
3		100.6	
4		165.5	
5	5.75	100.5	d (1.1)
6		160.3	
7	3.23	23.0	d (7.4)
8	5.31	120.6	m
9		140.9	
10	2.10	39.5	m
11	2.12	25.8	m
12	5.04	123.6	m
13		135.6	
14	1.99	39.4	m
15	2.08	26.1	m
16	5.15	125.1	m
17		134.7	
18	2.22	26.8	m
	2.06		m
19	1.40	29.5	dddd (14.0, 10.6, 8.4, 5.7)
	1.59		m
20	3.36	78.3	dd (10.6, 1.9)
21		73.1	
22	1.15	23.2	s
23	1.20	26.4	s
24	1.60	15.8	s
25	1.58	16.0	s
26	1.77	16.3	s
27	2.18	19.7	s

Table S2. Table of accurate masses for the OzID products of compound **1**

OzID-MS Products	Molecular Formula	Measured Value	Calculated Value	Accuracy (in ppm)
Parent Mass	$C_{26}H_{40}NaO_5^+$	455.2772	455.2773	0.2
B	$C_{18}H_{32}NaO_3^+$	319.2248	319.2249	0.3
a	$C_8H_8NaO_5^+$	207.0287	207.0269	8.7
b	$C_{18}H_{32}NaO_4^+$	335.2202	335.2198	1.2
A	$C_8H_8NaO_4^+$	191.0332	191.0320	6.3
B	$C_{13}H_{24}NaO_3^+$	251.1634	251.1623	4.4
a	$C_{13}H_{16}NaO_5^+$	275.0902	275.0895	2.5
b	$C_{13}H_{24}NaO_4^+$	267.1579	267.1573	2.2
A	$C_{13}H_{16}NaO_4^+$	259.0954	259.0946	3.1
B	$C_8H_{16}NaO_3^+$	183.0992	183.0997	2.7
a	$C_{18}H_{24}NaO_5^+$	343.1526	343.1522	1.2
b	$C_8H_{16}NaO_4^+$	199.0954	199.0946	4.0
A	$C_{18}H_{24}NaO_4^+$	327.1578	327.1573	1.5

Table S3. Table of accurate masses for the OzID products of compound **1** in an extract

OzID-MS Products	Molecular Formula	Measured Value	Calculated Value	Accuracy (in ppm)
Parent Mass	$C_{26}H_{40}NaO_5^+$	455.2780	455.2773	1.5
B	$C_{18}H_{32}NaO_3^+$	319.2239	319.2249	3.1
a	$C_8H_8NaO_5^+$	-	207.0269	-
b	$C_{18}H_{32}NaO_4^+$	335.2192	335.2198	1.8
A	$C_8H_8NaO_4^+$	191.0329	191.0320	4.7
B	$C_{13}H_{24}NaO_3^+$	251.1616	251.1623	2.8
a	$C_{13}H_{16}NaO_5^+$	275.0886	275.0895	3.3
b	$C_{13}H_{24}NaO_4^+$	267.1573	267.1573	0.0
A	$C_{13}H_{16}NaO_4^+$	259.0950	259.0946	1.5
B	$C_8H_{16}NaO_3^+$	183.0983	183.0997	7.6
a	$C_{18}H_{24}NaO_5^+$	343.1520	343.1522	0.6
b	$C_8H_{16}NaO_4^+$	199.0937	199.0946	4.5
A	$C_{18}H_{24}NaO_4^+$	327.1567	327.1573	1.8

Table S4. Table of accurate masses for the OzID products of compound **1** *in situ*

OzID-MS Products	Molecular Formula	Measured Value	Calculated Value	Accuracy (in ppm)
Parent Mass	$C_{26}H_{40}NaO_5^+$	455.2783	455.2773	2.2
B	$C_{18}H_{32}NaO_3^+$	319.2241	319.2249	2.5
a	$C_8H_8NaO_5^+$	-	207.0269	-
b	$C_{18}H_{32}NaO_4^+$	335.2200	335.2198	0.6
A	$C_8H_8NaO_4^+$	191.0332	191.0320	6.3
B	$C_{13}H_{24}NaO_3^+$	251.1619	251.1623	1.6
a	$C_{13}H_{16}NaO_5^+$	275.0885	275.0895	3.6
b	$C_{13}H_{24}NaO_4^+$	267.1567	267.1573	2.2
A	$C_{13}H_{16}NaO_4^+$	259.0952	259.0946	2.3
B	$C_8H_{16}NaO_3^+$	183.1024	183.0997	14.7*
a	$C_{18}H_{24}NaO_5^+$	343.1515	343.1522	2.0
b	$C_8H_{16}NaO_4^+$	199.0919	199.0946	13.6*
A	$C_{18}H_{24}NaO_4^+$	327.1583	327.1573	3.1

* The measured value was close to the noise level which caused the ppm shift to be out of the 10 ppm range.

Table S5. Table of accurate masses for the OzID products of compound **2**

OzID-MS Products	Molecular Formula	Measured Value	Calculated Value	Accuracy (in ppm)
Parent Mass	$C_{28}H_{40}O_5Na^+$	479.2784	479.2773	2.3
B	$C_{20}H_{32}O_3Na^+$	343.1948	343.2249	87.7
a	$C_8H_8O_5Na^+$	207.0283	207.0269	6.8
b	$C_{20}H_{32}O_4Na^+$	359.2246	359.2198	13.3
A	$C_8H_8O_4Na^+$	191.0335	191.0320	7.9
a	$C_{13}H_{16}O_5Na^+$	275.0936	275.0895	14.9
A	$C_{13}H_{16}O_4Na^+$	259.0984	259.0949	13.5

Table S6. Table of accurate masses for the OzID products of compound **3**

OzID-MS Products	Molecular Formula	Measured Value	Calculated Value	Accuracy (in ppm)
Parent Mass	$C_{14}H_{16}O_3Na^+$	255.1003	255.0997	2.4
A	$C_{10}H_{10}O_4Na^+$	217.0475	217.0477	0.9
A	$C_{12}H_{12}O_4Na^+$	243.0625	243.0634	3.7
●	$C_{14}H_{16}O_4Na^+$	271.0966	271.0946	7.4

Table S7. Table of accurate masses for the OzID products of compound **4**

OzID-MS Products	Molecular Formula	Measured Value	Calculated Value	Accuracy (in ppm)
Parent Mass	$C_{19}H_{28}O_4Na^+$	343.1891	343.1886	1.5
A	$C_{14}H_{22}O_3Na^+$	261.1485	261.1467	6.9
A	$C_{16}H_{24}O_3Na^+$	287.1632	287.1624	2.8
●	$C_{19}H_{28}O_5Na^+$	359.1840	359.1835	1.4
a	$C_{16}H_{24}O_4Na^+$	303.1581	303.1567	4.6

Table S8. Table of accurate masses for the OzID products of compound **5**

OzID-MS Products	Molecular Formula	Measured Value	Calculated Value	Accuracy (in ppm)
Parent Mass	$C_{26}H_{38}O_5Na^+$	453.2609	453.2617	1.8
a	$C_{20}H_{28}O_6Na^+$	387.1808	387.1784	6.2
A	$C_{20}H_{28}O_5Na^+$	371.1844	371.1835	2.4
a	$C_{22}H_{30}O_6Na^+$	413.2004	413.1940	15.5
A	$C_{22}H_{30}O_5Na^+$	397.1985	397.1991	1.5
●	$C_{26}H_{38}O_6Na^+$	469.2548	469.2566	3.8

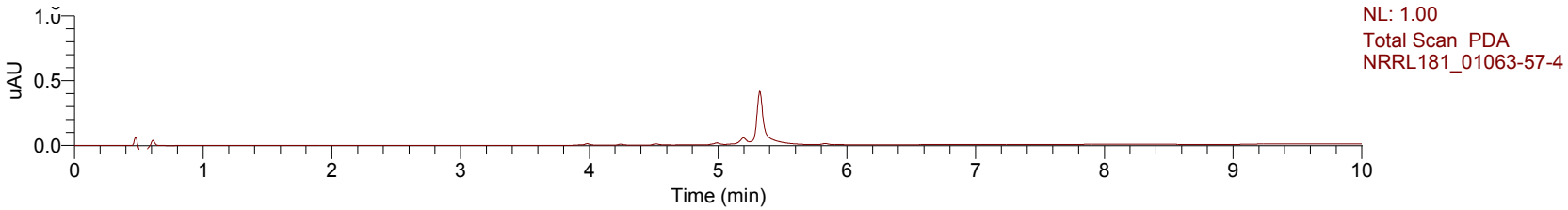


Figure S1. UPLC-PDA detector chromatogram of ent-sartorypyrone E (**1**)

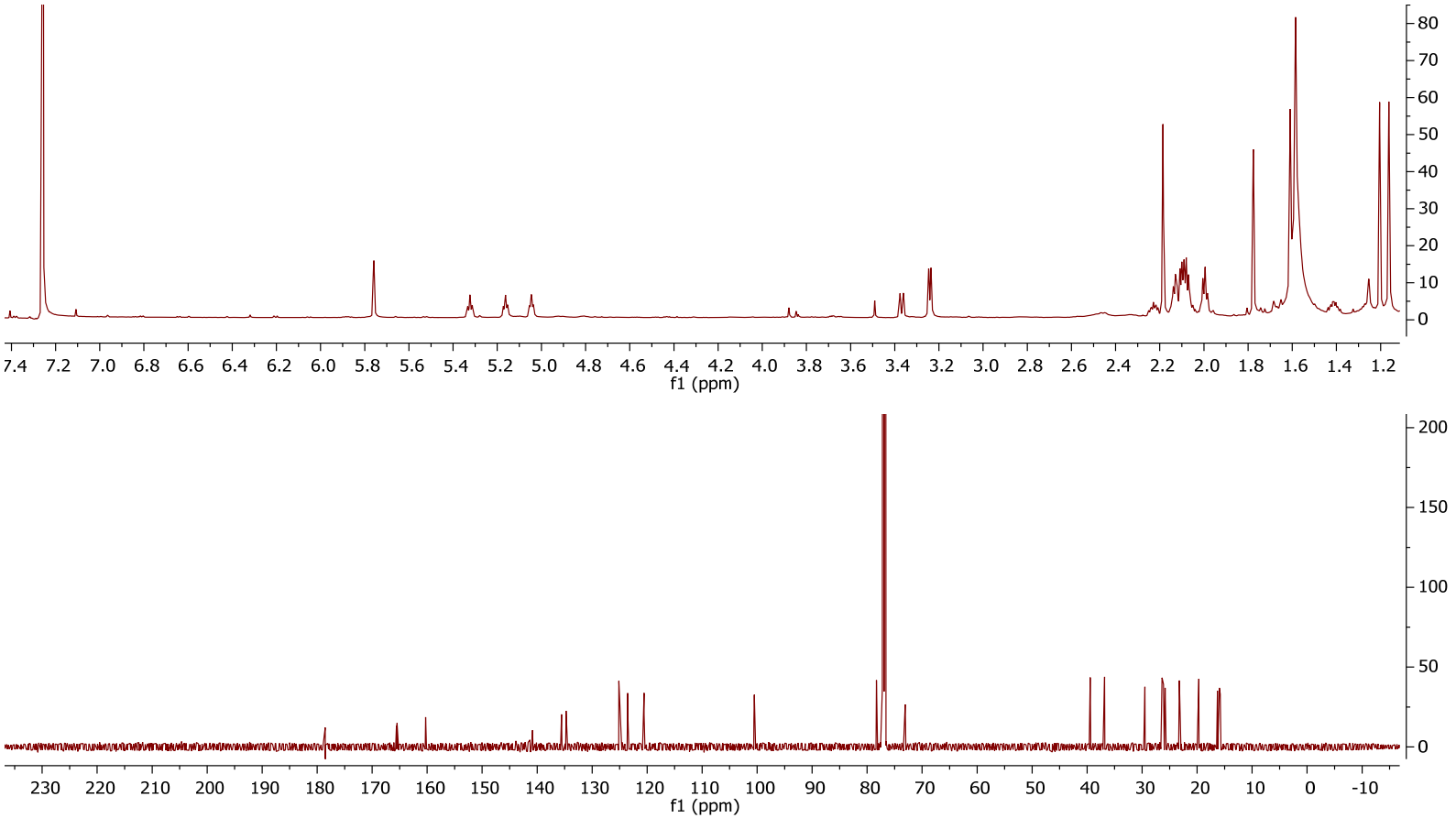


Figure S2. ^{1}H NMR spectrum (700 MHz, Top) and ^{13}C NMR spectrum (175 MHz, Bottom) both in $CDCl_3$, of ent-sartorypyrone E (**1**)

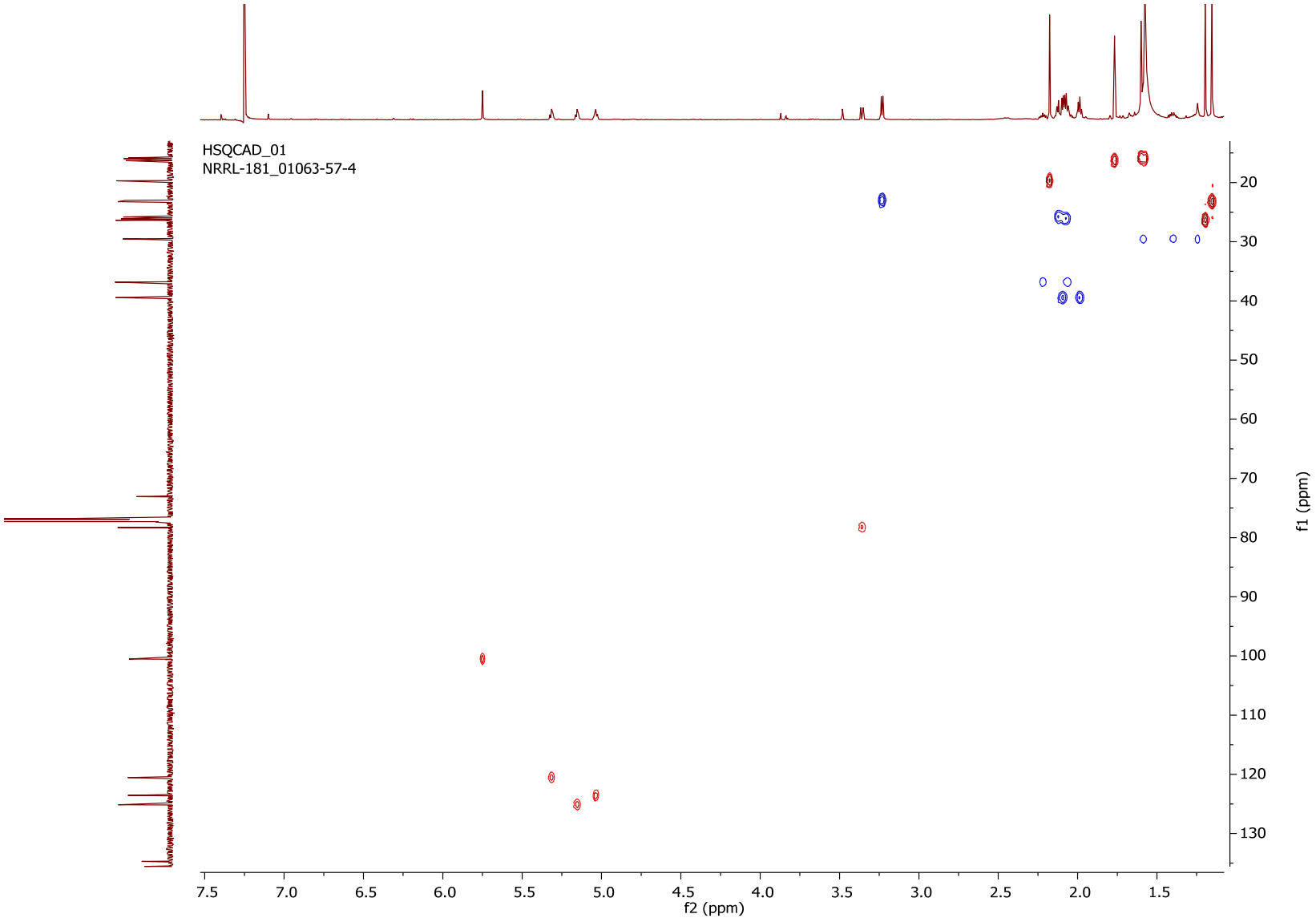


Figure S3. HSQC spectrum of ent-sartorypyrone E (**1**), $CDCl_3$, 700 MHz

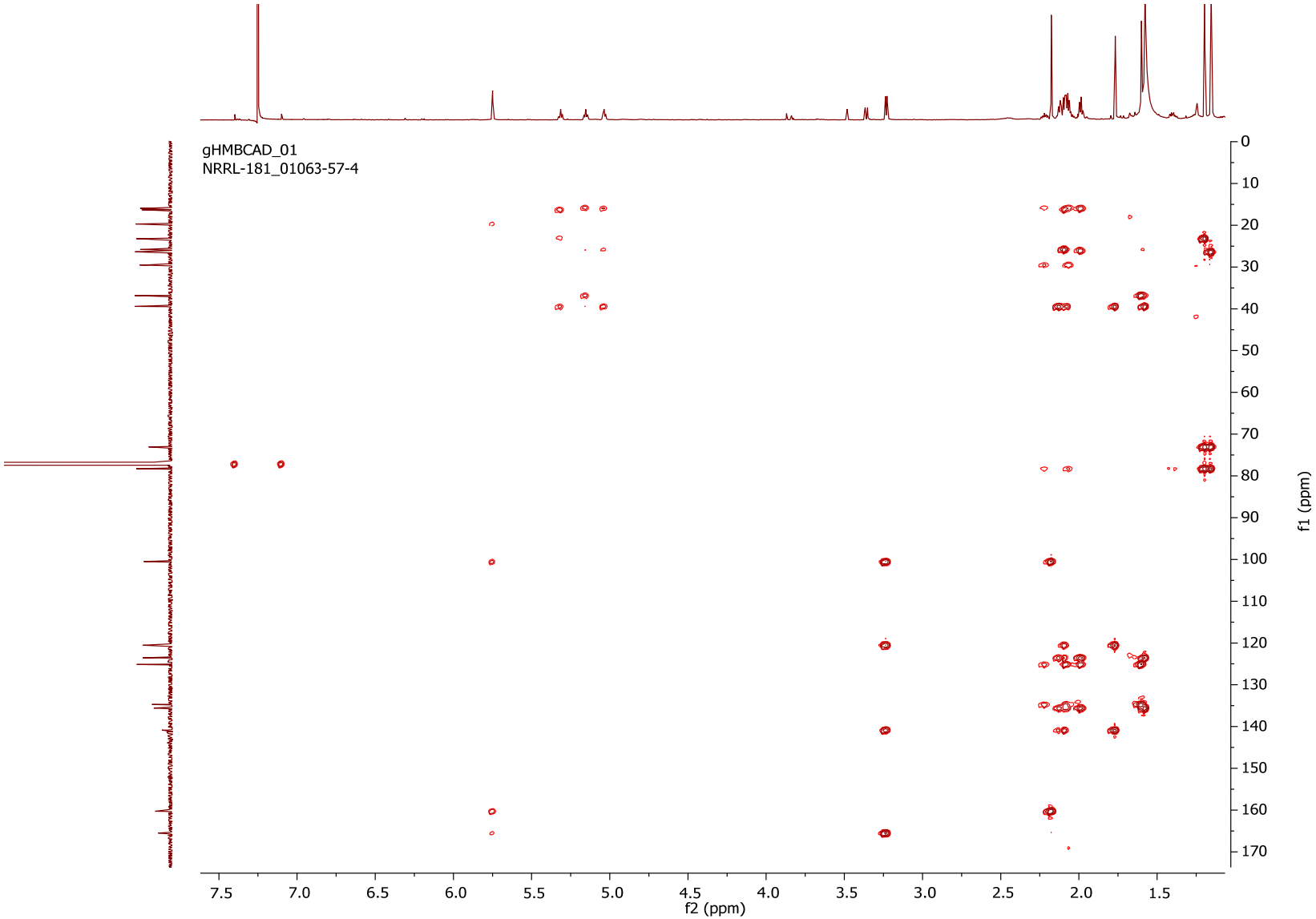


Figure S4. HMBC spectrum of ent-sartorypyrone E (**1**), $CDCl_3$, 700 MHz

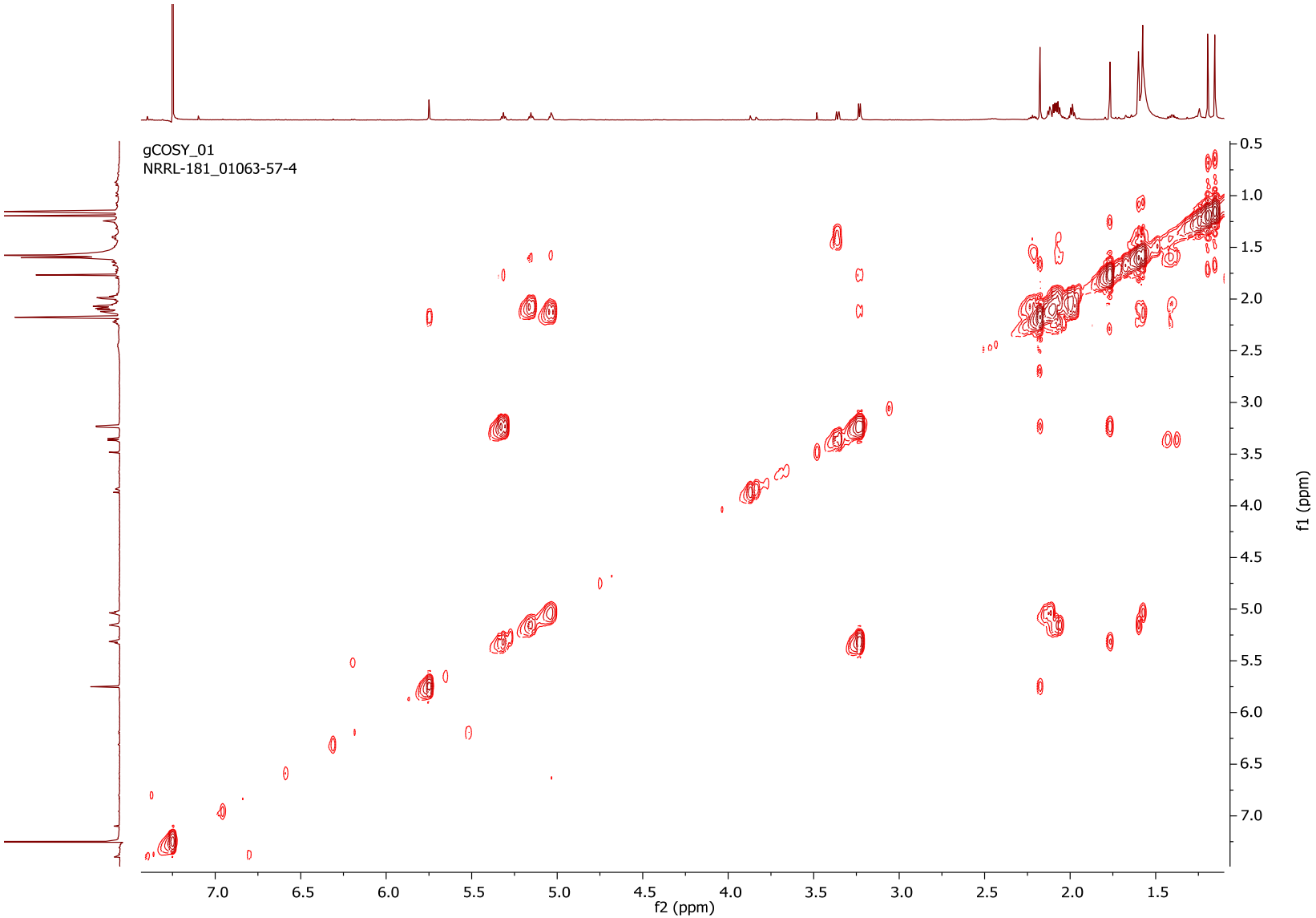


Figure S5. ^{1}H COSY spectrum of ent-sartorypyrone E (**1**), $CDCl_3$, 700 MHz

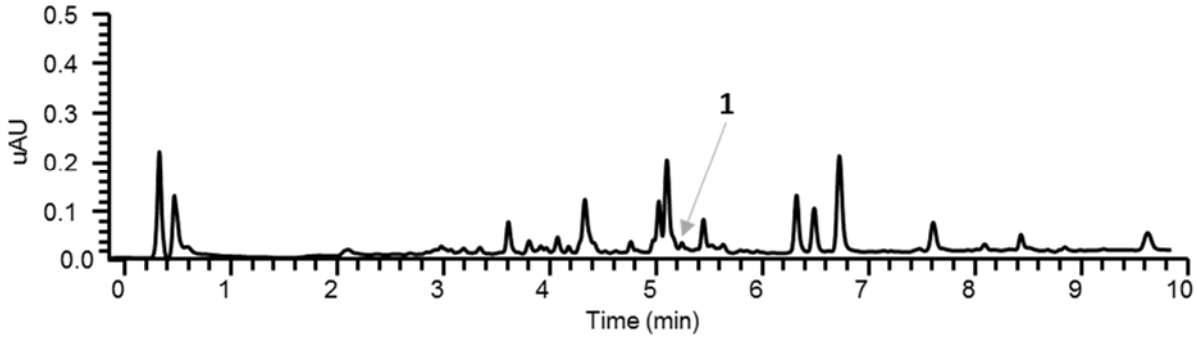


Figure S6. UPLC-PDA detector chromatogram of an extract of *A. fischeri* grown on solid oatmeal media

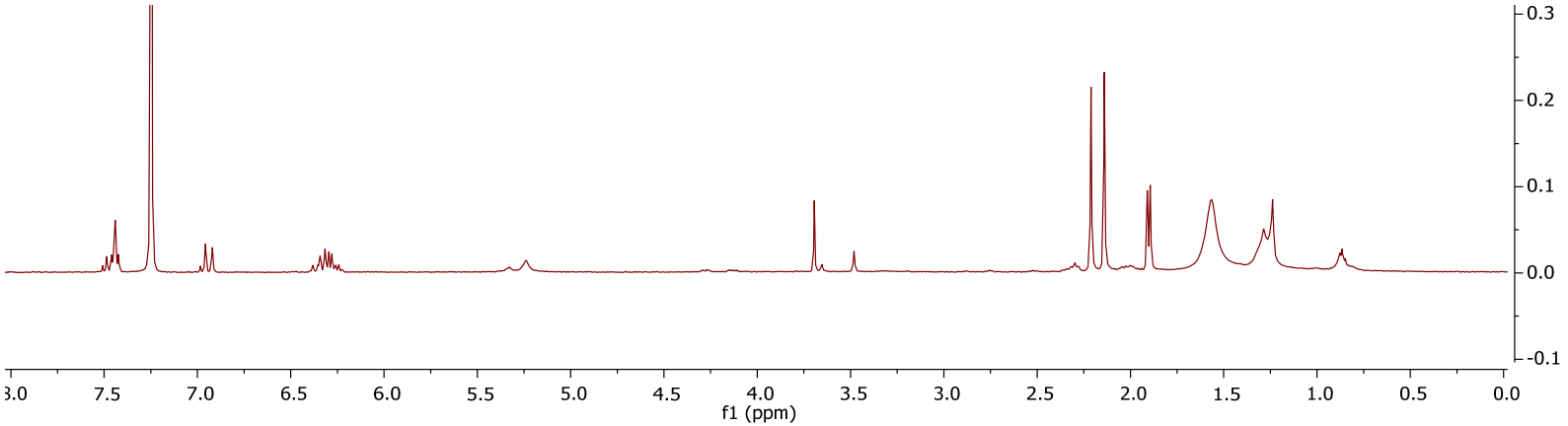


Figure S7. ^{1}H NMR spectrum (400 MHz) in $CDCl_3$, of sorbicillin (**3**)

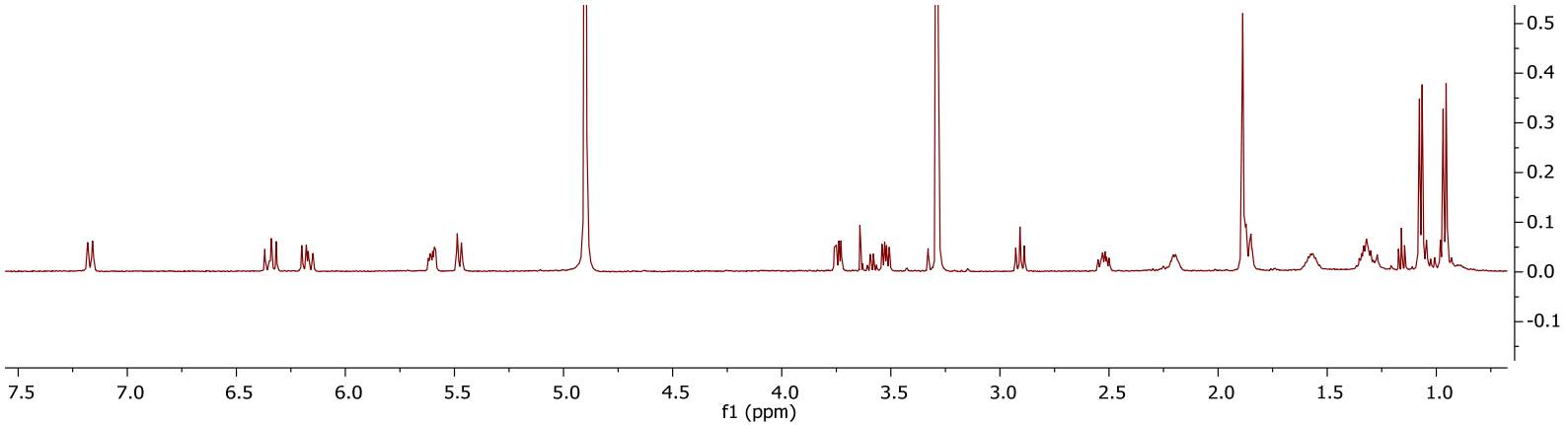


Figure S8. ^{1}H NMR spectrum (500 MHz) in MeOD, of trichodermic acid A (**4**)

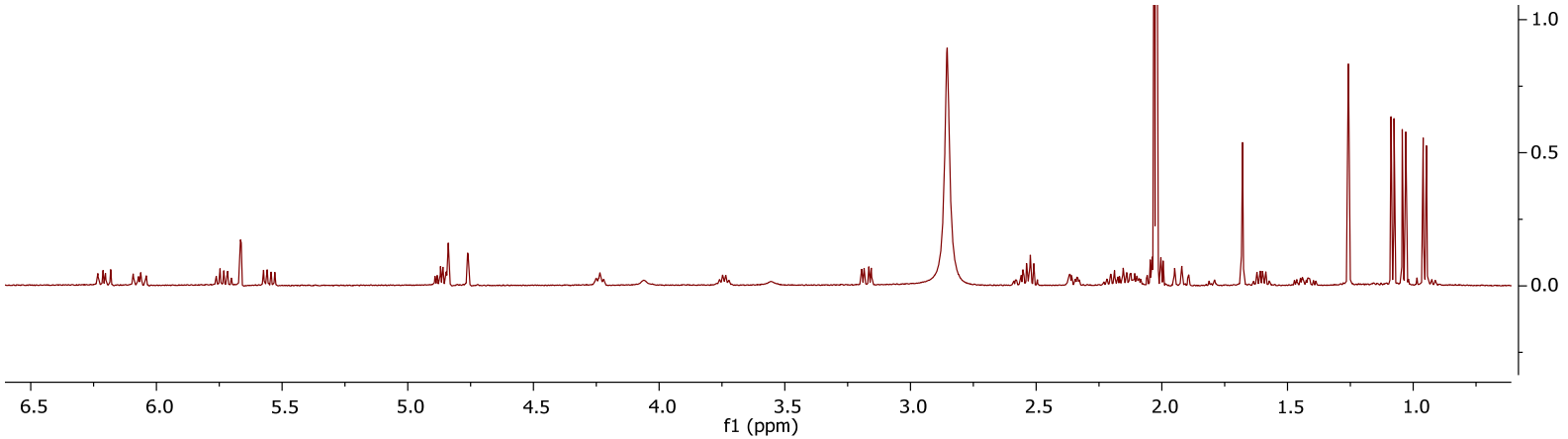
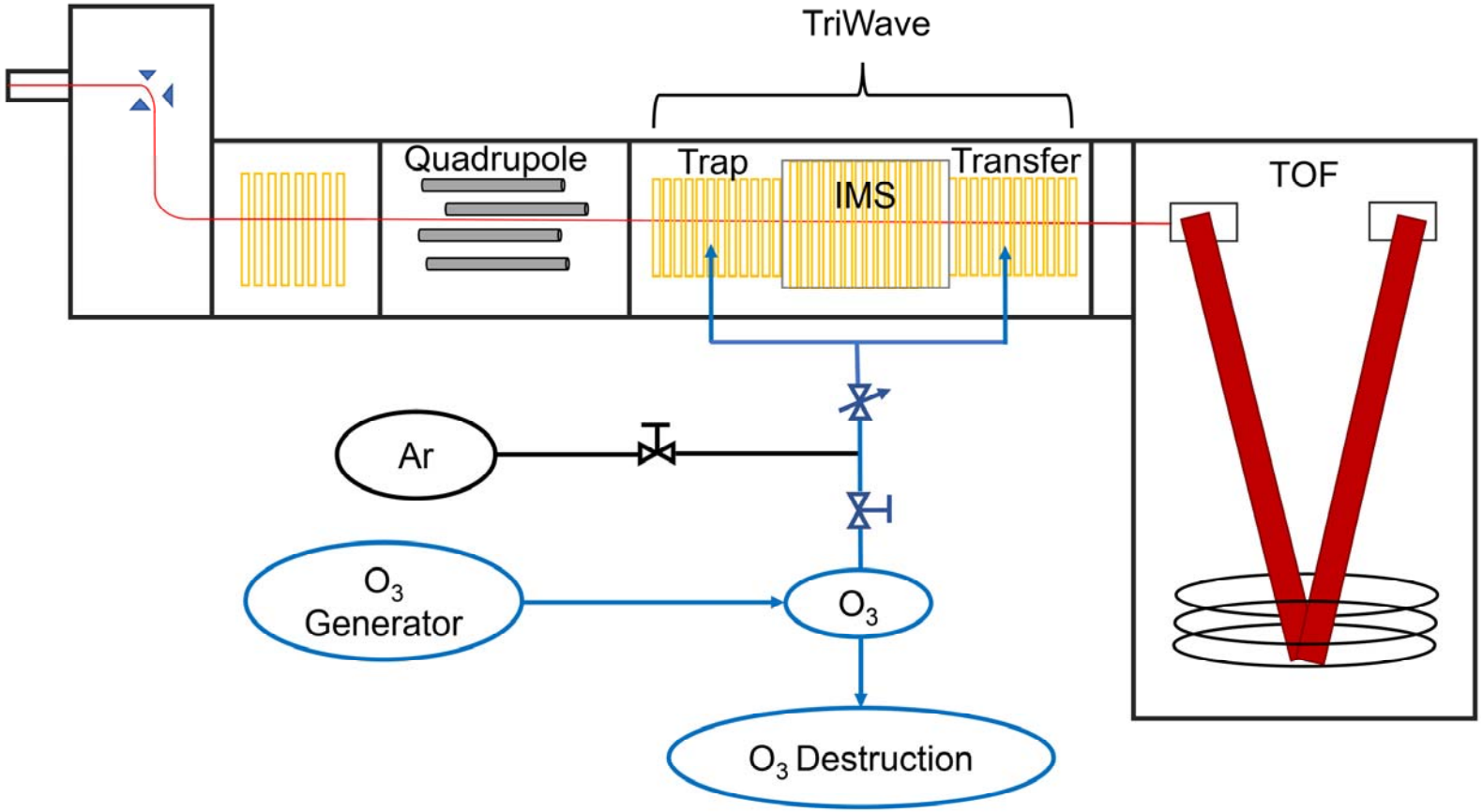


Figure S9. ^{1}H NMR spectrum (500 MHz) in $(CD_3)_2CO$, of AA03390 (**5**)

Figure S10. Schematic of the Waters Synapt G2 HDMS modified to allow ozone in the trap and transfer cells to perform OzID-MS. Adapted from Vu et al.[1]



References

(1) Vu, N.; Brown, J.; Giles, K.; Zhang, Q., *Rapid Commun. Mass Spectrom.* **2017,** *31*, 1415-1423.
(2) Eamvijarn, A.; Gomes, N. M.; Dethoup, T.; Buaruang, J.; Manoch, L.; Silva, A.; Pedro, M.; Marini, I.; Roussis, V.; Kijjoa, A., *Tetrahedron* **2013,** *69*, 8583-8591.