

## SUPPLEMENTARY INFORMATION

### Hypoxanthine-guanine phosphoribosyltransferase is dispensable for *Mycobacterium smegmatis* viability

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**Table S1.** List of primers, DNA constructs and strains used in this work.

<b>Deletion constructs</b>					
Construct name	Gene to delete	upstream region <sup>a</sup> (from – to)	primer pair	downstream region <sup>a</sup> (from – to)	primer pair
pYS2-Δhpt	<i>hgprt</i>	6,174,169 – 6,173,470	1; 2	6,172,887 – 6,172,188	3; 4
pYS2-ΔpurF	<i>purF</i>	5,869,979 – 5,869,280	5; 6	5,867,743 – 5,867,044	7; 8
<b>Expression constructs</b>					
Construct name	Description				reference
pSE200	Replicable vector in Msm, derivate of pSE100, constitutive expression in Msm				(1)
pSE200.Mtb.HGPRT	<i>Mtb.hgprt</i> coding sequence was inserted under control of constitutive promoter.				This study
pSE200.Msm.HGPRT	<i>Msm.hgprt</i> coding sequence was inserted under control of Msm constitutive promoter.				This study
<b>Primer list</b>					
primer	sequence (5' - to -3') <sup>b</sup>		orientation <sup>c</sup>	restriction site	
1	aaa <u>ACTAGT</u> aggccgcgcgcgacggggcgcccgt		F	<i>SpeI</i>	
2	ttt <u>ATTTAAAT</u> tagcgtgccacgtgaggtcgccgcaa		R	<i>SwaI</i>	
3	aaaa <u>TTAATTAA</u> acgctcaggtgagttcccagatcac		F	<i>PacI</i>	
4	ttt <u>ATGCAT</u> gcactgctggtcggcggtggccggcc		R	<i>NsiI</i>	
5	aaa <u>ACTAGT</u> gaacgacacctggatgctgacggtg		F	<i>SpeI</i>	

6	ttt <u>ATTTAAAT</u> gatatggctgctcccgatgggctg	R	<i>SwaI</i>
7	aaaa <u>TTAATTA</u> Atacgggctcaccgagaacagtgc	F	<i>PacI</i>
8	ttt <u>ATGCAT</u> gcctgggacgtcggcgggcccgcag	R	<i>NsiI</i>
9	Tggggccgagatcatccc	F	
10	aatgcgaagaagcgggtccg	R	
11	gtggacgtggctgtcgaatccaccg	F	
12	ctagtgcgtgtagacctcgggtc	R	
13	atgaccagcaccacgacacggccg	F	
14	tcagatgatgtgcaggcttcgtac	R	
15	<u>attaacagctgatt</u> atgacgccgcgttggtcgt	F	
16	<u>ccgggggatccatt</u> tctactgatagaccctgggtcc	R	
17	<u>attaacagctgatt</u> atggacgtggctgtcgaatcca	F	
18	<u>ccgggggatccatt</u> tctagtgcgtgtagacctcgg	R	

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<sup>a</sup>*M. smegmatis* mc<sup>2</sup> 155 reference genome sequence with NCBI accession number CP000480.1

<sup>b</sup>corresponding restriction sites and in In-Fusion sites are capitalized and underlined, respectively.

<sup>c</sup>F – forward direction, R – reverse direction

## Synthesis and characterization of ANP phosphoramidate prodrugs 30 and 31.

Prodrugs of ANP-based inhibitors were prepared as previously described by Eng *et al.* (2). The compounds were identical (prepared in the same batches by D.H.) as the compounds tested on *Mtb* previously:

### **Tetra-(L-phenylalanine ethyl ester) Prodrug of (2-((2-(Guanin-9-yl)ethyl)(2-((2-hydroxyethyl)(2-phosphonoethyl)amino)ethyl)amino)ethyl)phosphonic acid (30)**

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): 10.65 (s, 1H, NH); 7.67 (s, 1H, H-8); 7.20 (m, 20H, Ar); 6.55 (s, 2H, NH<sub>2</sub>); 5.33

(s, 1H, OH); 4.62 (m, 4H, NH); 3.99 (m, 14H, Et, CHNH and H-1'); 3.66 (m, 2H, H-10'); 2.89 (m, 8H, CH<sub>2</sub>Ph), 2.80 (m, 4H), 2.67 (m, 4H), 2.54 (m, 2H) and 2.46 (m, 2H, CH<sub>2</sub>-N); 1.7 (m, 4H, CH<sub>2</sub>-P); 1.09 (m, 12H, Et).

<sup>13</sup>C NMR (DMSO-d<sub>6</sub>): 172.99 m, 4 C (CO); 156.72 (C-6); 153.49 (C-2); 151.02 (C-4); 137.41 (C-8); 137.12 (m, 4C), 129.29 (m, 8C), 128.04 (m, 8C) and 126.40 (m, 4C, Ar); 116.36 (C-5); 60.30 (m, 4C, Et); 60.21 (C-OH); 53.98 (m, 4C, NHCH); 55.94, 50.48, 50.22, 49.59, and 47.95 (2C C-N); 41.49 (C-1'); 27.67 (d, JP,C = 279.6, C-P); 27.60 (d, JP,C = 271.4, C-P); 13.87 (m, 4C, Et).

MS (ESI): m/z = 1199 [M + H]<sup>+</sup>.

HRMS (ESI) C<sub>59</sub>H<sub>82</sub>O<sub>12</sub>N<sub>11</sub>P<sub>2</sub> [M + H]<sup>+</sup> calcd. 1198.56142, found 1198.56209.

### **Tetra-(L-phenylalanine ethyl ester) Prodrug of (2-((2-(Hypoxanthin-9-yl)ethyl)(2-((2-hydroxyethyl)(2-phosphonoethyl)amino)ethyl)amino)ethyl)phosphonic acid (31)**

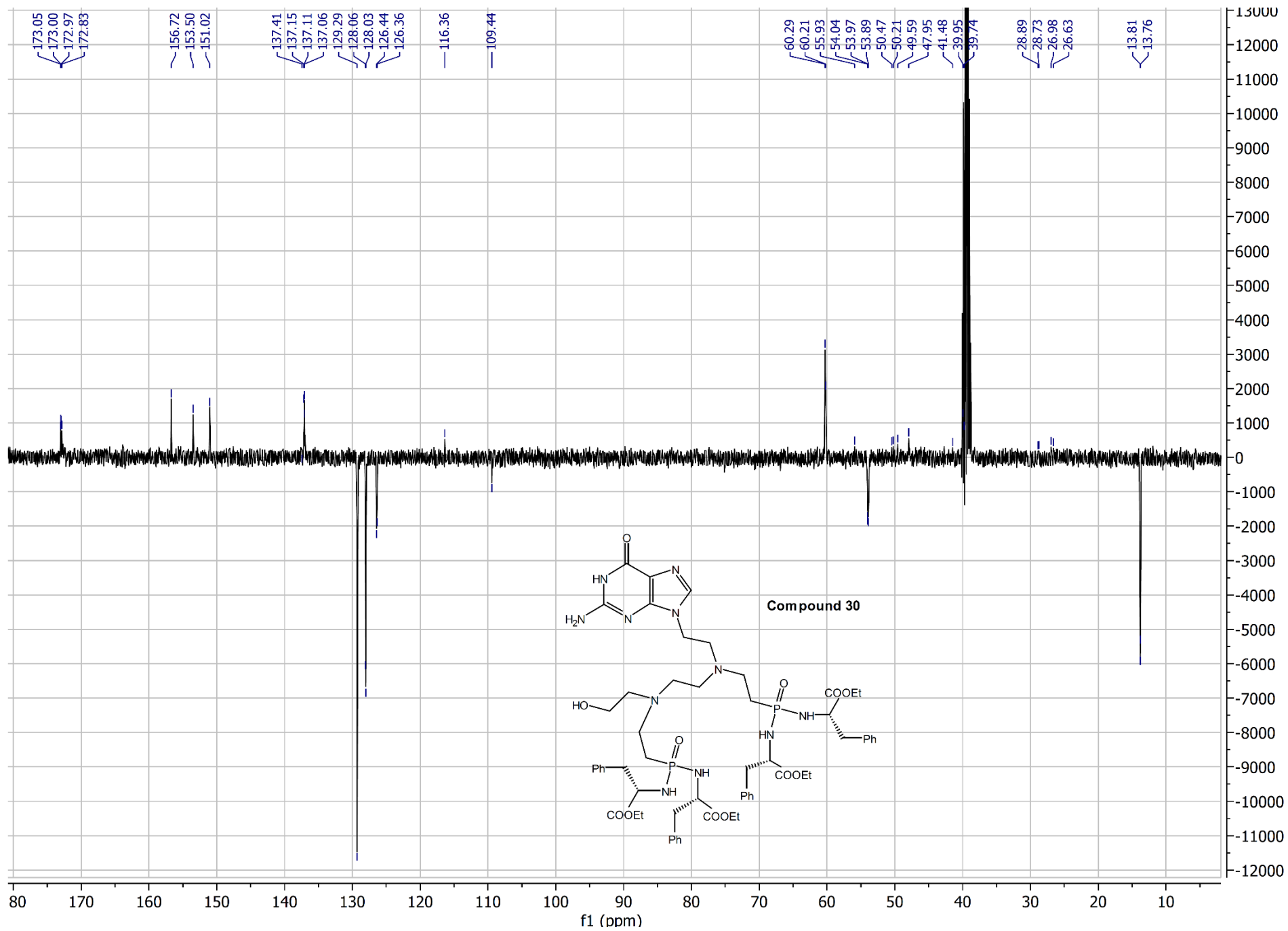
<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): 12.33 (s, 1H, NH); 8.08 (s, 1H) and 8.04 (s, 1H, H-2 and H-8); 7.20 (m, 16H) and 7.12 (m, 4H, Ar); 5.32 (s, 1H, OH); 4.60 (m, 4H, NH); 4.16 (m, 2H, H-1'); 4.00 (m, 10H, Et and CHNH); 3.88 (m, 2H, CHNH); 3.66 (m, 2H, H-10'); 2.89 (m, 8H, CH<sub>2</sub>Ph), 2.81 (m, 4H), 2.67 (m, 2H), 2.55 (m, 2H), 2.45 (m, 2H) and 2.33 (m, 2H, CH<sub>2</sub>-N); 1.61 (m, 4H, CH<sub>2</sub>-P); 1.09 (m, 12H, Et).

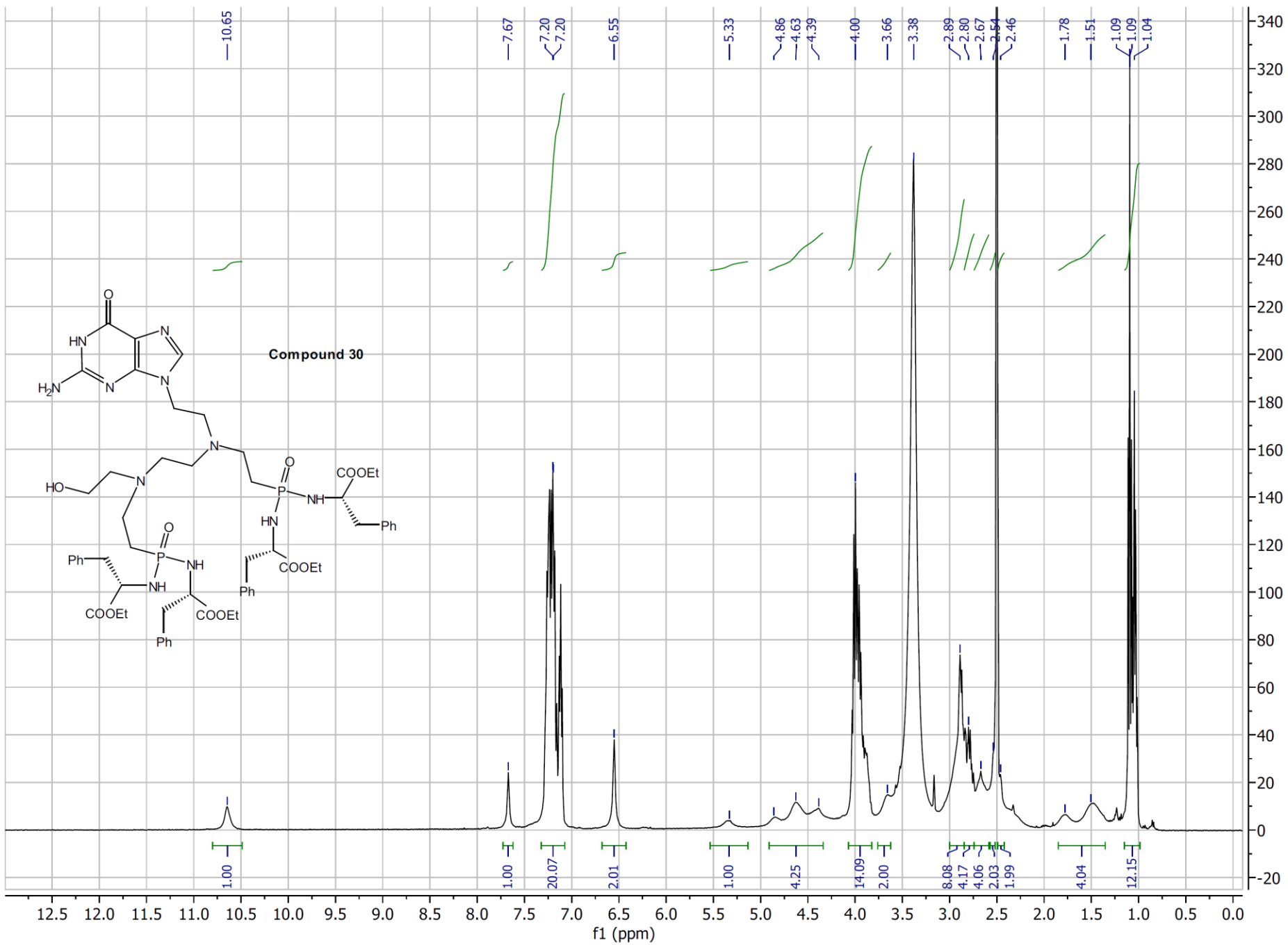
<sup>13</sup>C NMR (DMSO-d<sub>6</sub>): 172.89 (m, 4C, CO); 156.58 (C-6); 148.22 (C-4); 145.43 (C-2); 140.44 (C-8); 137.10 (m, 4C), 129.27 (m, 8C), 128.05 (m, 8C) and 126.42 (m, 4C, Ar); 123.77 (C-5); 60.27 (m, 4C,

Et); 60.13 (C–OH); 53.93 (m, 4C, NHCH); 57.03, 56.23, 52.13, 49.30 and 45.45 (C–N); 41.22 (C-1'); 27.59 (d, JP,C = 266.6, C–P); 27.55 (d, JP,C = 267.1 (C–P); 13.82 (m, 4C, Et).

MS (ESI):  $m/z = 1184 [M + H]^+$ .

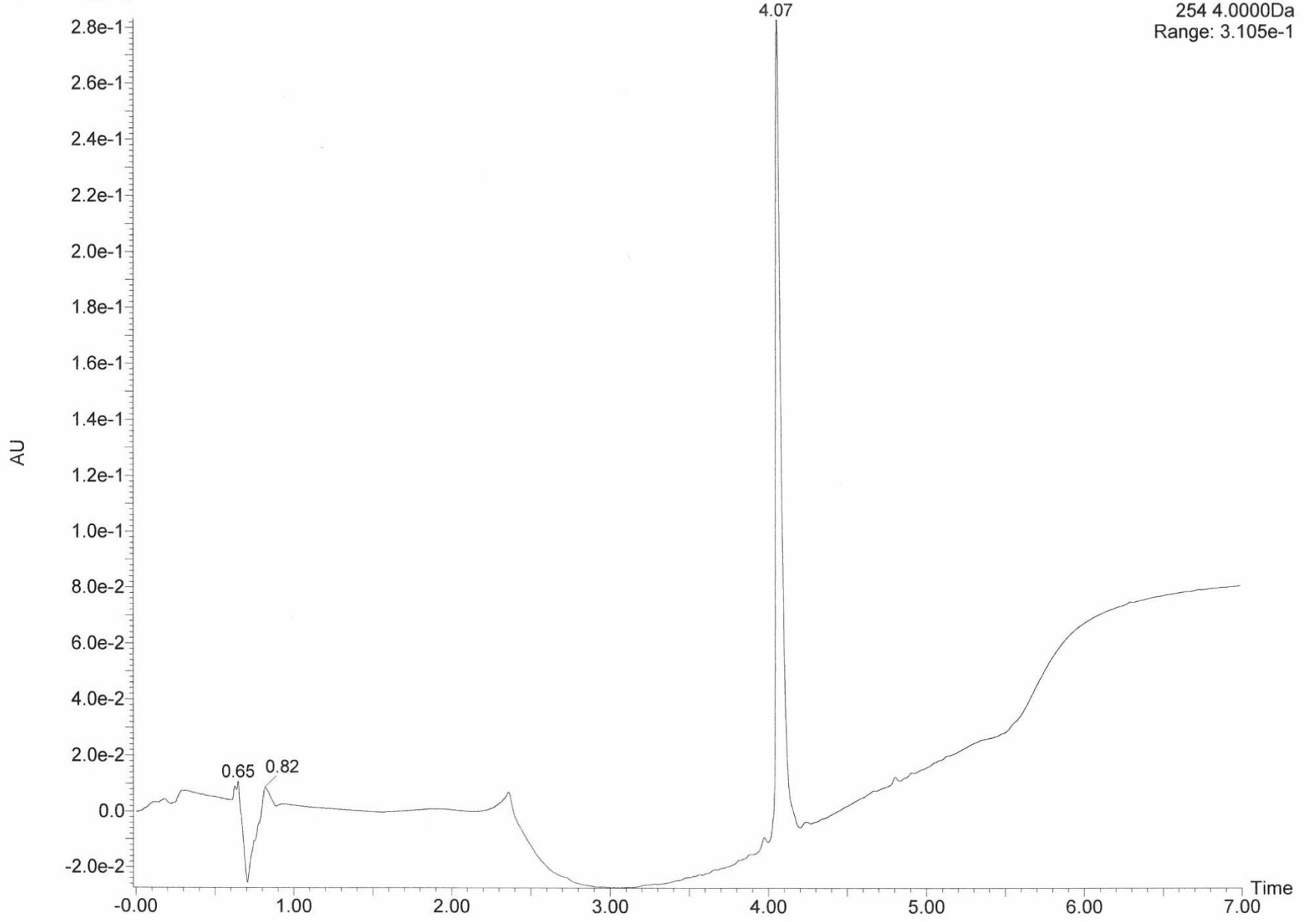
HRMS (ESI) C<sub>59</sub>H<sub>81</sub>O<sub>12</sub>N<sub>10</sub>P<sub>2</sub> [M + H]<sup>+</sup> calcd. 1183.55052, found 1183.55129





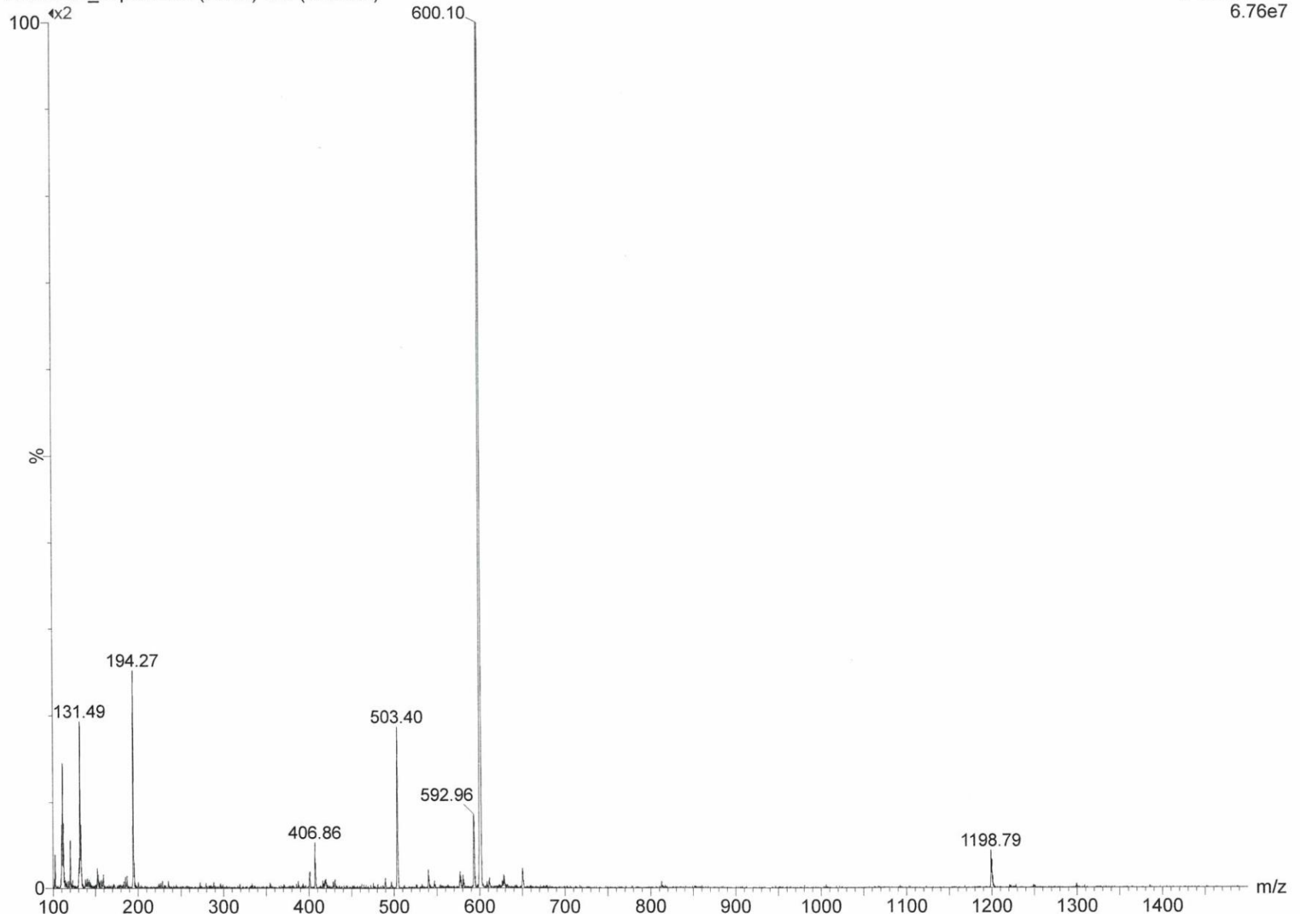
DA-XV-62\_6hplcB

3: Diode Array  
254 4.0000Da  
Range: 3.105e-1



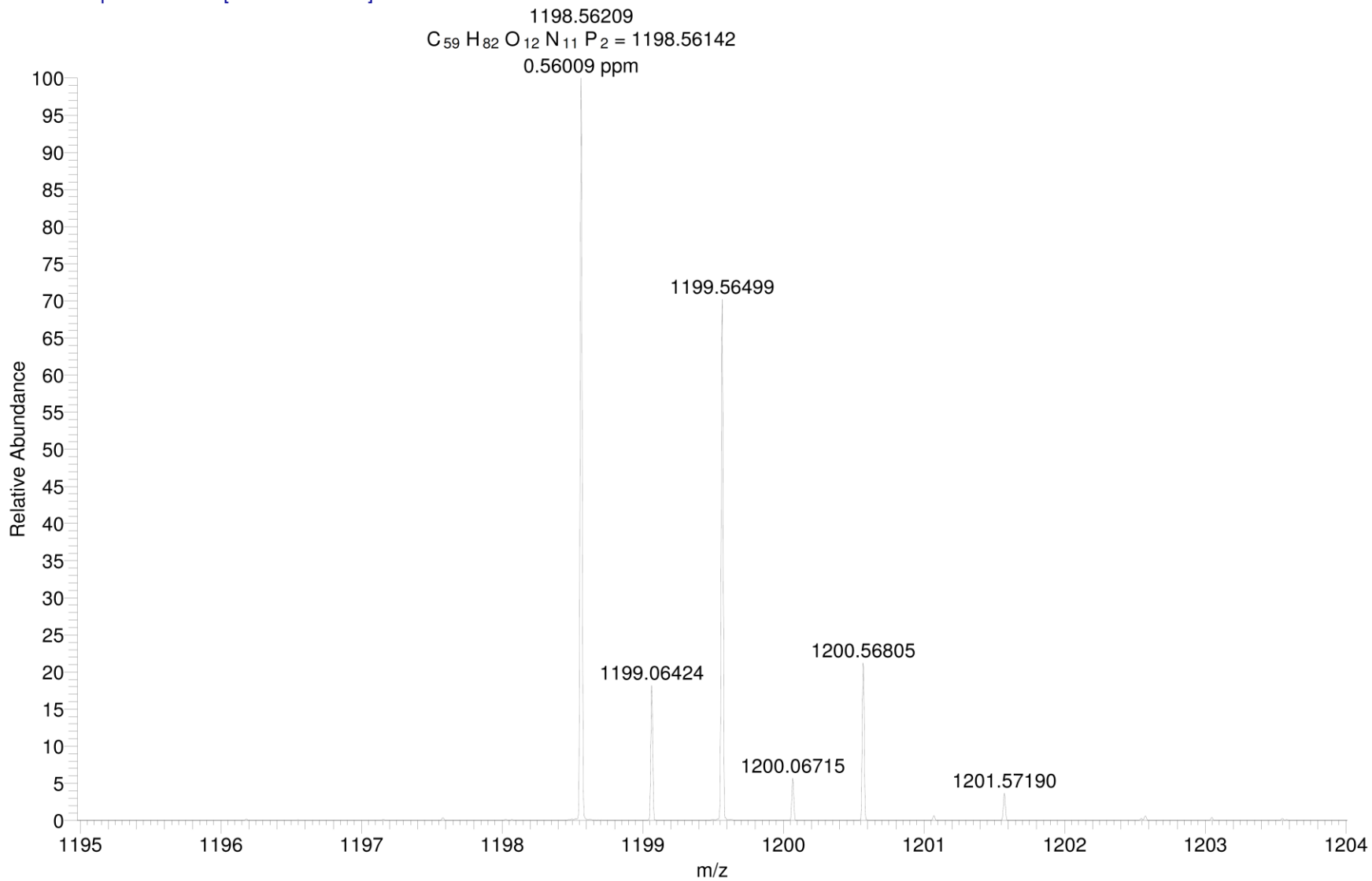
DA-XV-62\_6hplcB 455 (4.088) Cm (448:469)

1: Scan ES+  
6.76e7

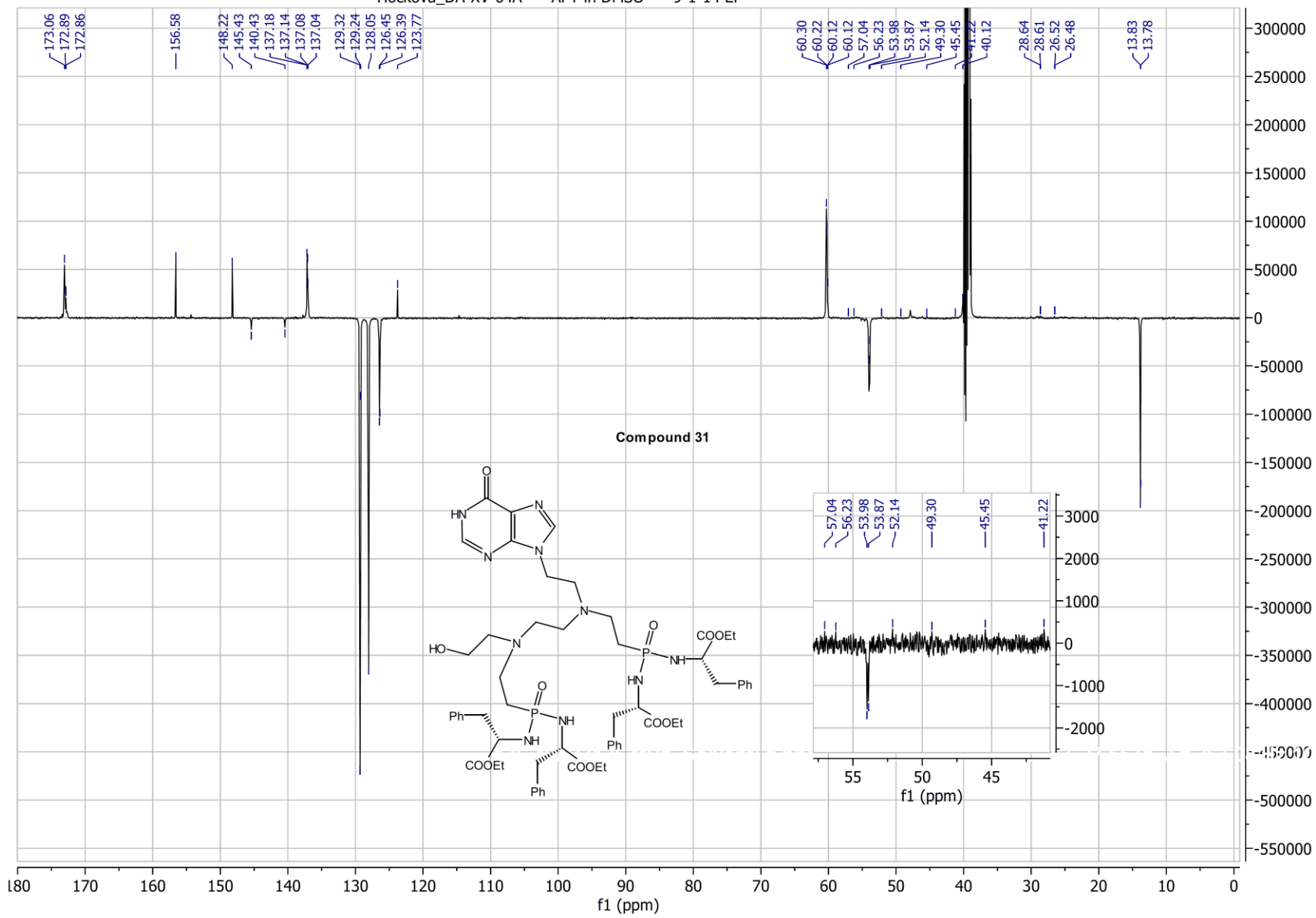


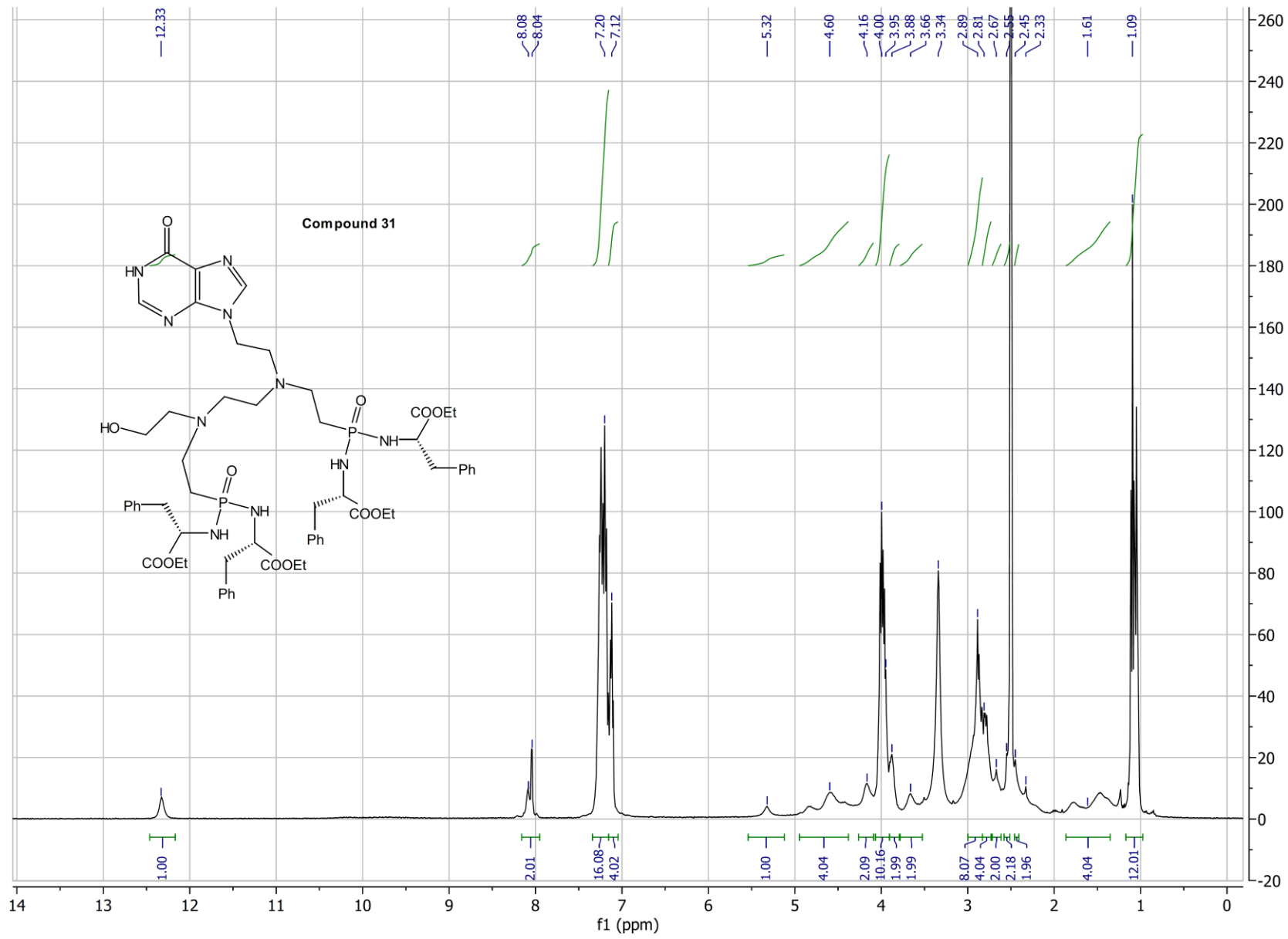


300115servisHR\_15 #59-65 RT: 1.65-1.81 AV: 7 NL: 1.03E7  
T: FTMS + p ESI Full ms [200.00-2000.00]



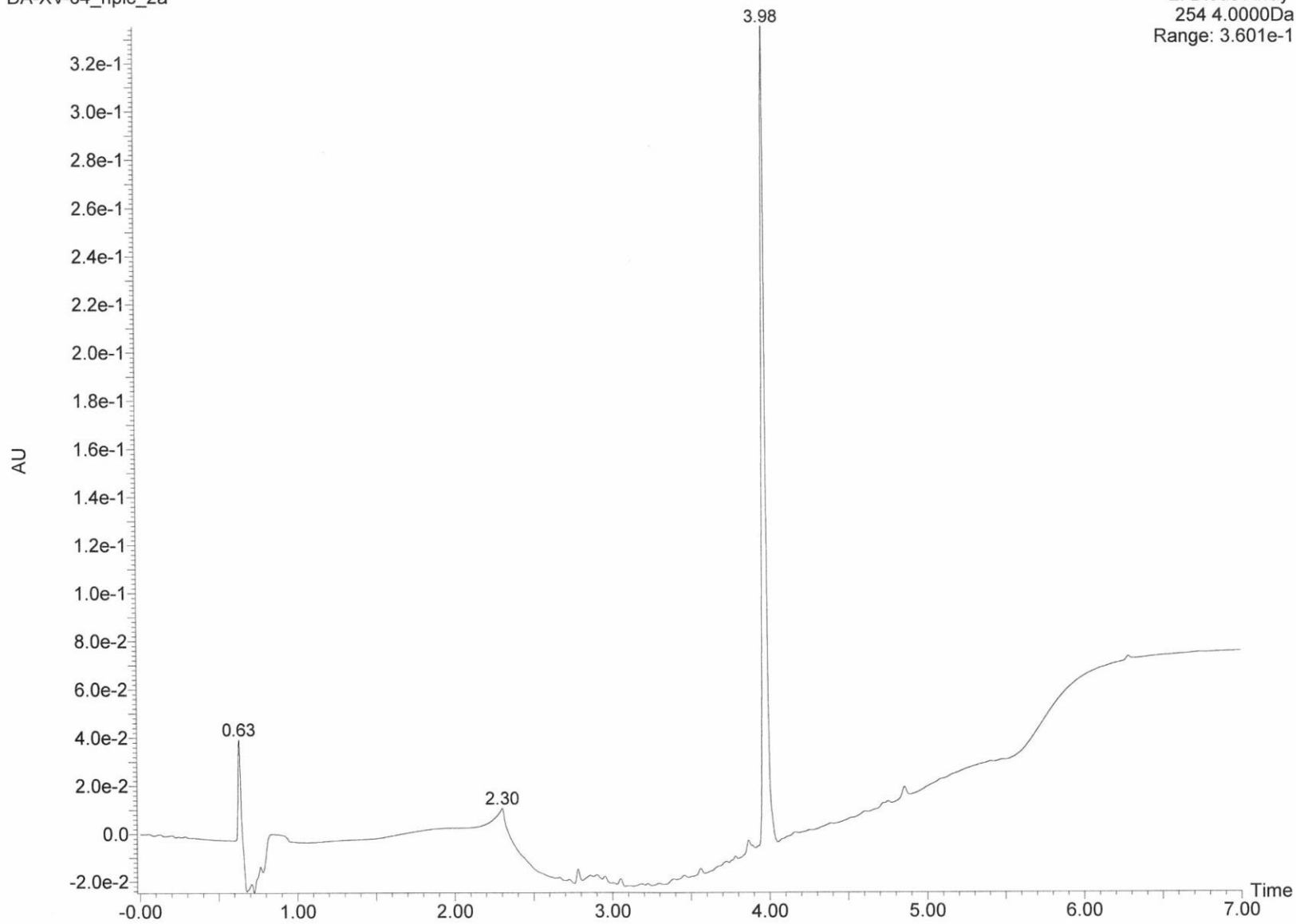
Hockova\_DA-XV-64A — APT in DMSO — 9-1-14 EP — \*\*\*\*\* —





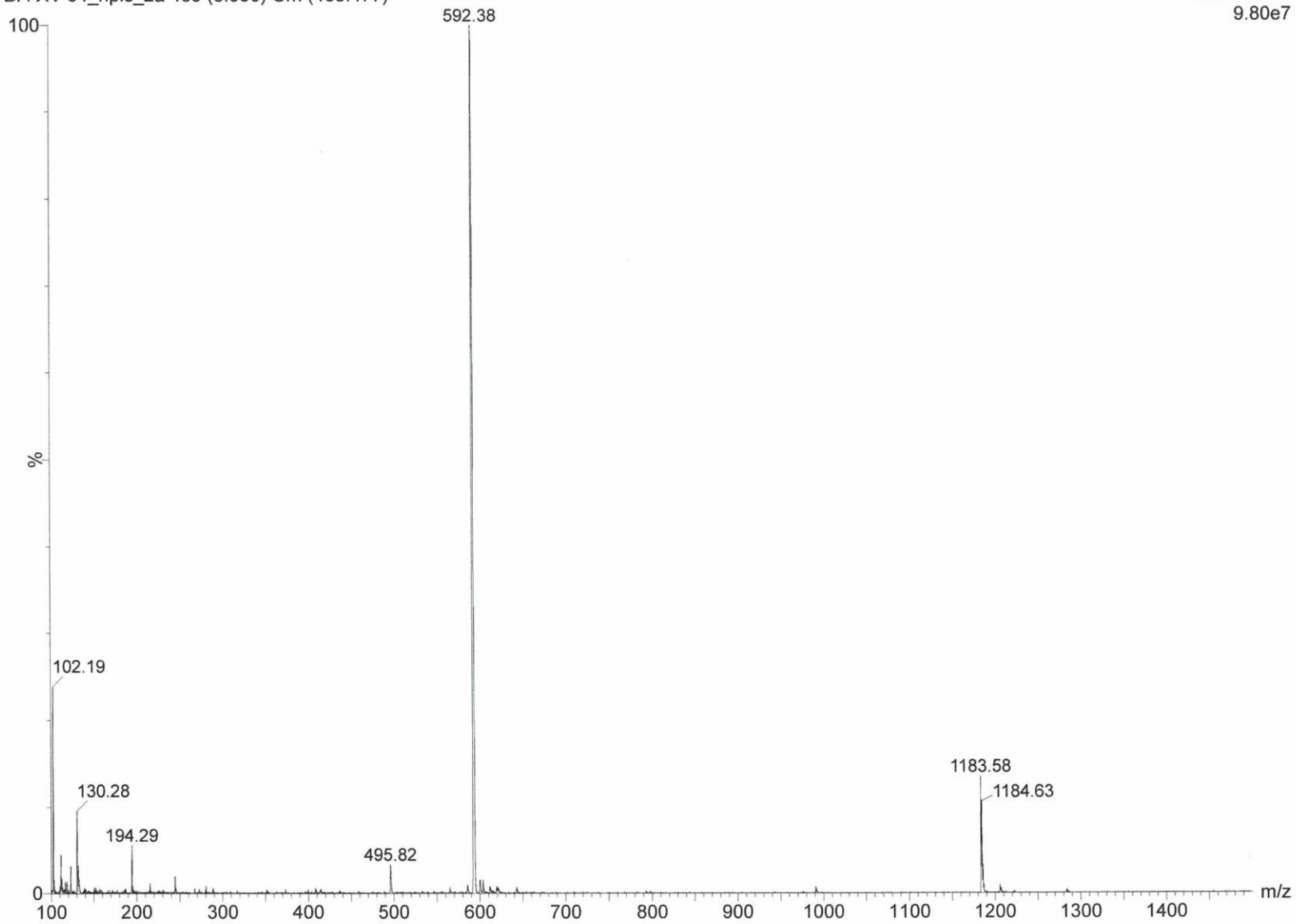
DA-XV-64\_hplc\_2a

2: Diode Array  
254 4.000Da  
Range: 3.601e-1

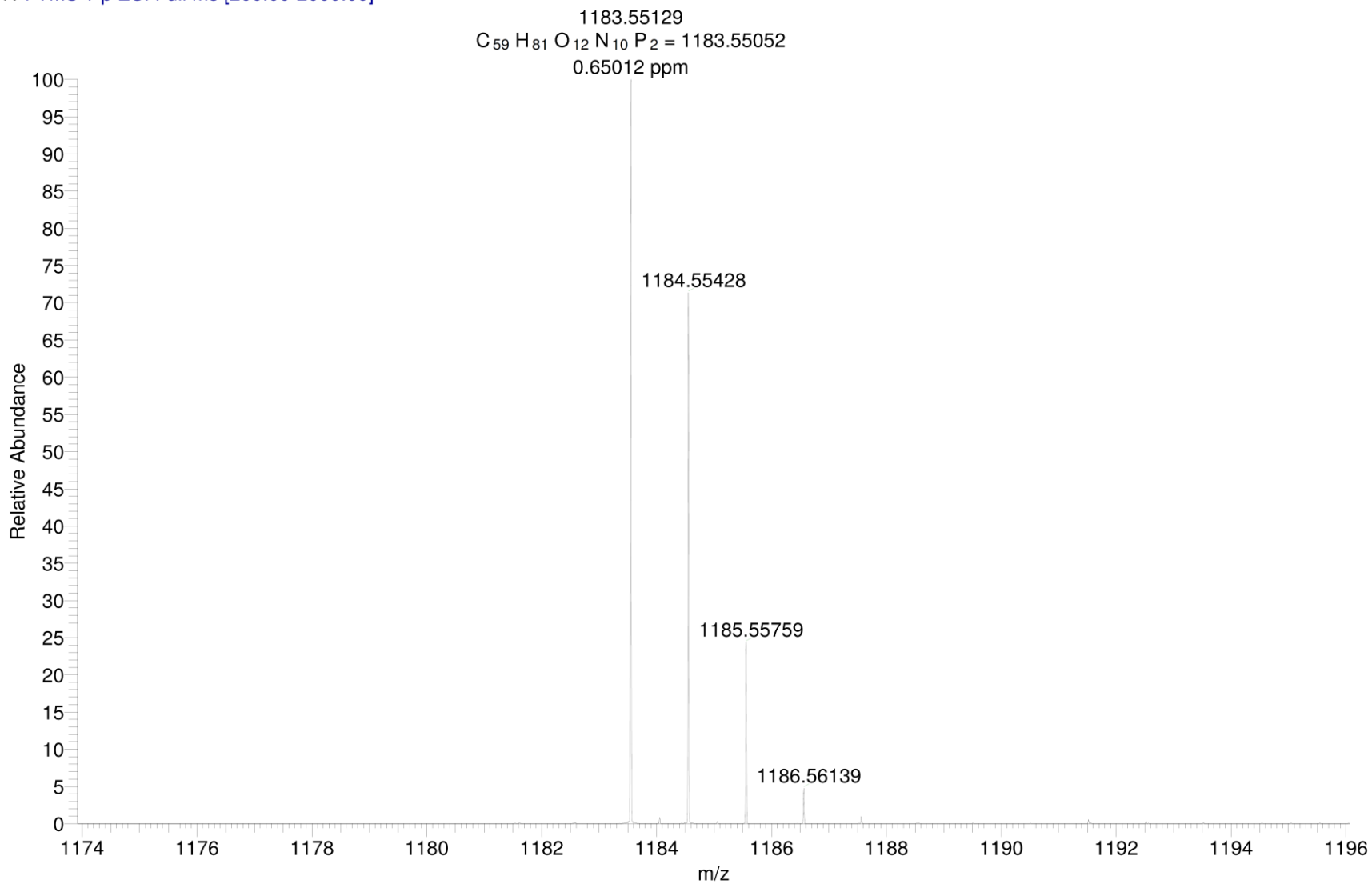


DA-XV-64\_hplc\_2a 469 (3.990) Cm (465:477)

1: Scan ES+  
9.80e7



300115servisHR\_16 #60-64 RT: 1.67-1.78 AV: 5 NL: 9.46E6  
T: FTMS + p ESI Full ms [200.00-2000.00]



**References:**

1. Knejzlik Z, Herkommerova K, Pichova I. 2019. Catabolism of 8-oxo-purines is mainly routed via the guanine to xanthine interconversion pathway in *Mycobacterium smegmatis*. *Tuberculosis (Edinb)* 119:101879.
2. Eng WS, Hockova D, Spacek P, Janeba Z, West NP, Woods K, Naesens LM, Keough DT, Guddat LW. 2015. First Crystal Structures of *Mycobacterium tuberculosis* 6-Oxopurine Phosphoribosyltransferase: Complexes with GMP and Pyrophosphate and with Acyclic Nucleoside Phosphonates Whose Prodrugs Have Antituberculosis Activity. *J Med Chem* 58:4822-38.