Supporting Information

Selective Peptidomimetic Inhibitors of NTMT1/2: Rational design, synthesis, characterization, and crystallographic studies

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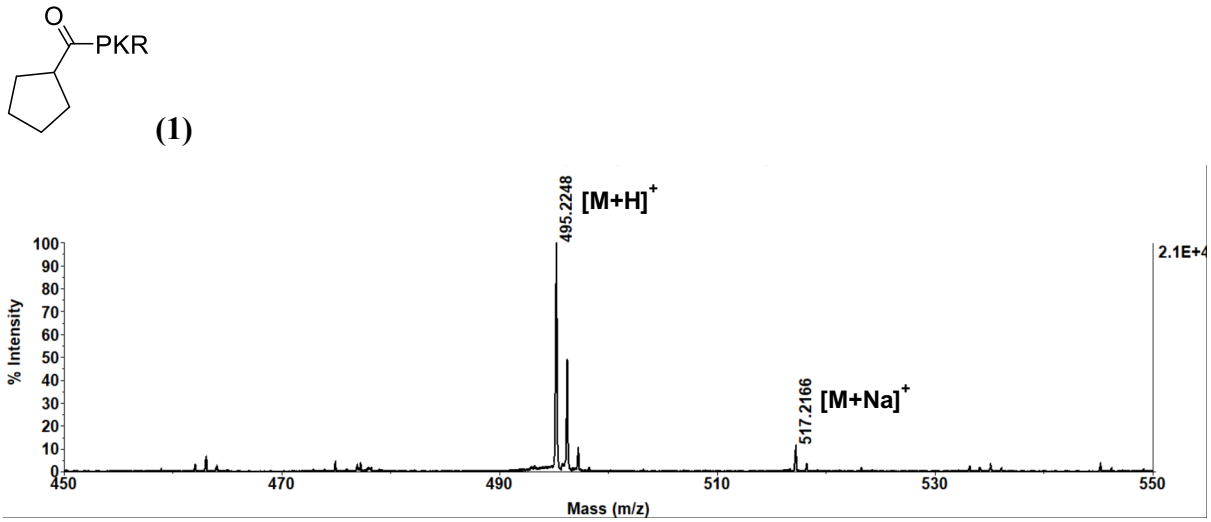
Figure S1. MALDI-MS and HPLC analysis of compounds **1-24**, **DC431** and **DC432**



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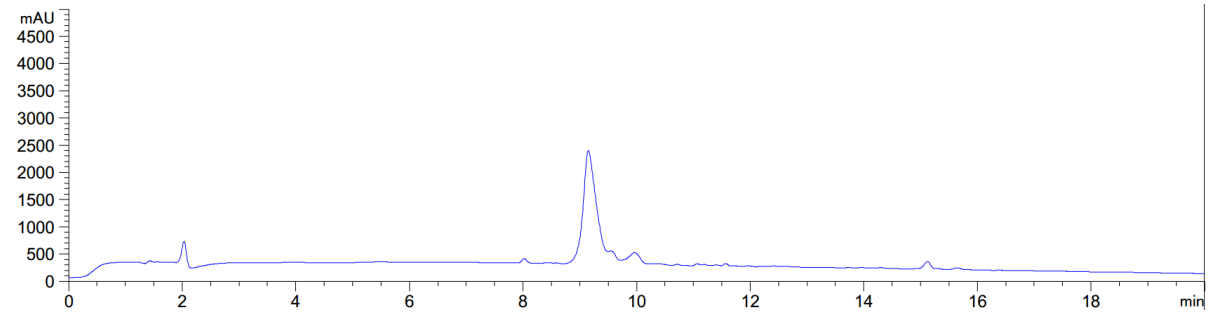

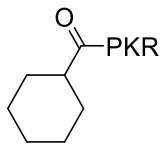

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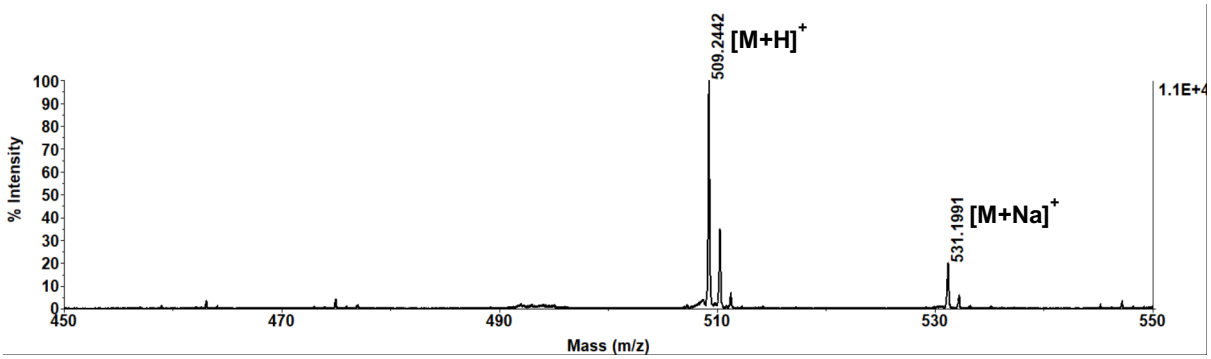

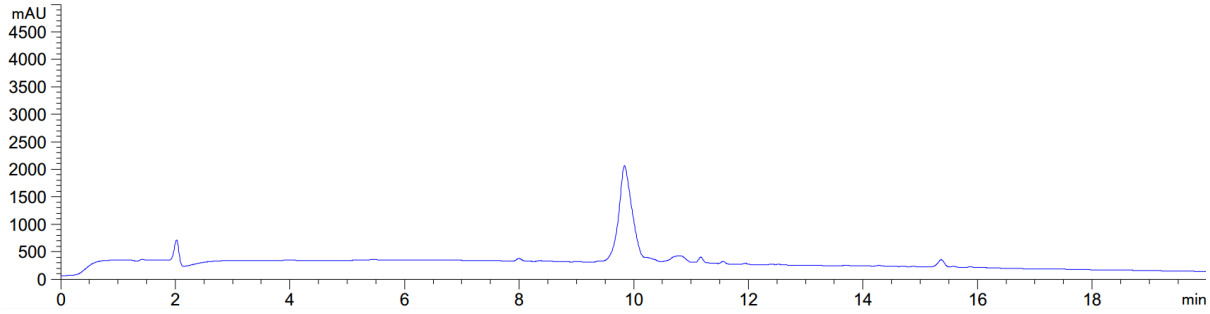

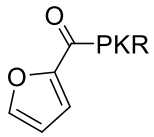
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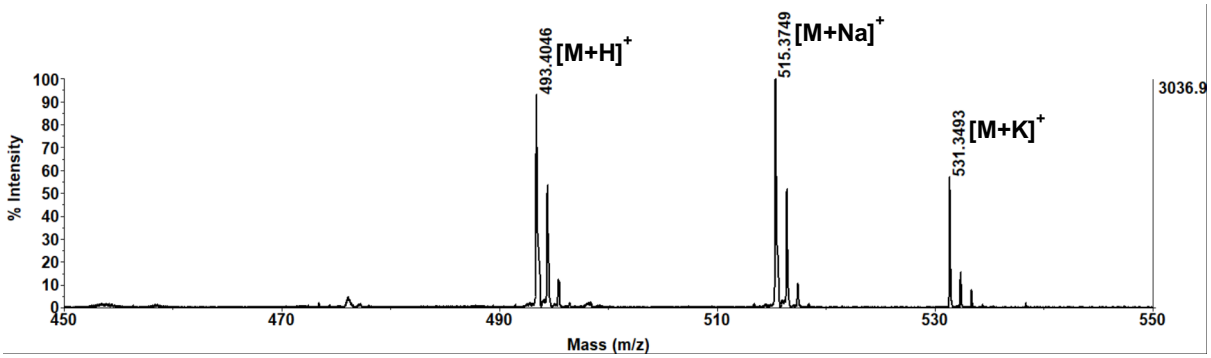
PKR
O

(3)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[M+Na]+
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[M+K]+
3036.9
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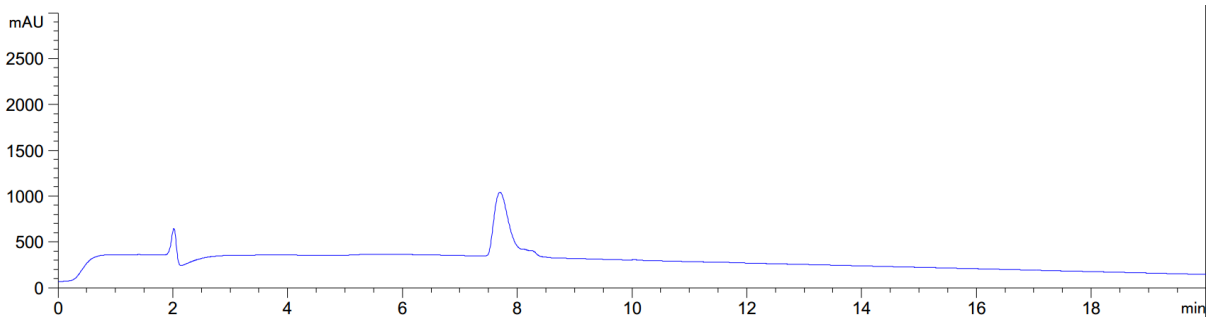
Mass (m/z)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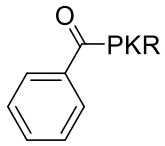
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PKR

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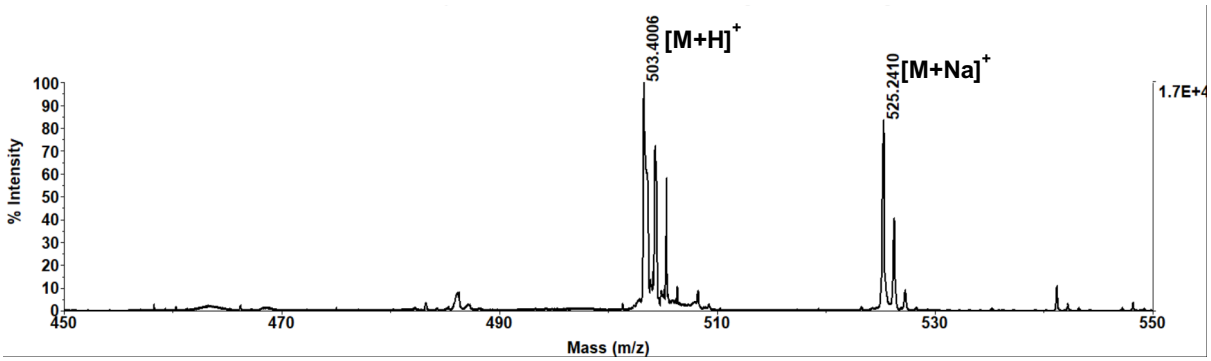
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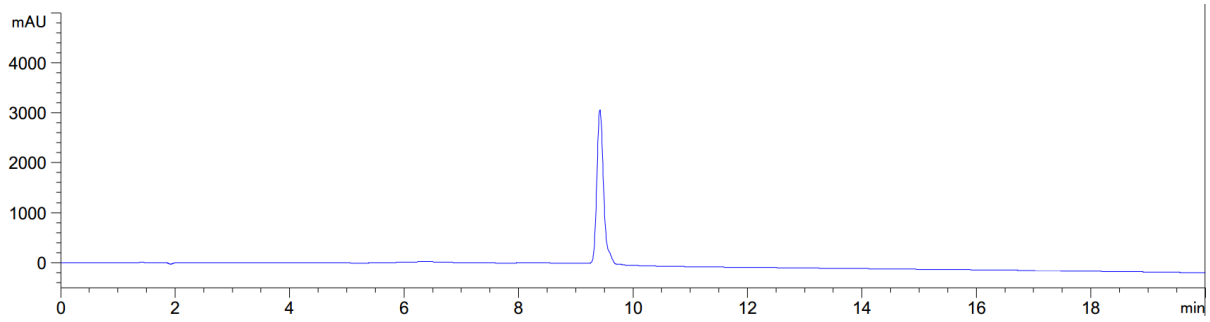
525.2410
[M+Na]+
1.7E+4
% Intensity
Mass (m/z)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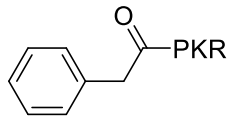
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PKR

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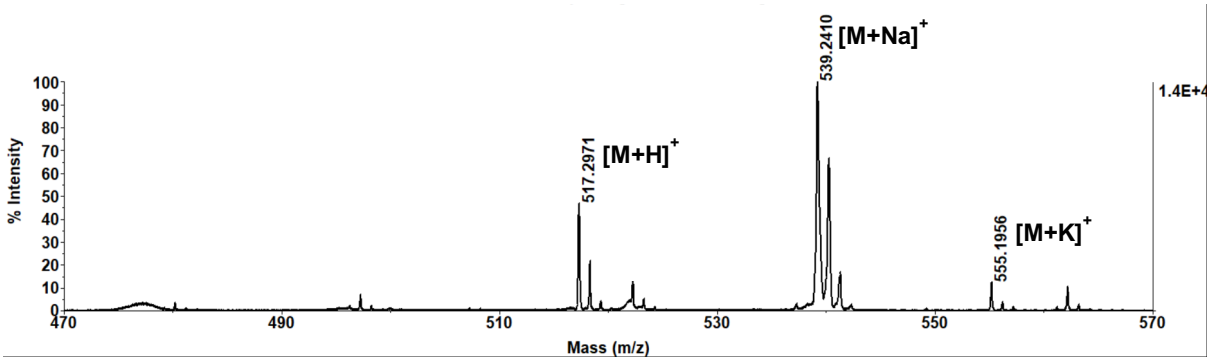
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Mass (m/z)
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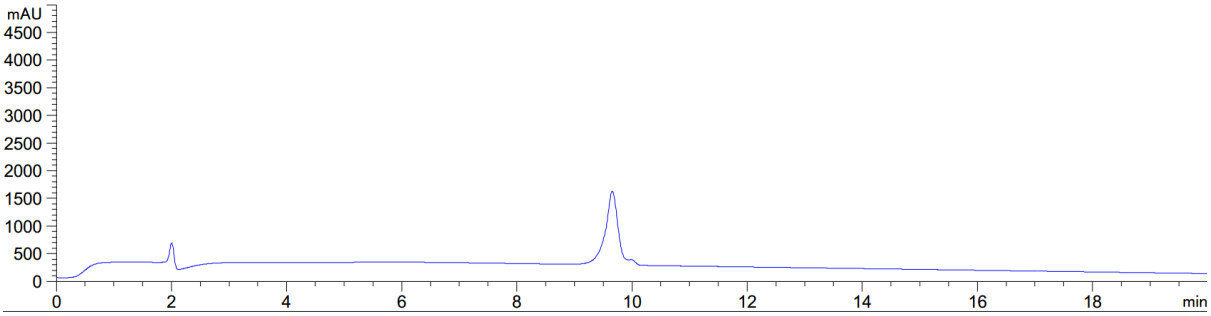
539.2410 [M+Na]+
555.1956 [M+K]+
1.4E+4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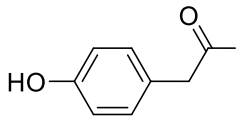
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PKR
HO

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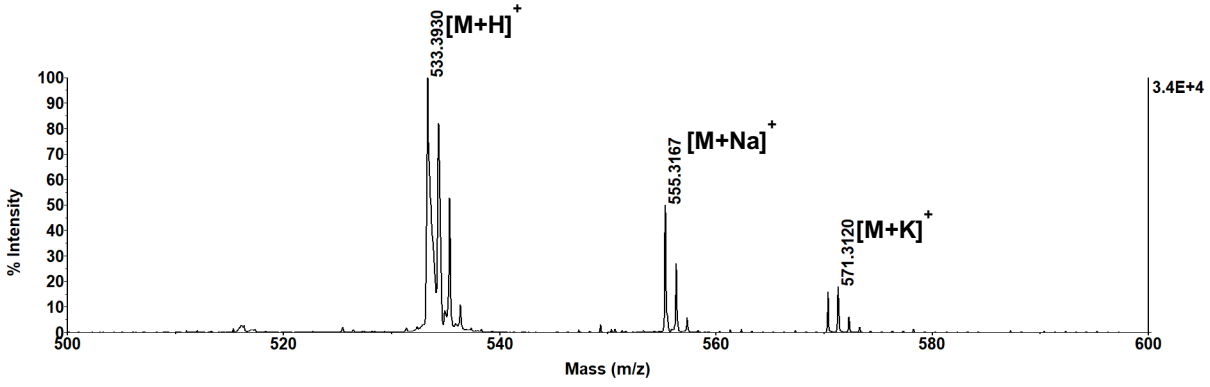
% Intensity
Mass (m/z)
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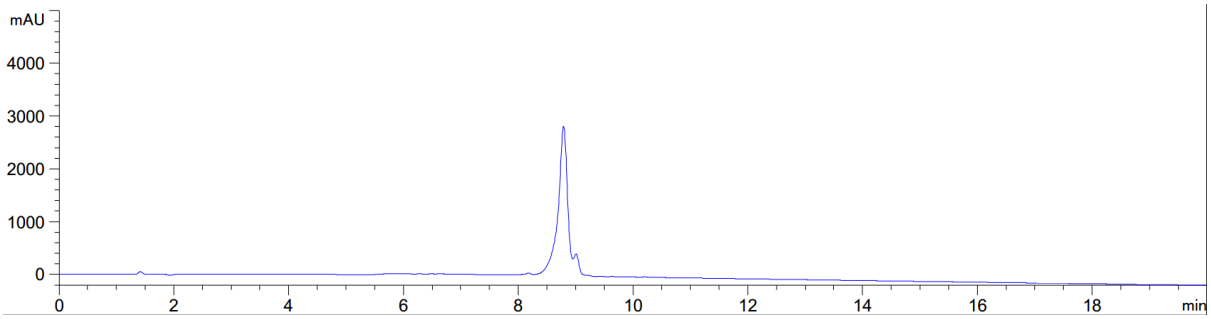
555.3167 [M+Na]+
571.3120 [M+K]+
3.4E+4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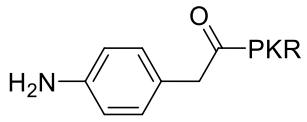
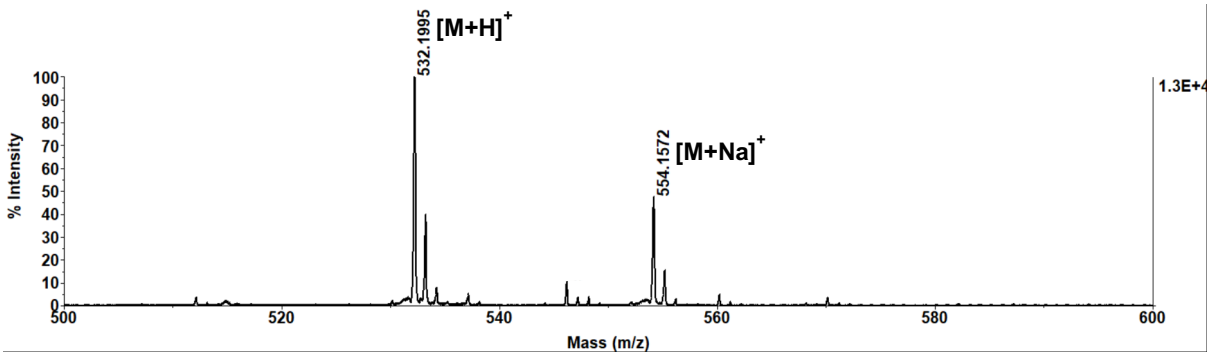

O
PKR
H_2N

(7)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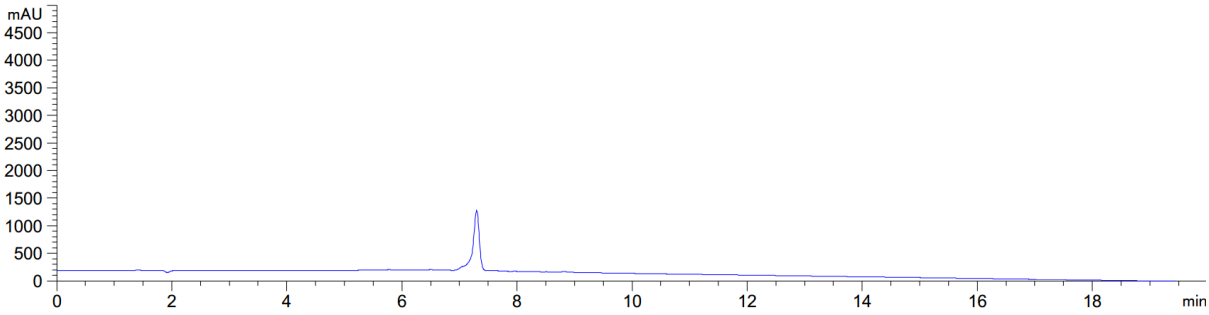
[M+H]+
554.1572
[M+Na]+
1.3E+4
% Intensity
Mass (m/z)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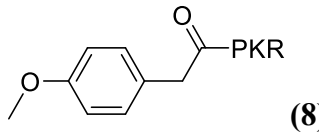
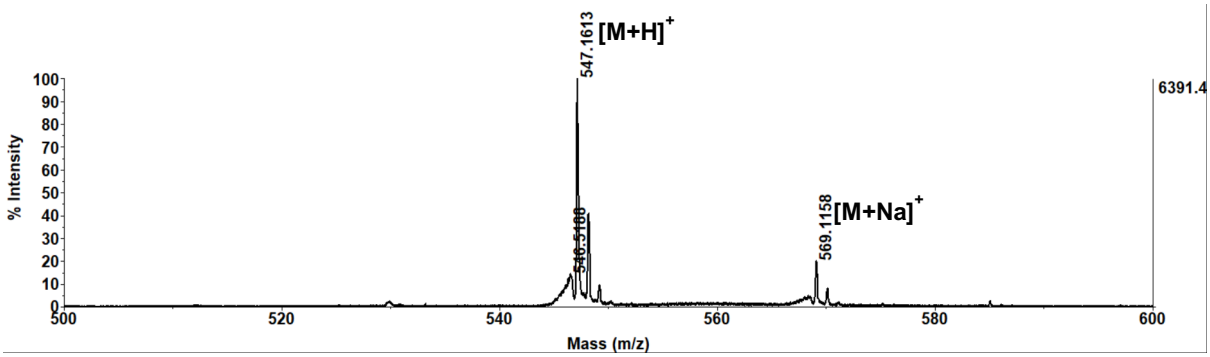

O
PKR
O

(8)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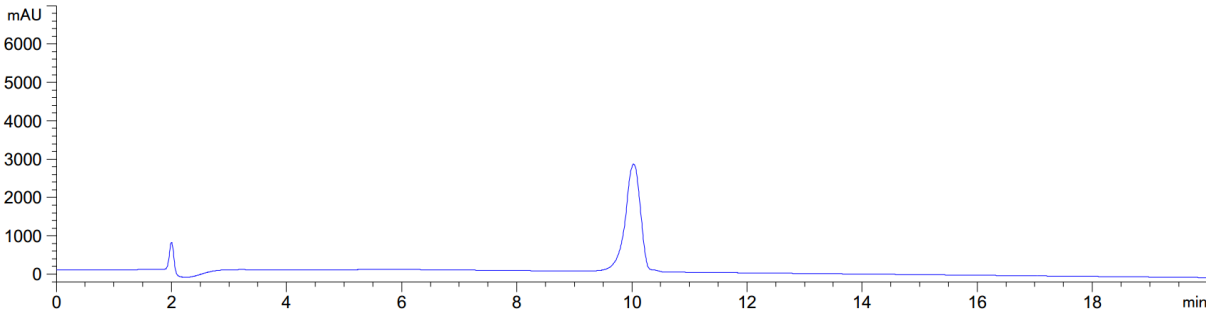
[M+H]+
569.1158
[M+Na]+
6391.4
% Intensity
Mass (m/z)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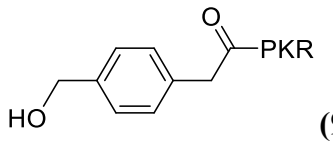
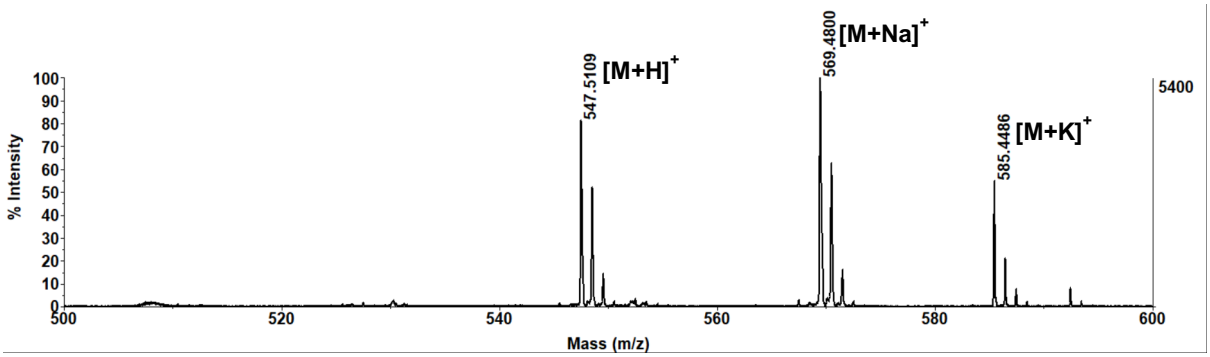

O
PKR
HO

(9)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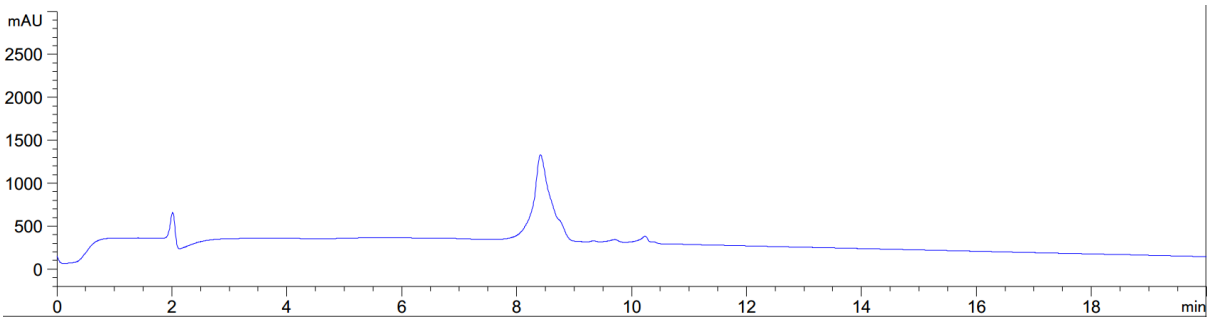
[M+H]+
569.4800
[M+Na]+
585.4486
[M+K]+
5400
% Intensity
Mass (m/z)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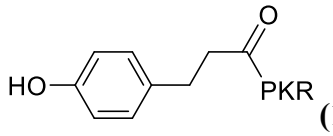
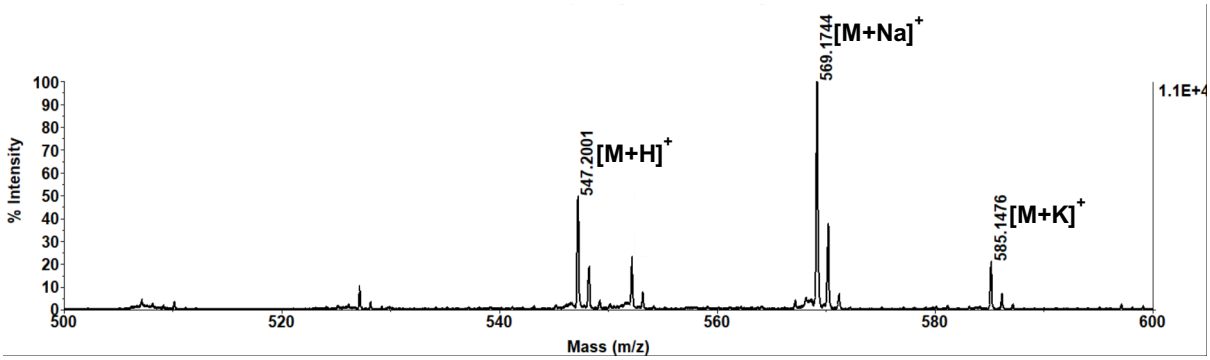
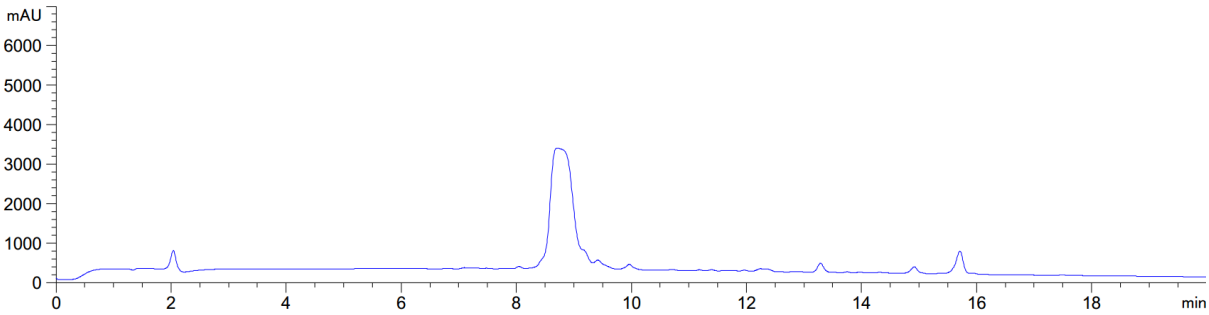

O
HO
PKR

(10)


547.2001
[M+H]+
569.1744
[M+Na]+
585.1476
[M+K]+
1.1E+4
% Intensity
Mass (m/z)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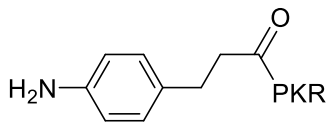
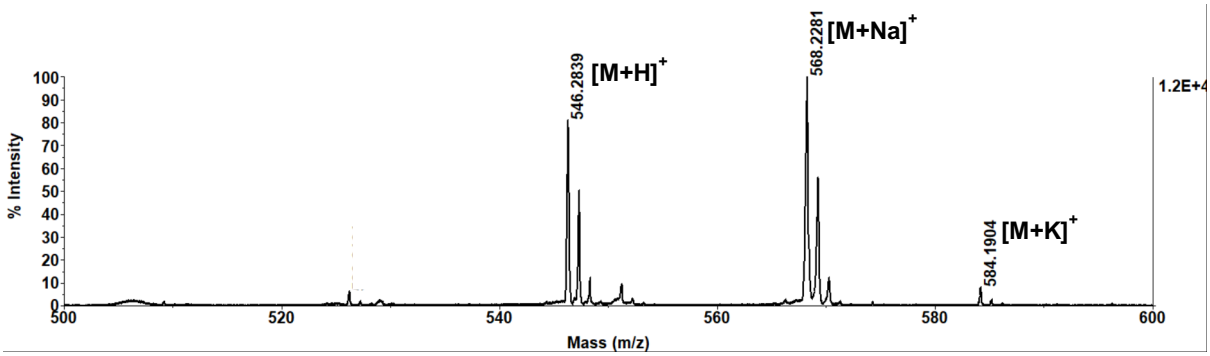

O
H2N
PKR

(11)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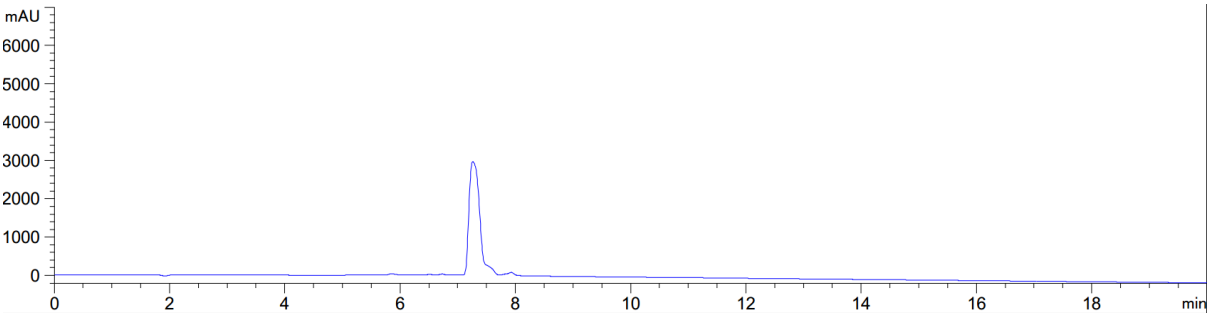
568.2281
[M+Na]+
584.1904
[M+K]+
% Intensity
Mass (m/z)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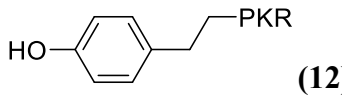
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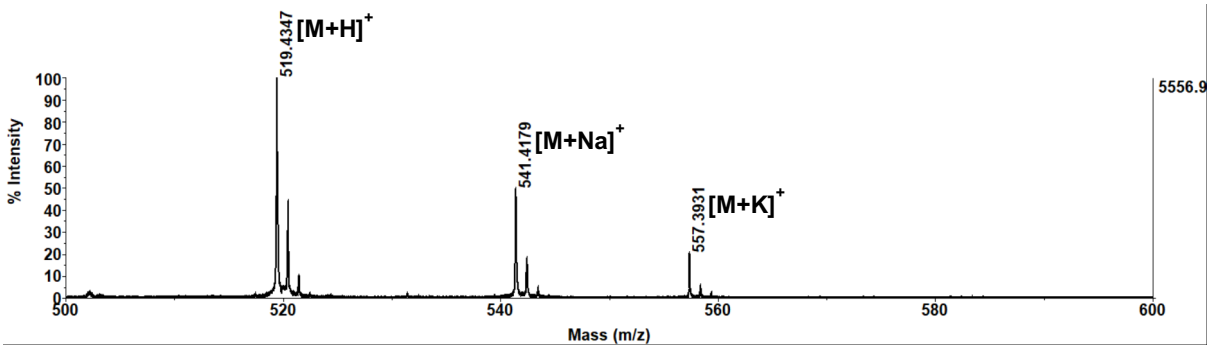
PKR

(12)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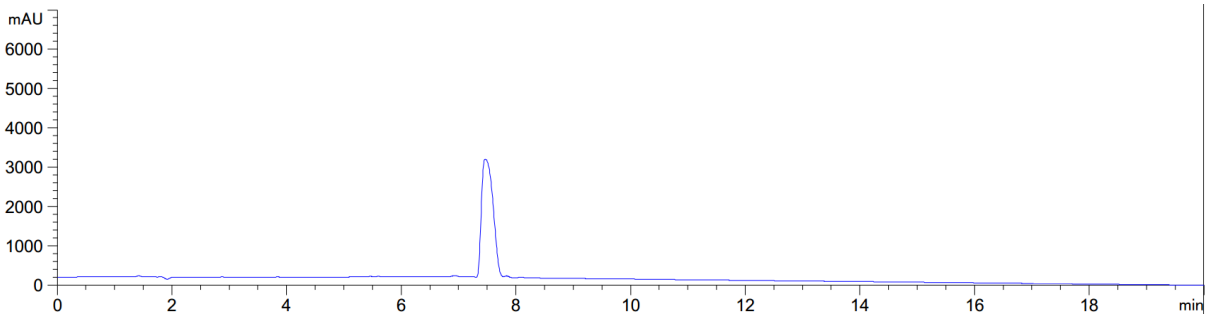
[M+H]+
541.4179
[M+Na]+
557.3931
[M+K]+
% Intensity
Mass (m/z)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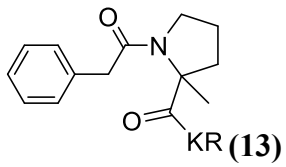
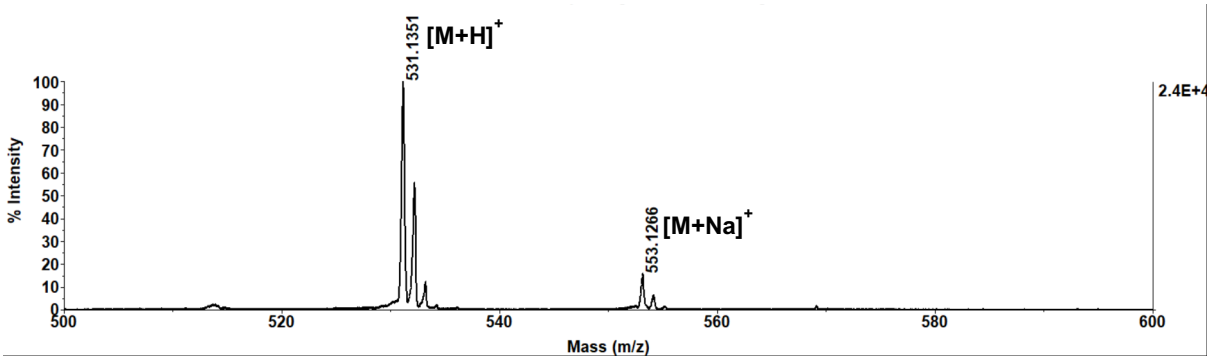
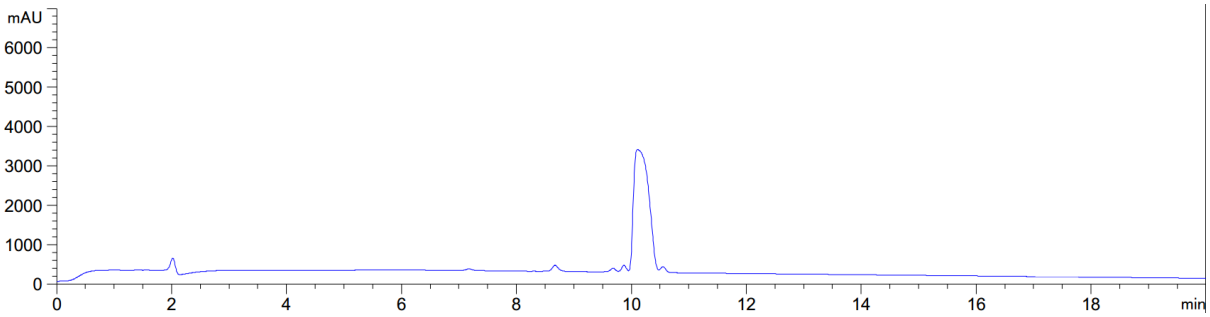

O
N
O
KR

(13)


531.1351
[M+H]+
553.1266
[M+Na]+
2.4E+4
% Intensity
Mass (m/z)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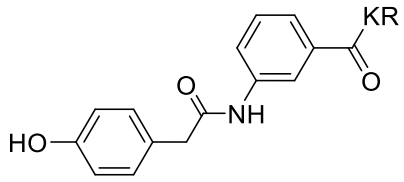
KR
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NH
HO

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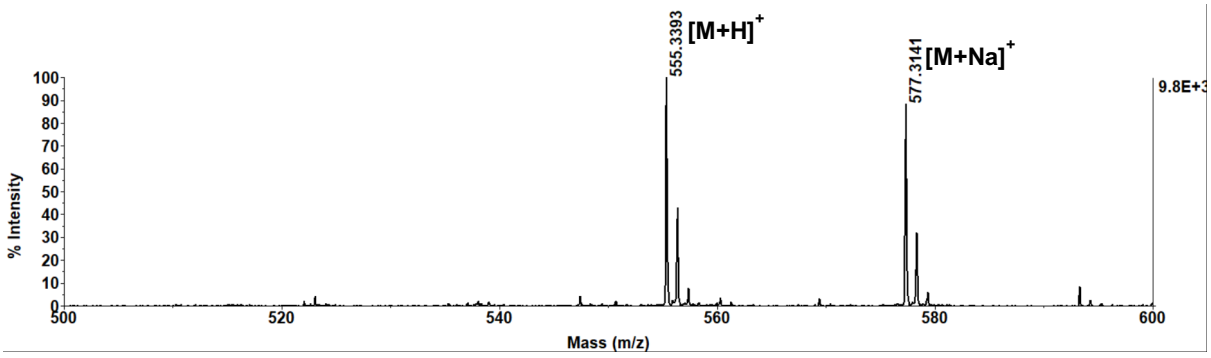
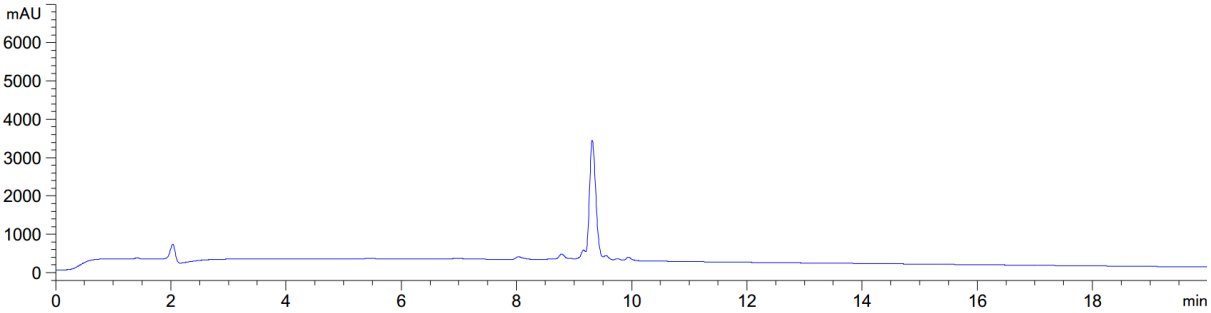

555.3393
[M+H]+
577.3141
[M+Na]+
9.8E+3
% Intensity
Mass (m/z)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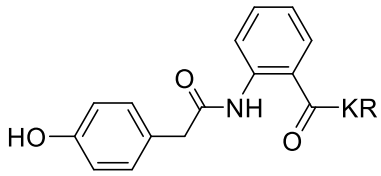
HO
O
NH
O
KR

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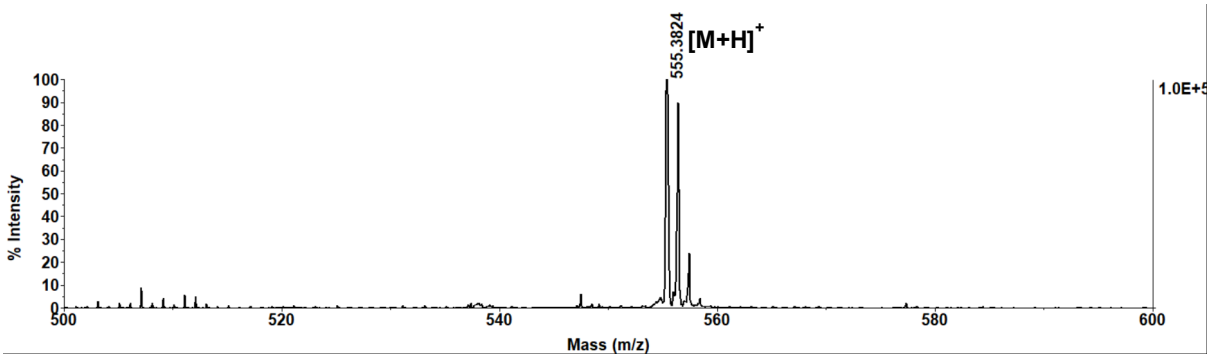
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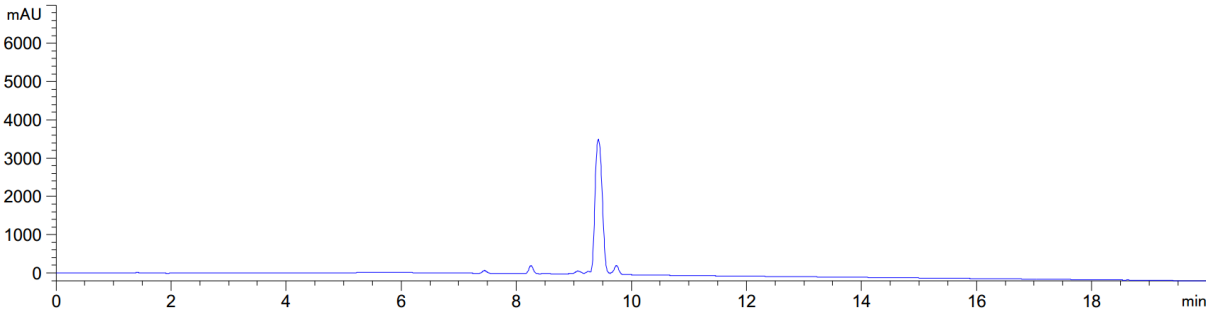
[M+H]+
1.0E+5
% Intensity
Mass (m/z)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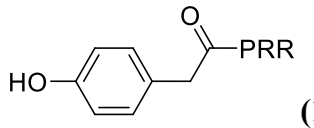
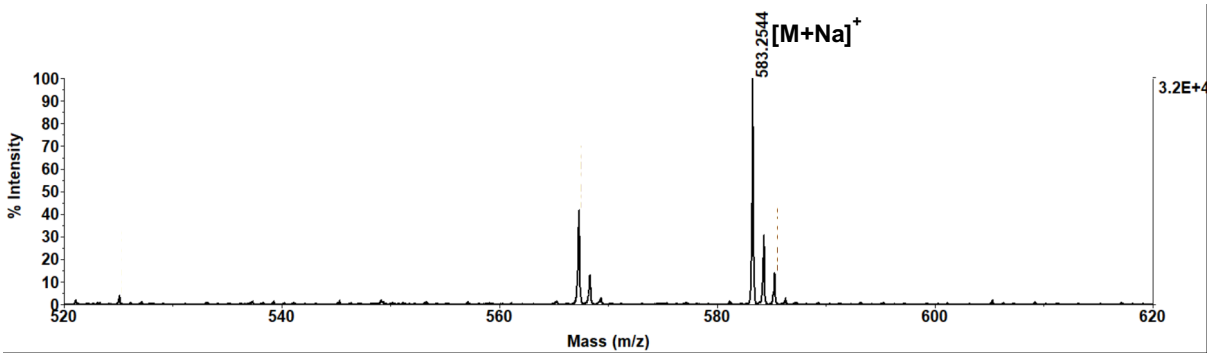

HO
O
PRR

(16)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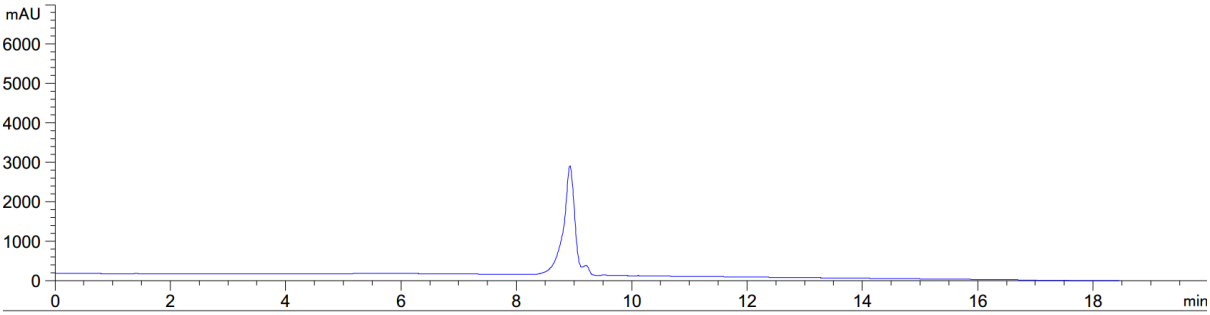
[M+Na]+
3.2E+4
% Intensity
Mass (m/z)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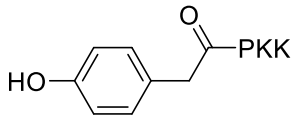
O
PKK
HO

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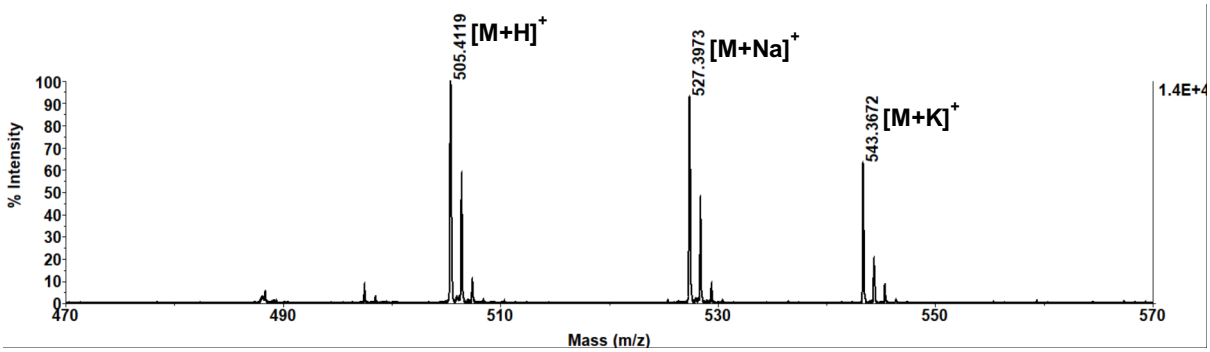
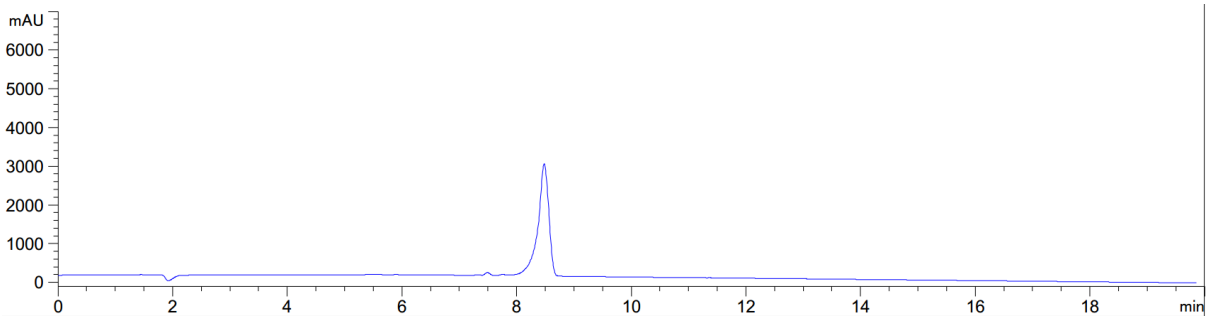
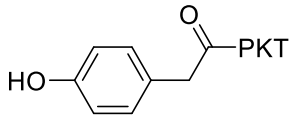
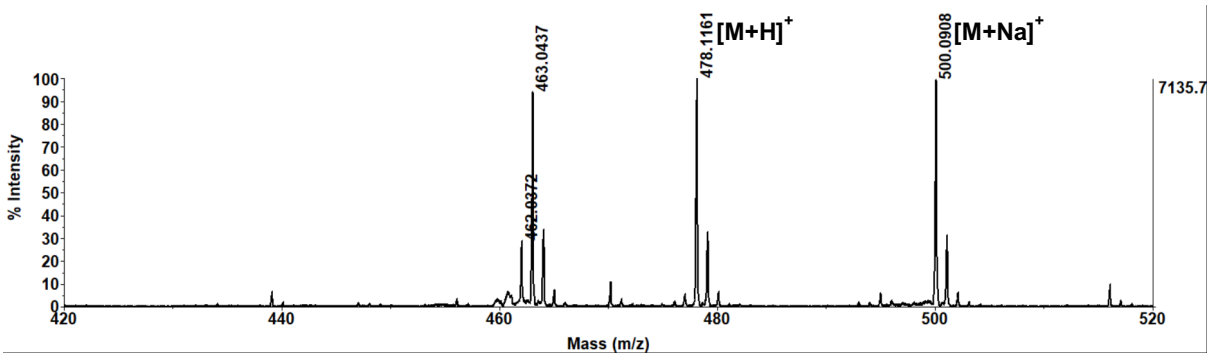
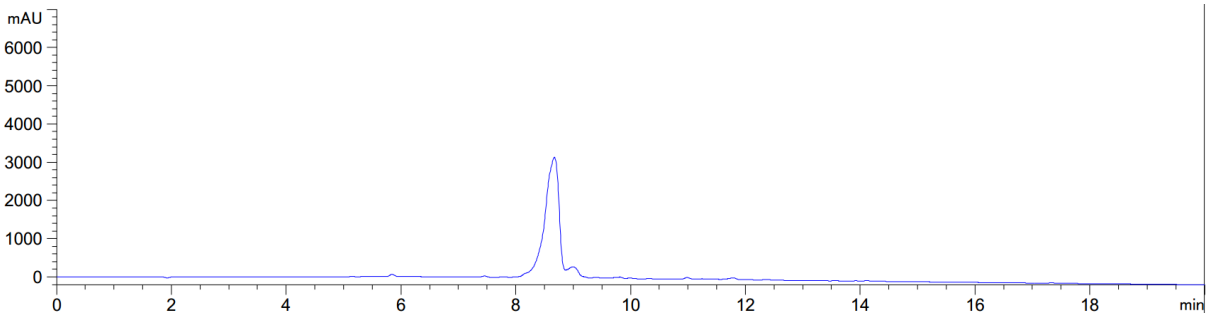

505.4119
[M+H]+
527.3973
[M+Na]+
543.3672
[M+K]+
1.4E+4
% Intensity
Mass (m/z)

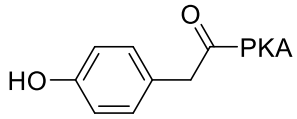

mAU
min


O
PKT
HO

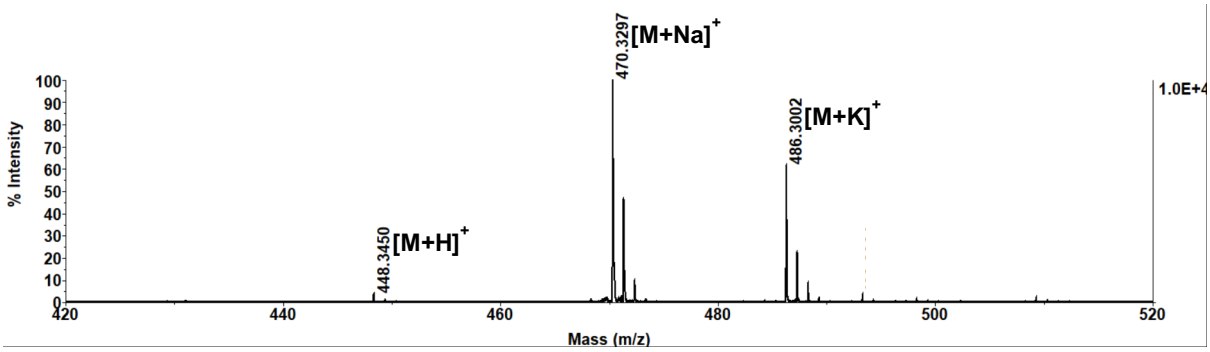
(18)

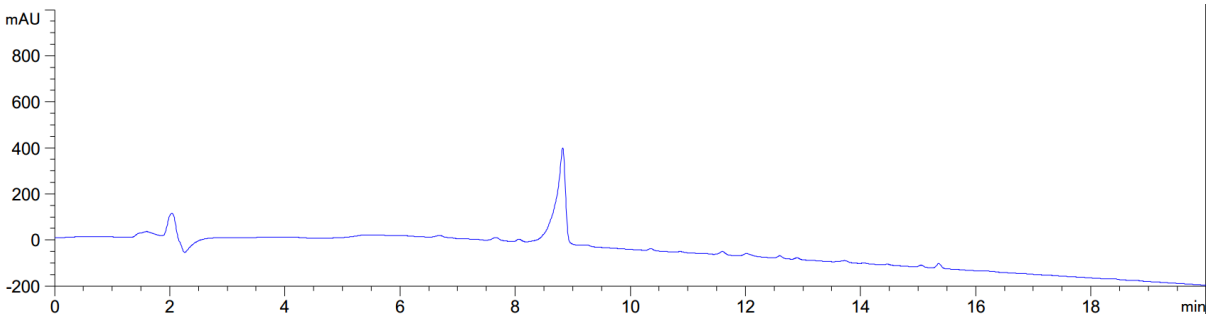

463.0437
462.0372
478.1161
[M+H]+
500.0908
[M+Na]+
7135.7
% Intensity
Mass (m/z)

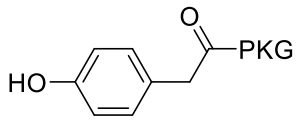

mAU
min


HO
O
PKA

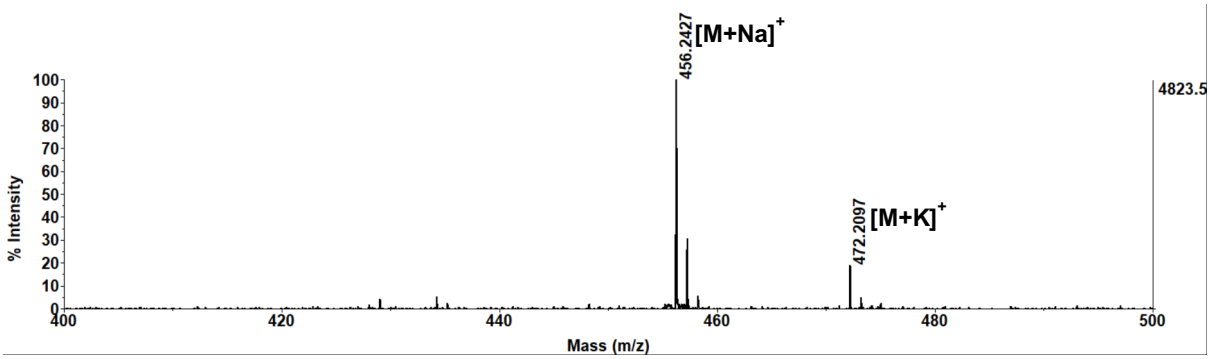
(19)

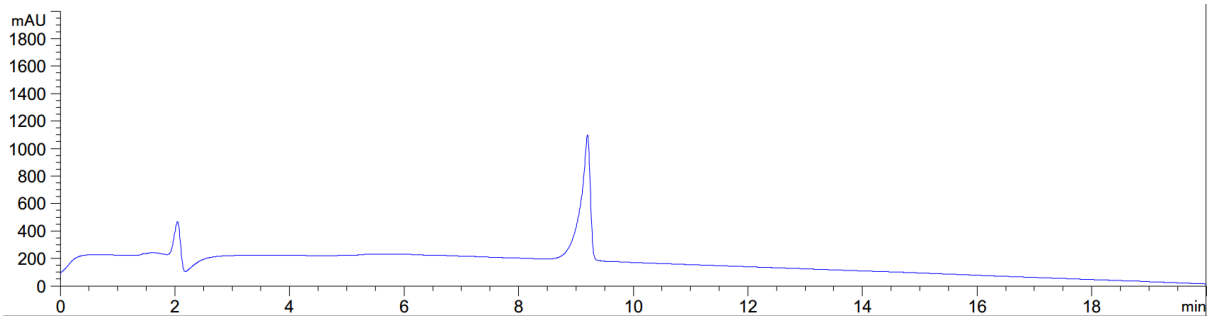

470.3297
[M+Na]+
486.3002
[M+K]+
448.3450
[M+H]+
1.0E+4
% Intensity
Mass (m/z)

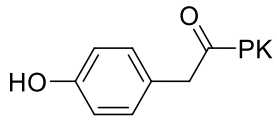

mAU
min


HO
O
PKG

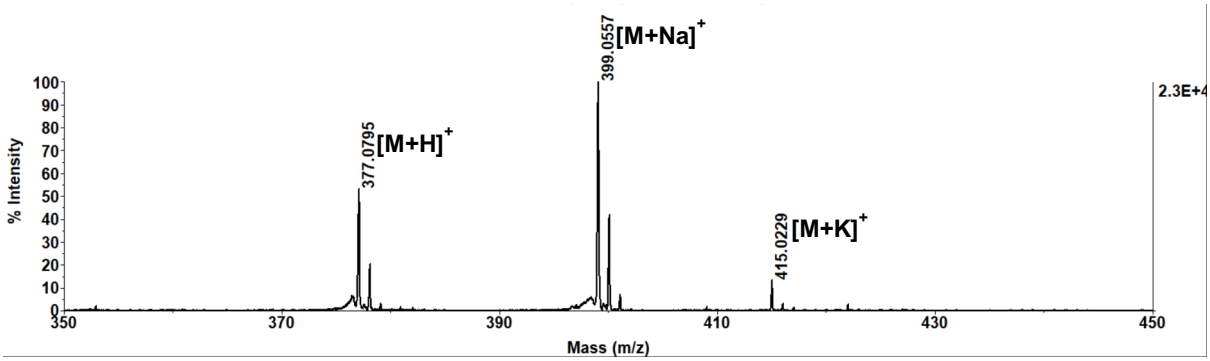
(20)

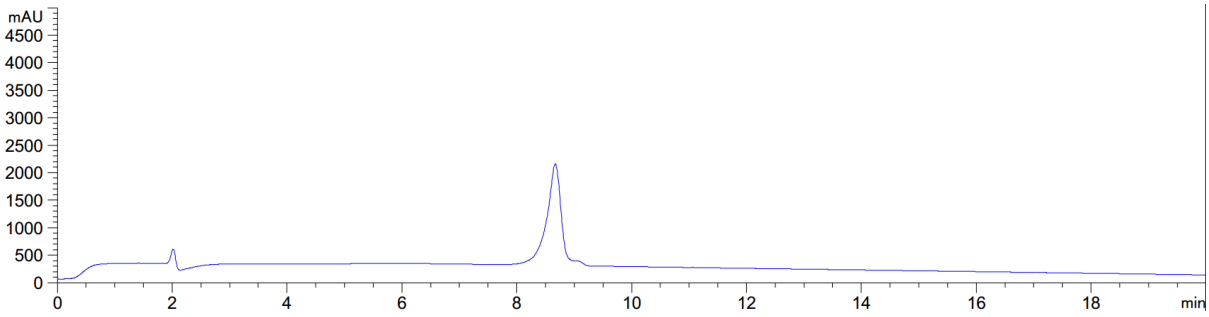

456.2427
[M+Na]+
4823.5
472.2097
[M+K]+
% Intensity
Mass (m/z)

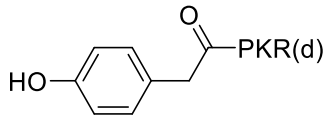

mAU
min


HO
O
PK

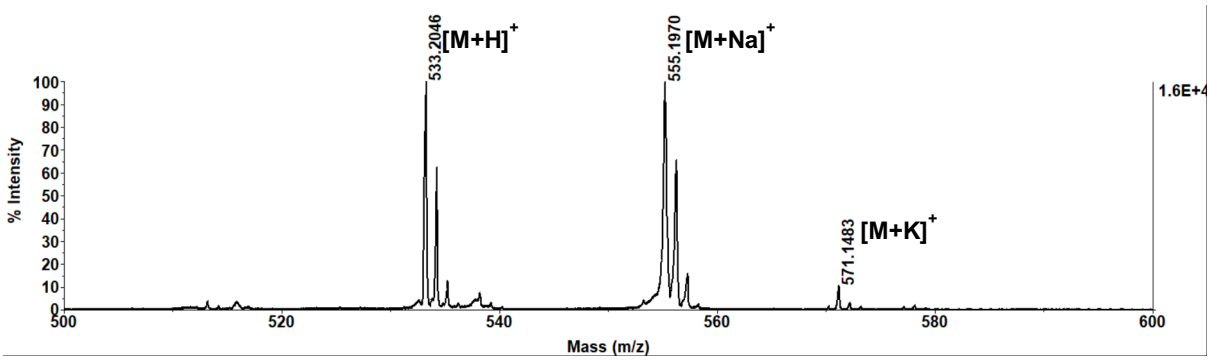
(21)

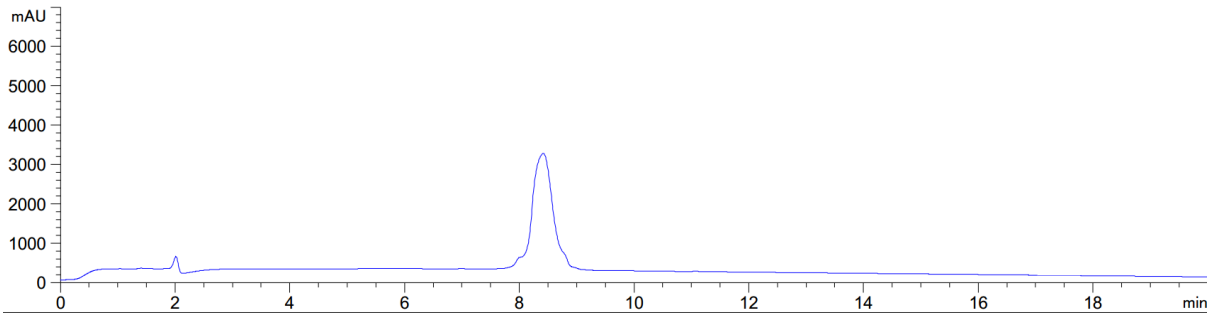

399.0557
[M+Na]+
377.0795
[M+H]+
415.0229
[M+K]+
2.3E+4
% Intensity
Mass (m/z)

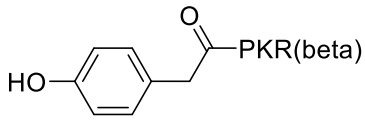

mAU
min


HO
O
PKR(d)

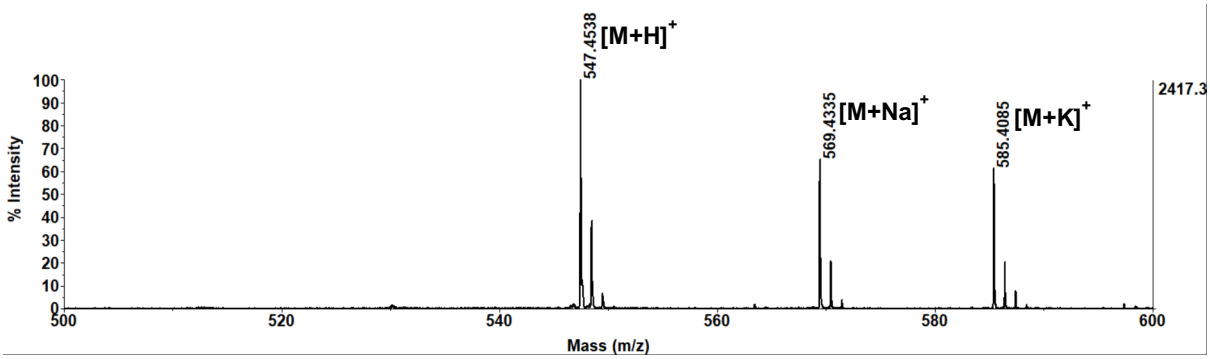
(22)

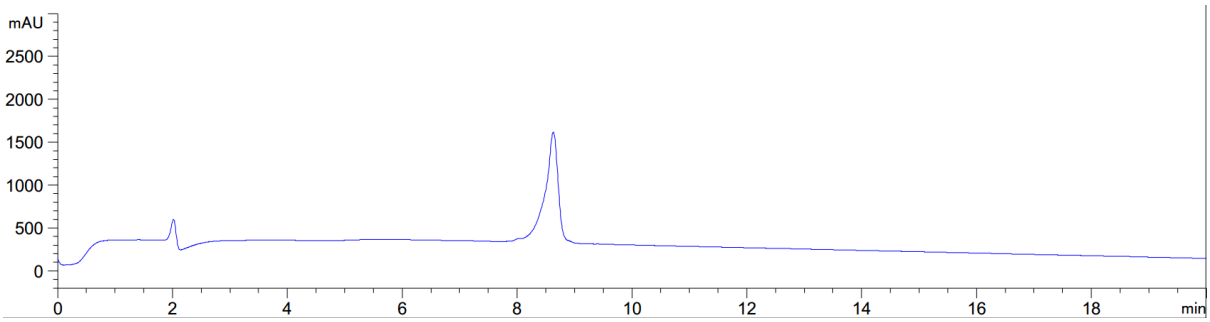

533.2046
[M+H]+
555.1970
[M+Na]+
571.1483
[M+K]+
1.6E+4
% Intensity
Mass (m/z)

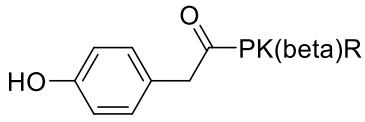

mAU
min


HO
O
PKR(beta)

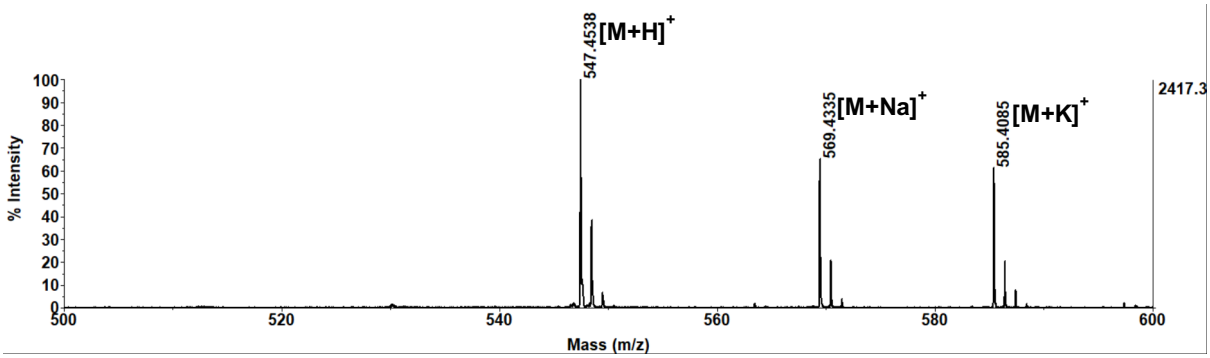
(23)

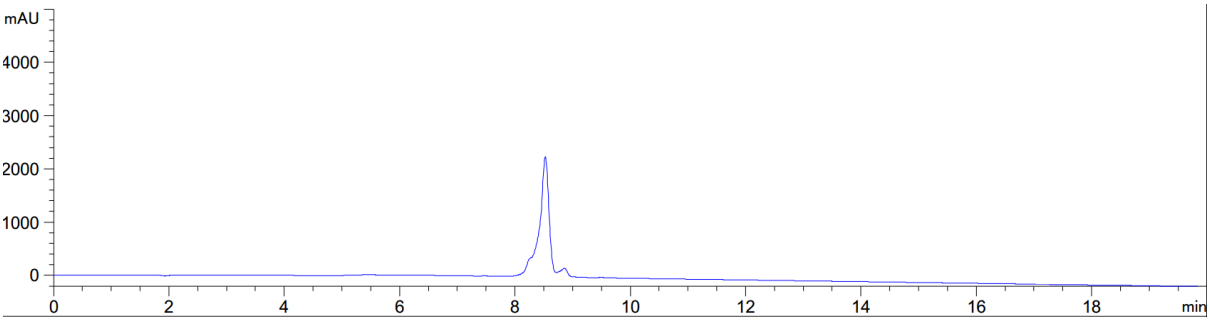

547.4538
[M+H]+
569.4335
[M+Na]+
585.4085
[M+K]+
2417.3
% Intensity
Mass (m/z)

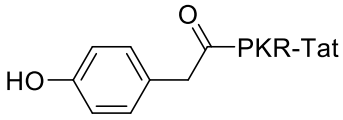

mAU
min


HO
O
PK(beta)R

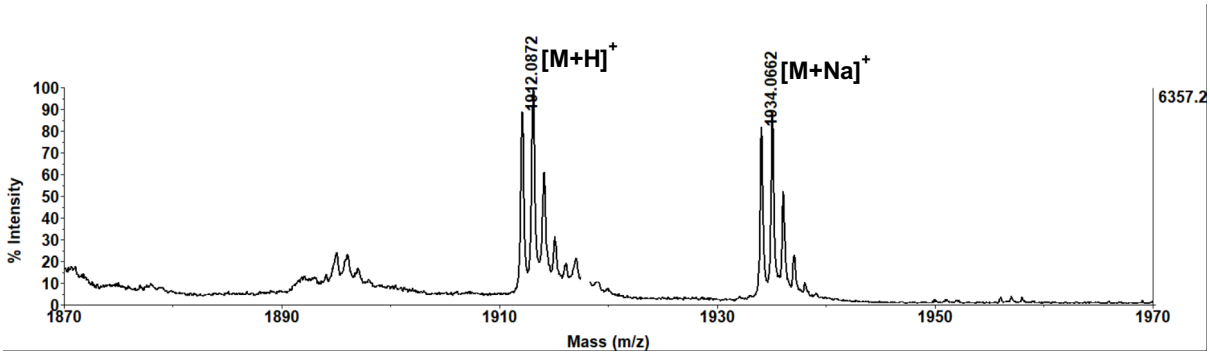
(24)

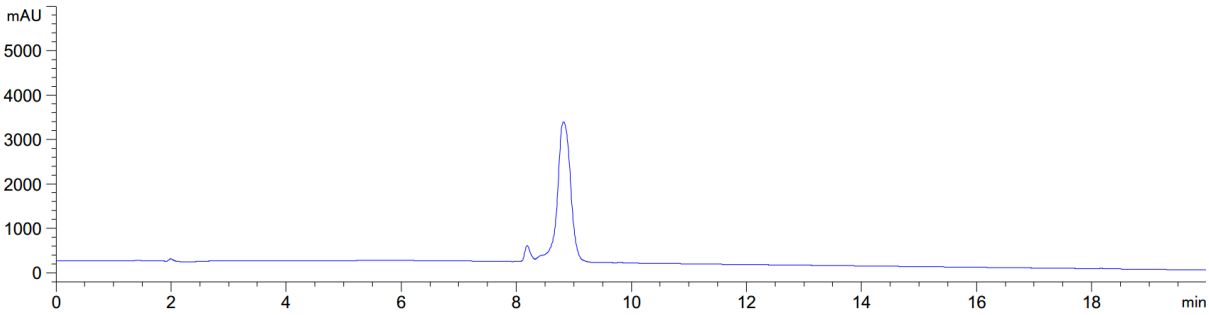

547.4538
[M+H]+
569.4335
[M+Na]+
585.4085
[M+K]+
2417.3
% Intensity
Mass (m/z)

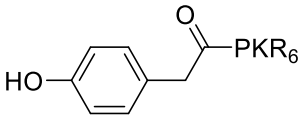

mAU
min


HO
O
PKR-Tat

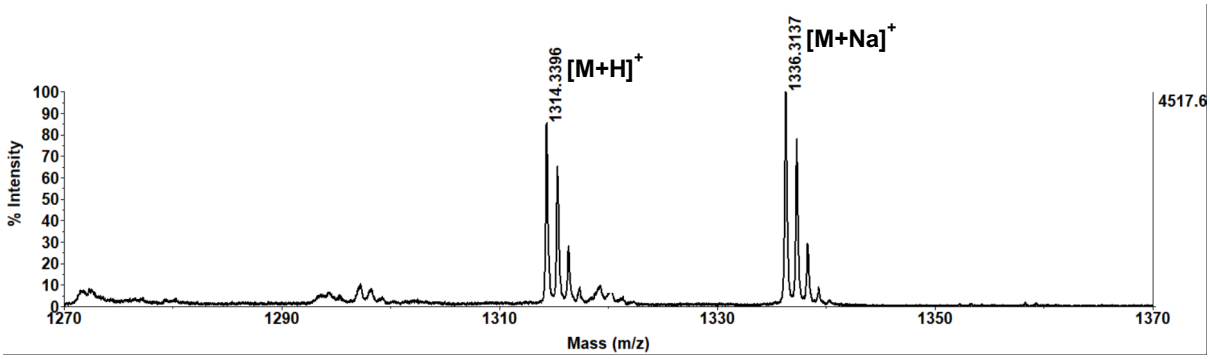
(DC431)


1942.0872
[M+H]+
1964.0662
[M+Na]+
6357.2
% Intensity
Mass (m/z)


mAU
min


HO
O
PKR6

(DC432)


1314.3396
[M+H]+
1336.3137
[M+Na]+
4517.6
% Intensity
Mass (m/z)

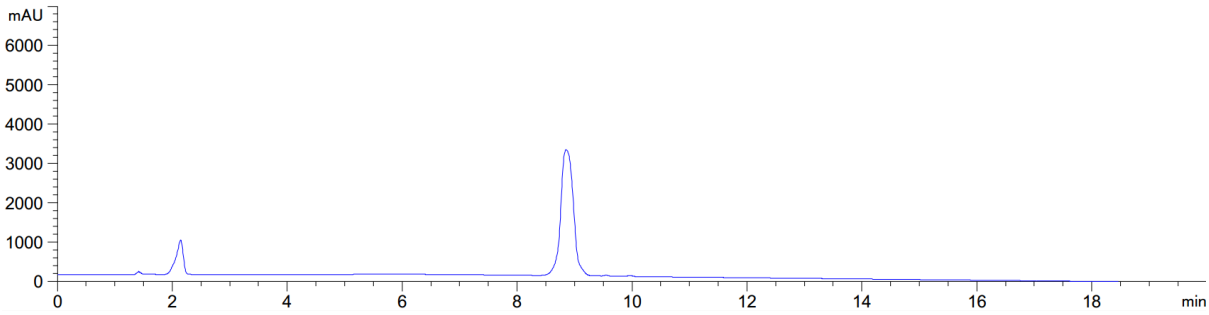

mAU
min

Figure S2. IC_{50} curves of compounds **5.** IC_{50} values were determined in triplicates (n = 3) and presented as mean ± SD

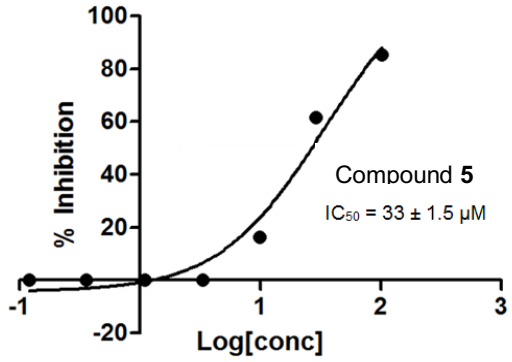


Figure S3. IC_{50} curve of **BM30** (**6**). IC_{50} values were determined in triplicates (n = 3) and presented as mean ± SD

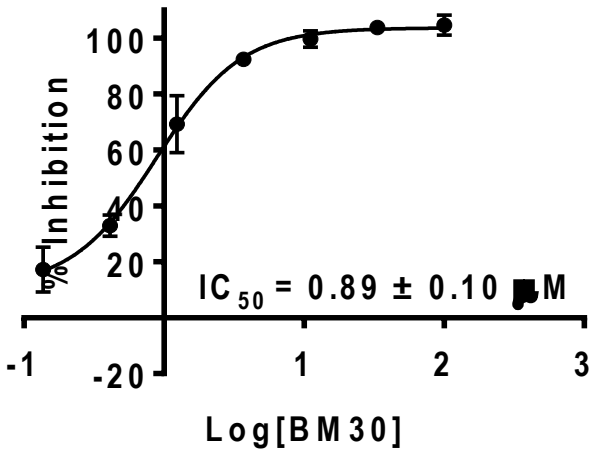
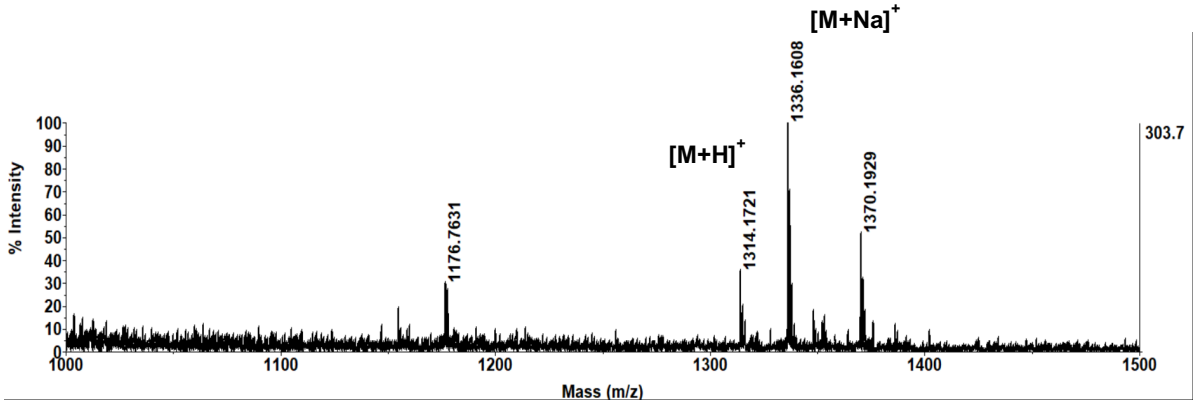
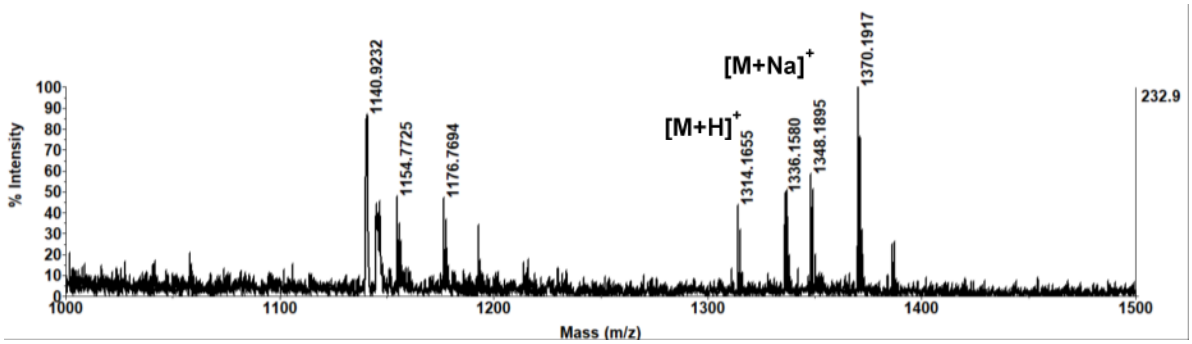


Figure S4. Cell permeability evaluation

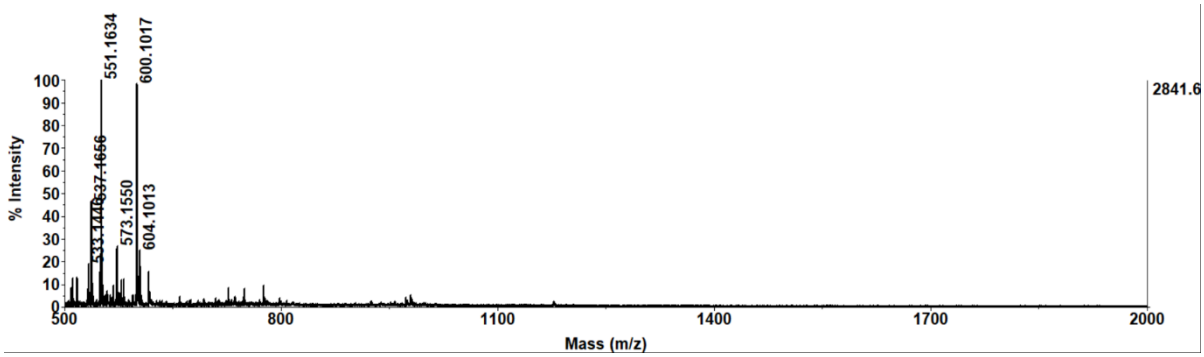
10 μM DC432



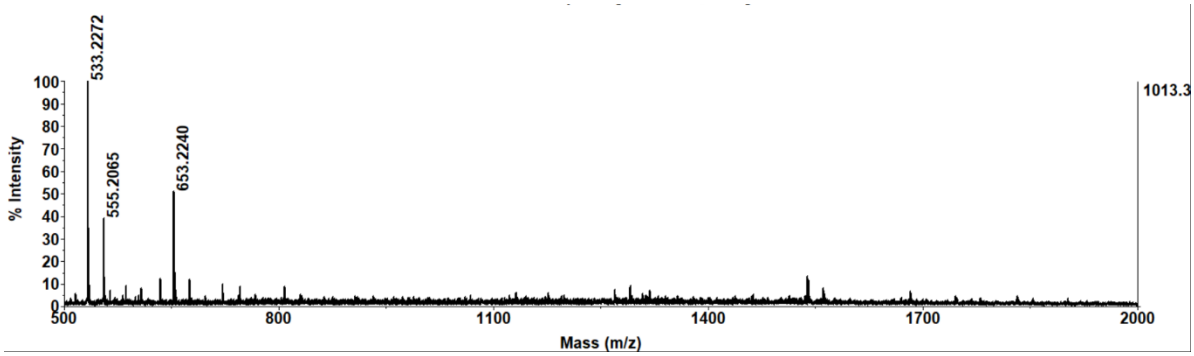
1 μM DC432



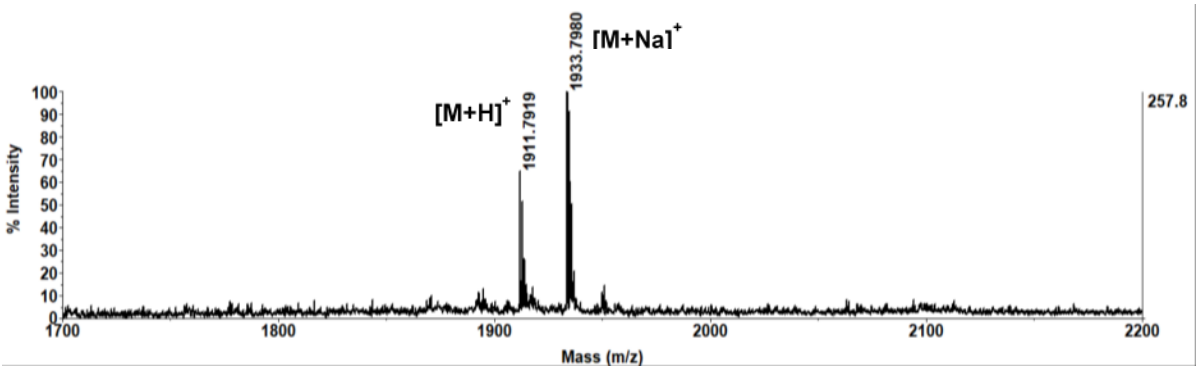
Last wash of cells incubated with 10 μM DC432



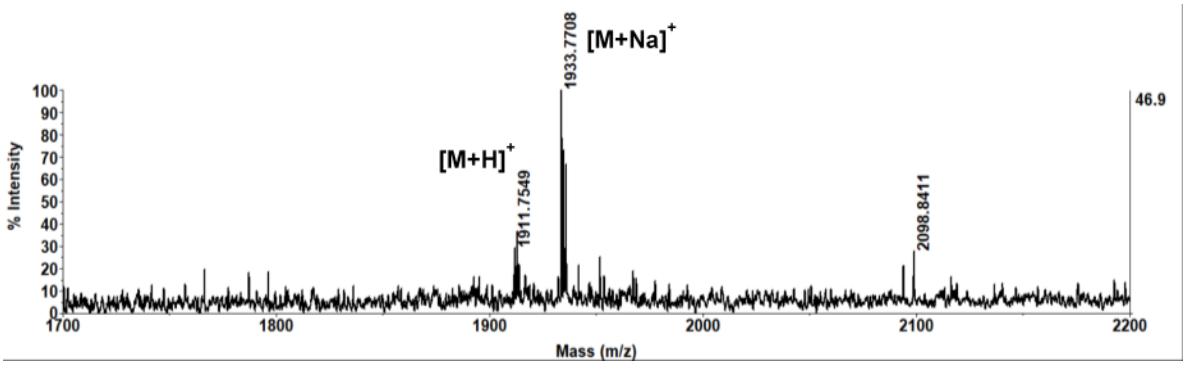
Last wash of cells incubated with 1 μM DC432



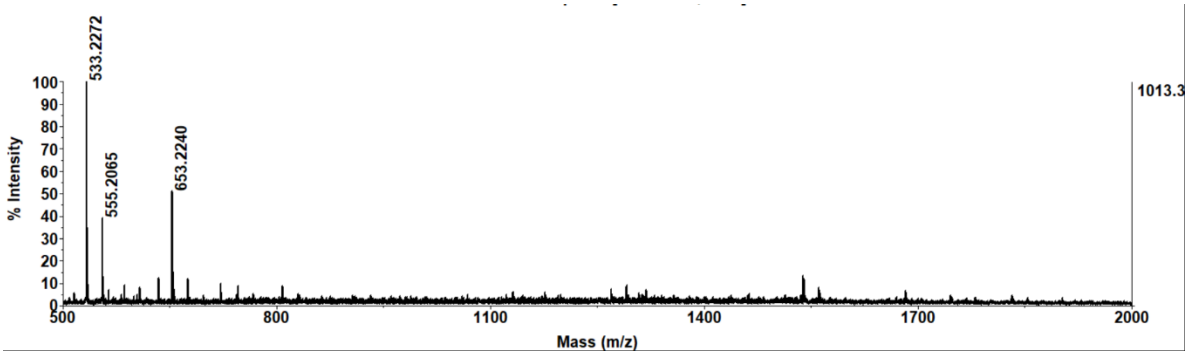
10 μM DC431



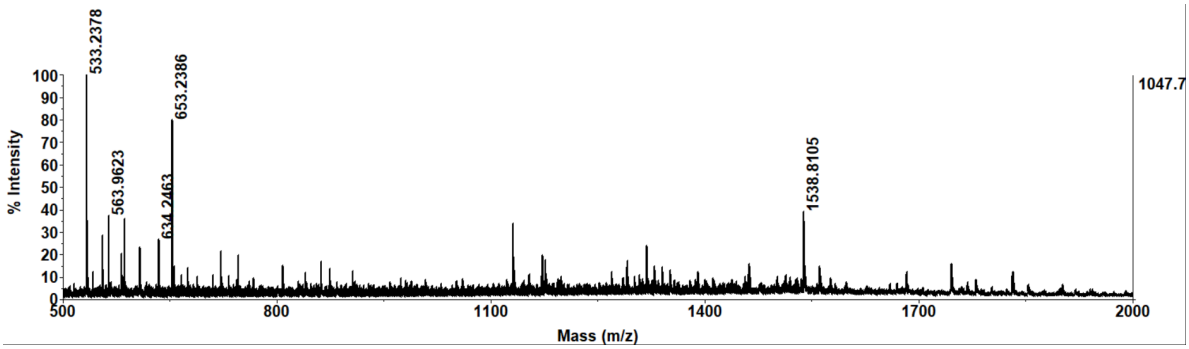
1 μM DC431



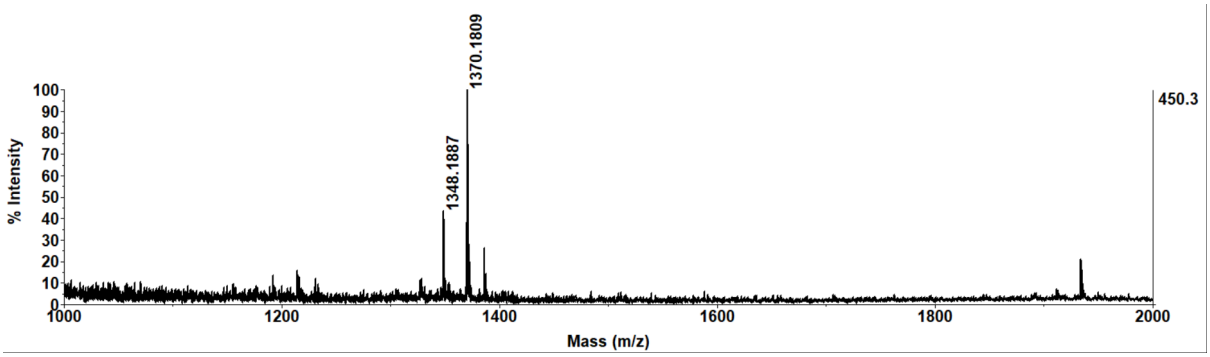
Last wash of cells incubated with 10 μM DC431



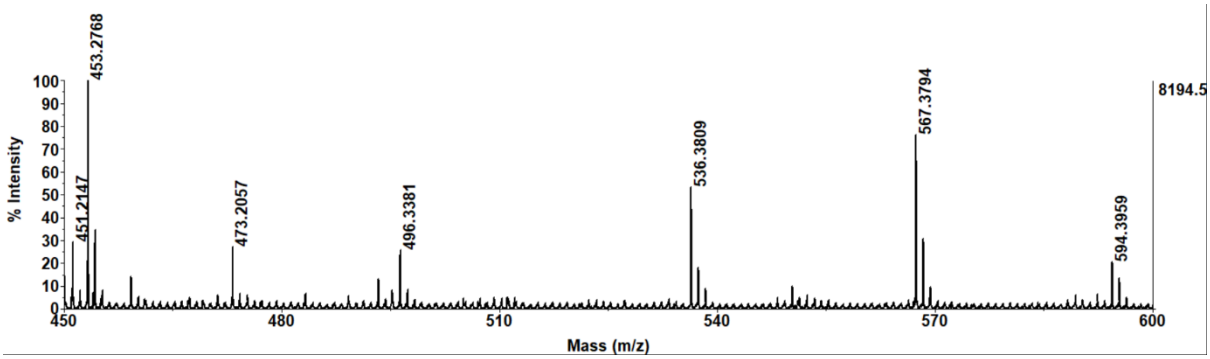
Last wash of cells incubated with 1 μM DC431



Cell lysate alone



100 μM BM-30



Cell lysate alone

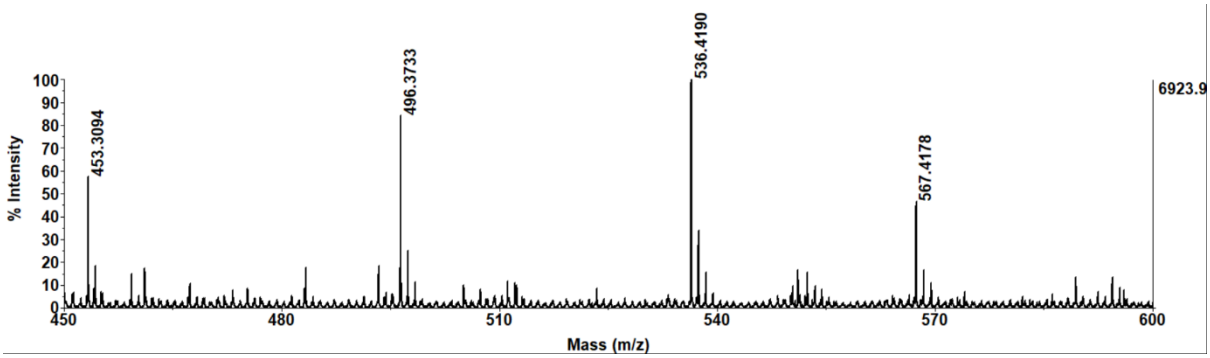


Table S1. Selectivity study of BM**30** and **22**

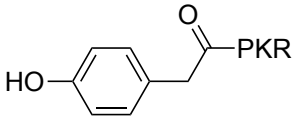
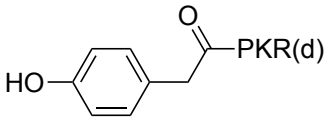
Compound	Structure	IC_{50} (μM)		
		NTMT1	**G9a**	**PRMT1**
BM30		0.32±0.06	>100	>100
22		4.2±1.2	13±4.1	>100

Table S2. Selectivity study of compound BM30 against a panel of 41 MTases (Reaction Biology)[a]

Enzyme	Activity (mean, %)	SD (%)	n
ASH1L	92	4.6	2
DNMT1	100	0.4	2
DNMT3a	100	3.4	2
DNMT3b	103	2.2	2
DNMT3b/3L	118	0.6	2
DOT1L	92	1.9	2
EZH1 Complex	112	0.9	2
EZH2 Complex	104	2.1	2
EZH2 (Y641F)	100	1.0	2
G9a	101	0.1	2
GLP	97	6.8	2
METTL21A-GST	95	10.6	2
MLL1 Complex	75	0.9	2
MLL2 Complex	85	1.0	2
MLL3 Complex	96	0.4	2
MLL4 Complex	84	2.4	2
NTMT1	11	0.1	2
NTMT2	12	0.1	2
NSD1	95	0.4	2
NSD2	102	0.7	2
NSD2 (E1099K)	98	2.7	2
NSD2 (T1150A)	97	5.4	2
NSD3	91	1.2	2
PRDM9	100	0.3	2
PRMT1	112	0.3	2
PRMT3	98	2.4	2
PRMT4	104	3.7	2
PRMT5	106	7.6	2
PRMT5/MEP50 Complex	106	2.3	2
PRMT6	98	8.0	2
PRMT7	104	7.6	2
PRMT8	100	10.8	2
SET1b Complex	94	5.5	2
SET7/9	90	4.2	2
SET8	96	5.1	2
SETD2	100	3.2	2
SMYD1	97	0.7	2
SMYD2	96	0.6	2
SUV39H1	99	6.1	2
SUV39H2	93	1.8	2
SUV420H1TV2	99	3.4	2

[a] Selectivity of **BM30** was determined at concentration of 100 μM for duplicate (n=2).

Table S3. Crystallography data and refinement statistics

Data Collection	**NTMT1-BM30**
λ (Å)	1.0332
Space group	$P6_522$
a, b, c (Å)	106.9, 106.9, 205.4
α, β, γ (º)	90, 90, 120
Resolution (Å)*	30 – 1.73 (1.76 – 1.73)
Completeness (%)*	99.9 (98.1)
Redundancy*	19.4 (18.7)
R_{sym} †*	0.21 (4.5)
I / σ (I)*	12.8 (0.7)
$CC_{1/2}$	1.0 (0.36)
Refinement	
Resolution (Å)	30 – 1.73
No. reflections	72,828
R§/R_{free}¶	0.18/0.20
r.m.s. deviations	
Bonds (Å)	0.008
Angles (º)	0.879
No. Protein atoms	7088
No. Ligand atoms	246
No. Waters	564
B-factors (Å^2)	
Wilson B	26.81
Protein	32.53
Ligands	41.43
Waters	40.42
Ramachandran Analysis¥	
Favored (%)	98.9
Allowed (%)	1.1
Outliers (%)	0
PDB code	6WH8

† $R_{sym} = \Sigma_{hkl,j} (|I_{hkl} - \langle I_{hkl} \rangle|) / \Sigma_{hkl,j} I_{hkl}$, where $\langle I_{hkl} \rangle$ is the average intensity for a set of j symmetry related reflections and I_{hkl} is the value of the intensity for a single reflection within a set of symmetry-related reflections.

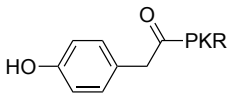
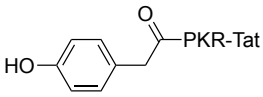
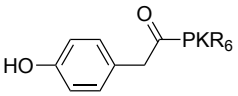
§ R factor $= \Sigma_{hkl} (||F_o| - |F_c||) / \Sigma_{hkl}|F_o|$ where F_o is the observed structure factor amplitude and F_c is the calculated structure factor amplitude.

¶ $R_{free} = \Sigma_{hkl,T} (||F_o| - |F_c||) / \Sigma_{hkl,T}|F_o|$, where a test set, T (5% of the data), is omitted from the refinement.

¥ Performed using Molprobity within PHENIX.

* Indicates statistics for last resolution shell shown in parenthesis.

Table S4. IC_{50} values and binding affinities of BM30 and its cell-permeable analogs[a]

Compound	Structure	IC_{50} (μM) for NTMT1	K_d (μM) for NTMT1	K_d (μM) for NTMT2
BM30		0.89±0.10	3, 2.8	3.7
DC431		0.23± 0.03	0.6, 0.8	3.4
DC432		0.054± 0.004	0.3, 0.4	1

[a] IC_{50} = mean±SD (n=3)