Supporting Information

Increased Selectivity towards Cytoplasmic *versus* Mitochondrial Ribosome Confers Improved Efficiency of Synthetic Aminoglycosides in Fixing Damaged Genes: A Strategy for Treatment of Genetic Diseases Caused by Nonsense Mutations

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Content of the Supporting Information (SI)

Table S1. Comparative cell toxicity and antibacterial activity data of gentamicin, G418 and synthetic compounds **1-12**[a]

Aminoglycoside	Cell toxicity LC_{50} (mM)[b]	Antibacterial activity MIC (μM)[c]
Gentamicin	3.2 ± 0.3	<0.75
Paromomycin	3.1 ± 0.4	1.2
G418	1.6 ± 0.1	<1.25
1	22.4 ± 0.9	100
2	7.0 ± 0.4	70
3	21.3 ± 1.7	42
4	16.3 ± 0.5	70
(*S*)-**5**	21.8 ± 0.5	83
(*R*)-**6**	20.1 ± 0.7	78
(*S*)-**7**	8.1 ± 1.4	33
(*R*)-**8**	19.3 ± 1.5	33
(*S*)-**9**	4.8 ± 0.3	96
(*R*)-**10**	7.6 ± 0.2	96
(*S*)-**11**	6.5 ± 0.3	192
(*R*)-**12**	2.8 ± 0.1	96

[a] All tested AGs were in their sulfate salt forms and the concentrations reported refer to that of the free amine form of each AG. All assays were performed in duplicate and analogous results were obtained in at least two or three experiments. [b] Cell toxicity was measured in human foreskin fibroblast (HFF) cells, and calculated as a ratio between the numbers of living cells in cultures grown in the presence of the tested compound, versus cultures grown without compound. The half-maximal lethal concentration (LC_{50}) values were obtained from fitting concentration-response curves using GraFit5 software. [c] The minimal inhibitory concentration (MIC) values were measured in *B. subtilis* ATCC6633 and determined by using the double-microdilution method, with two different starting concentrations of each tested compound (384 μg/mL and 6,144 μg/mL).

Table S2. Correlation between eukaryotic inhibition of translation (IC_{50}^{Euk}) and *in vitro* readthrough activity at 1.4 μM concentration of a series of aminoglycosides tested [a]

Aminoglycoside	IC_{50}^{Euk} (μM) [b]	R3X [c]	R245X [c]	G542X [c]	W1282X [c]	Q70X [c]	R3381X [c]
Gentamicin	62±9	0.4	0.08	0.15	0.11	0.12	0.12
Paromomycin	57±4	0.6	0.112	0.11	0.15	0.16	0.13
1	31±4	0.2	0.072	0.09	0.09	0.09	0.07
(*R*)-**6**	28±1.1	0.3	0.068	0.05	0.07	0.13	0.22
2	24±1	**1.85**	0.23	0.21	0.35	0.17	0.2
3	17±0.6	0.85	0.143	0.13	0.24	0.29	0.16
(*S*)-**5**	16±1	0.95	0.132	0.09	0.15	0.2	0.4
(*R*)-**10**	7.96±0.27	2	0.2	0.24	0.26	0.26	0.32
(*S*)-**7**	5.2±0.7	3.8	1.7	1.18	1.6	0.67	1.2
(*R*)-**8**	4.6±0.6	5	1.33	0.86	1.8	0.49	0.8
4	2.8±0.3	2.1	1	0.87	1.5	0.58	0.7
G418	2.0±0.3	7.3	2.6	3.6	2.4	1.4	3.5
(*S*)-**9**	1.49±0.08	7.4	**0.67**	1.4	**1**	0.78	1.1
(*R*)-**12**	0.89±0.07	3.8	1.15	2	3	1.7	1.6
(*S*)-**11**	0.73±0.07	6	1.2	4.4	4.2	1.1	2.4

[a] In all biological tests, all tested aminoglycosides were in their sulfate salt forms. The concentrations reported refer to that of the free amine form of each aminoglycoside. [b] Eukaryotic translation inhibition (IC_{50}^{Euk} values) was quantified as described in the experimental part. [c] *In vitro* stop codon suppression levels are at a single cocentration, 1.4 μM, of each compund tested and were taken from the data in Fig. 3 and 4. All the experiments were performed in duplicates and analogous results were obtained in three different experiments. The exceptional data points are labeled in red.

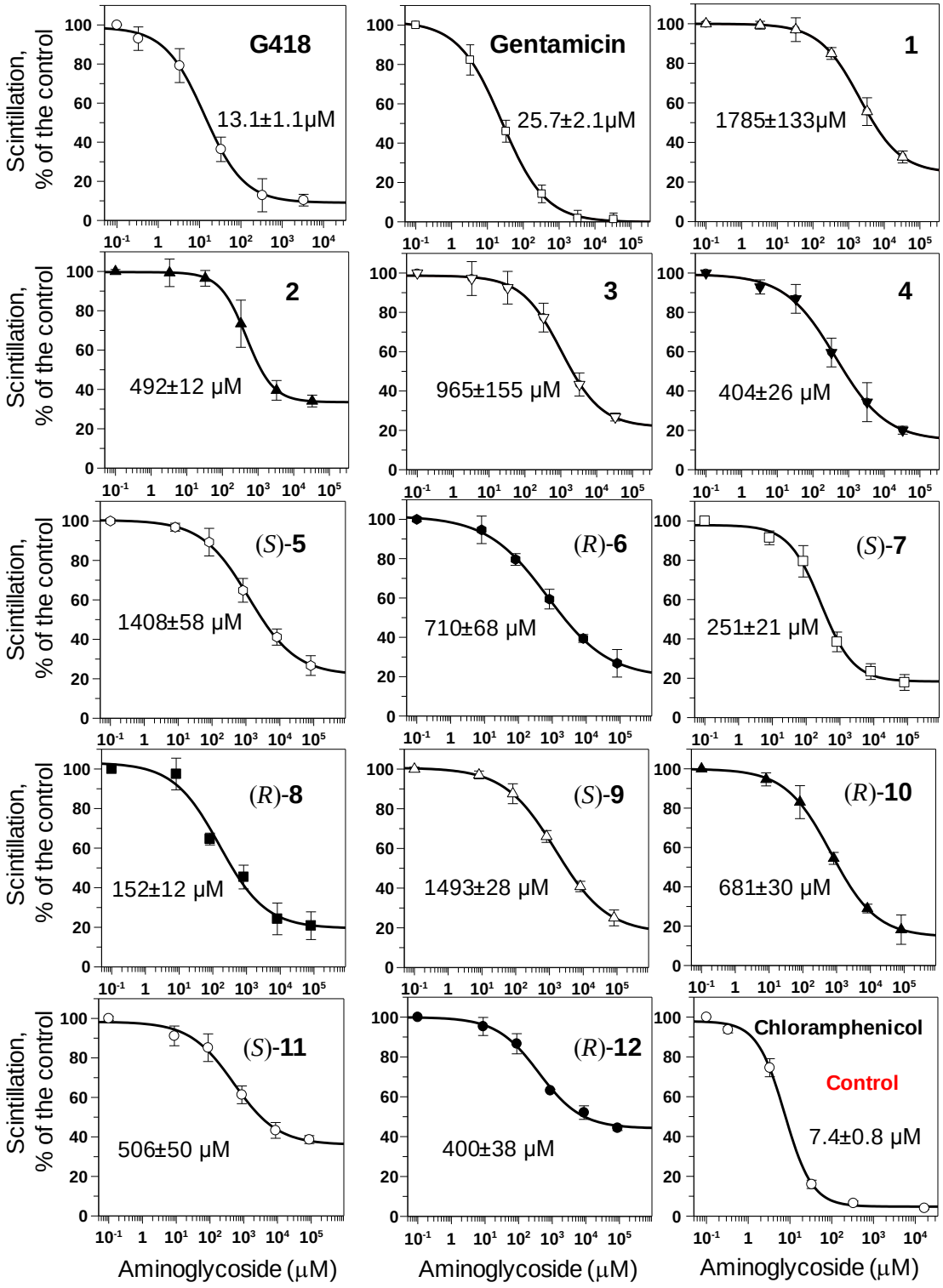


Figure S1. Semi logarithmic plots representing dose-response effect of G418, gentamicin, **1**, **2**, **3**, **4**, (*S*)-**5**, (*R*)-**6**, (*S*)-**7**, (*R*)-**8**, (*S*)-**9**, (*R*)-**10**, (*S*)-**11** and (*R*)-**12** on the inhibition of intact mitochondrial protein translation. Chloramphenicol was used as a control and is highlighted in red. The results are averages of at least three independent experiments.

Purity determination of the novel compounds 9-12.

Purity of the new compounds **9-12** were determined by using HPLC-ESI-MS analysis. The chromatographic separation of all the compounds was achieved by using the HPLC system (Acquity UPLC, Waters) and a 1.7µ (50 mm x 2.1 mm) column (Phenomenex Finetex Hilic). Sample aliquots of 3 µL were injected onto the column at a flow-rate of 300 µL/min. The HPLC separation conditions and the MS data (LCT Premier mass spectrometer, Waters) were as follow:

HPLC-ESI-MS conditions:

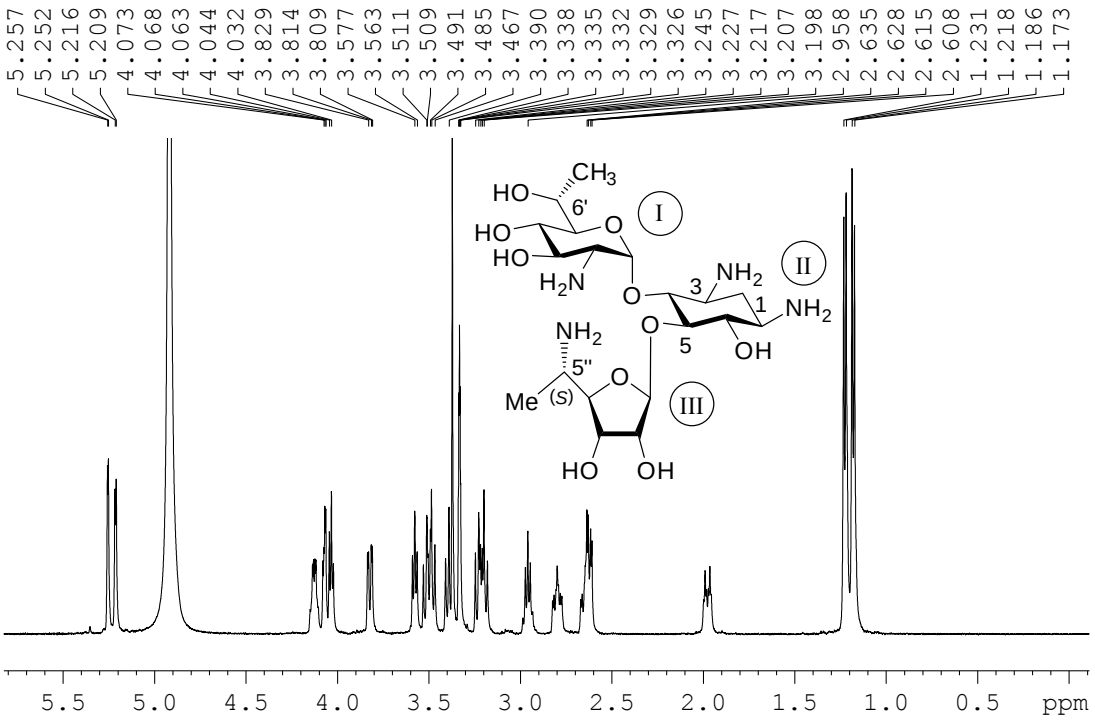
Time Duration (min)	0.1% Formic acid in Acetonitrile (%)	30mM ammonium formate pH=3.5 in Water (%)
0	75	25
3	75	25
7	50	50
10	50	50
11	75	25
15	75	25

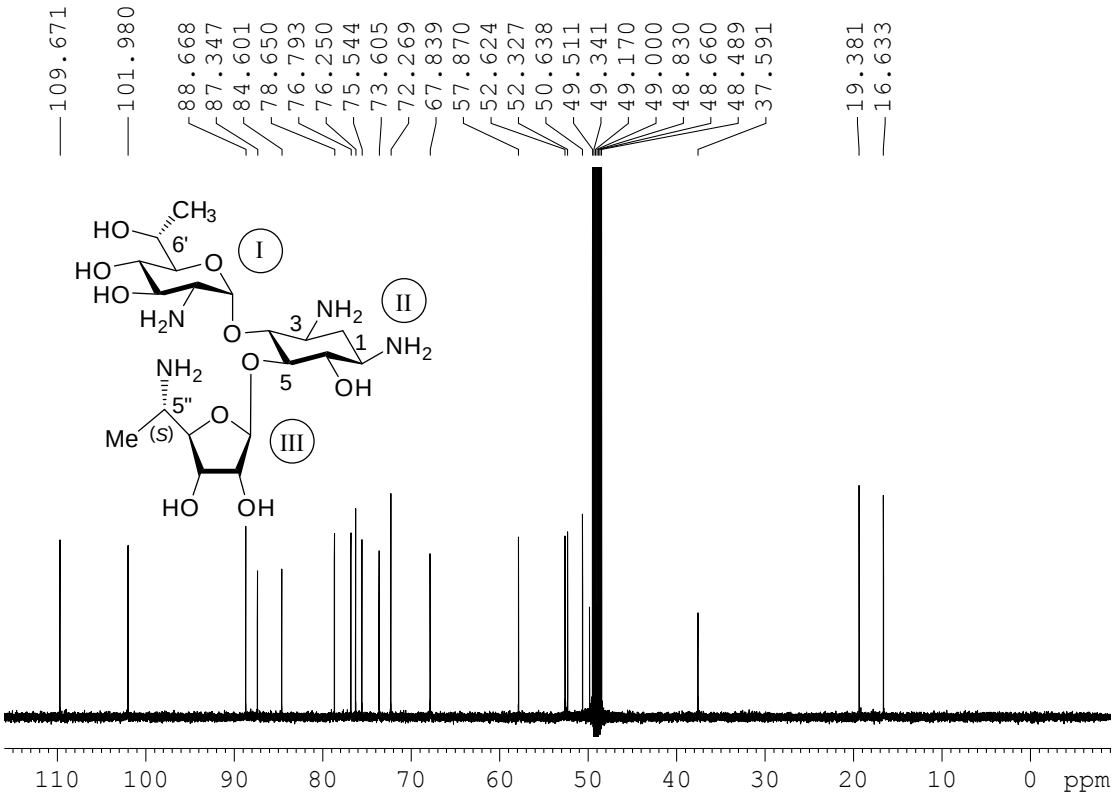
Retention time, purity and HRMS data:

Compound	Retention Time (min)	Purity (%)	HRMS (m/z)
(*S*)-**9**	6.12	99.54	483.2667
(*R*)-**10**	5.89	99.21	483.2639
(*S*)-**11**	7.14	95.21	583.3041
(*R*)-**12**	6.04	97.30	584.3124

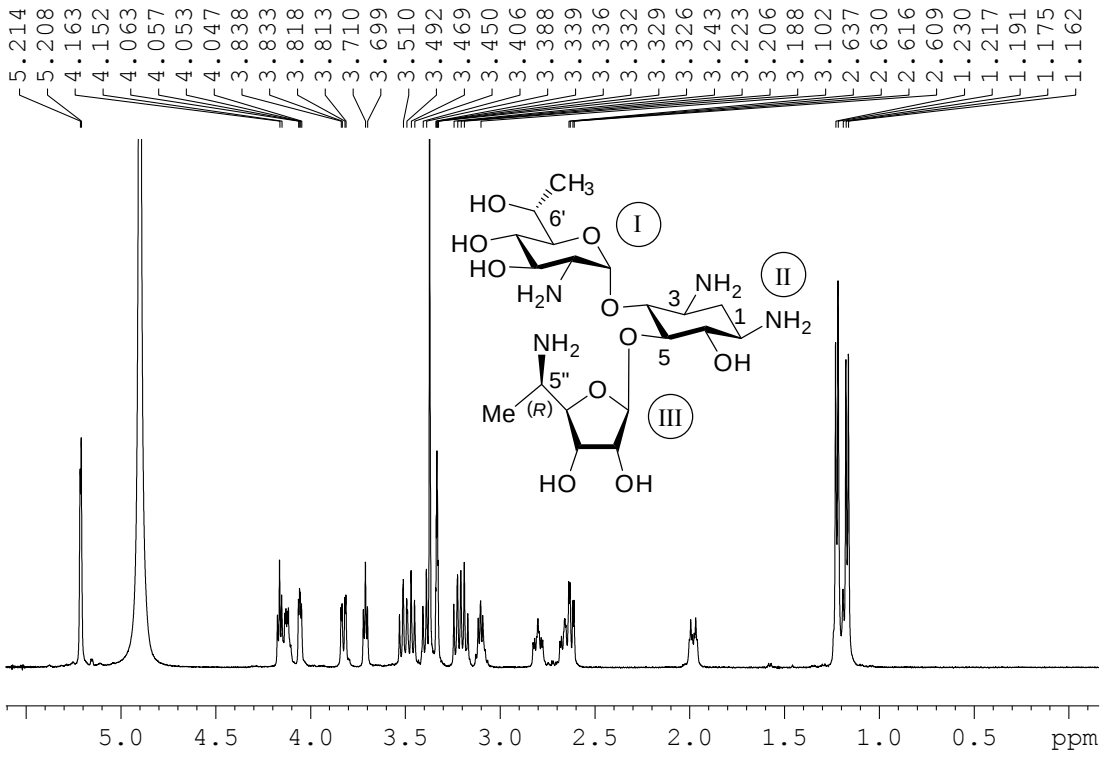
The ^{1}H and ^{13}C NMR spectra, HPLC purification profiles, along with the observed mass spectral analysis data of **9-12** are attached.

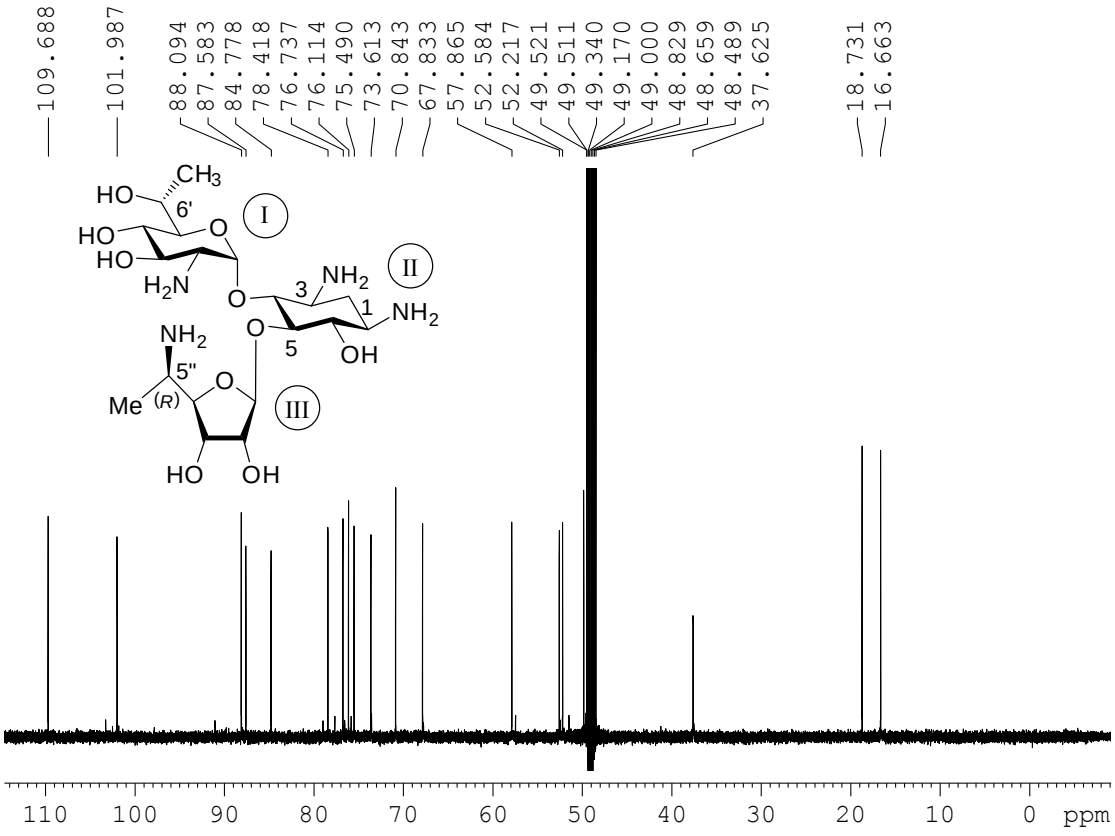
^{1}H and ^{13}C NMR spectra of (*S*)-**9** in CD_3OD



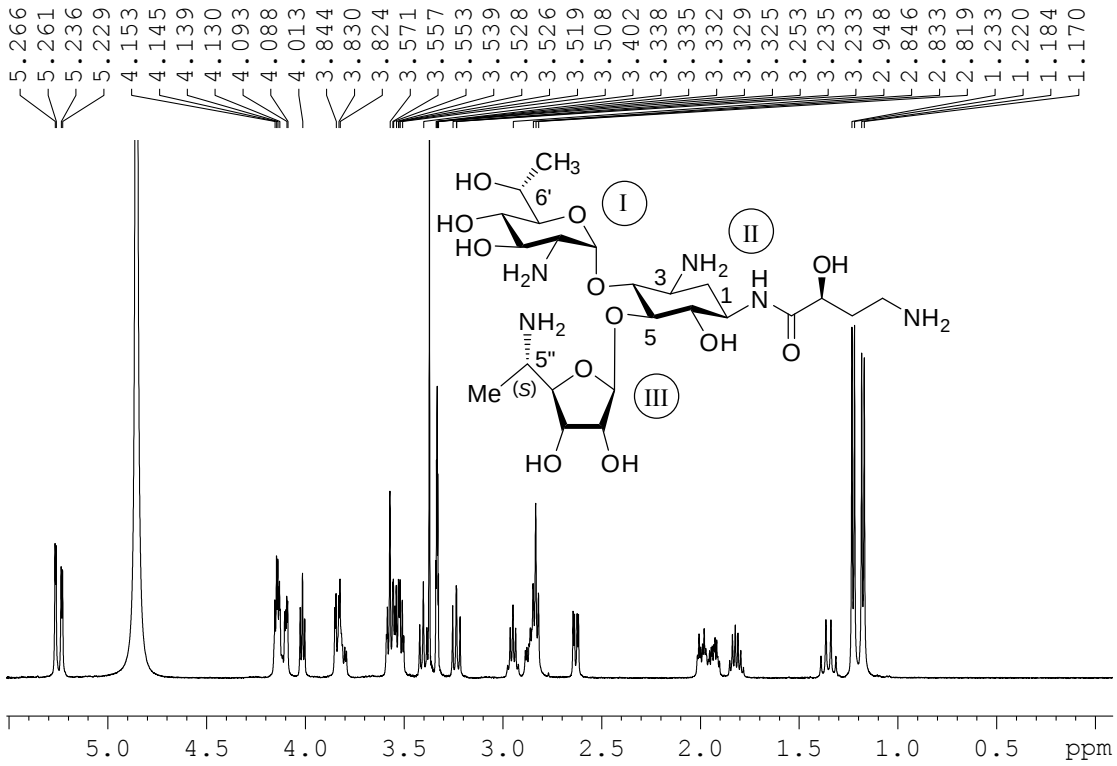


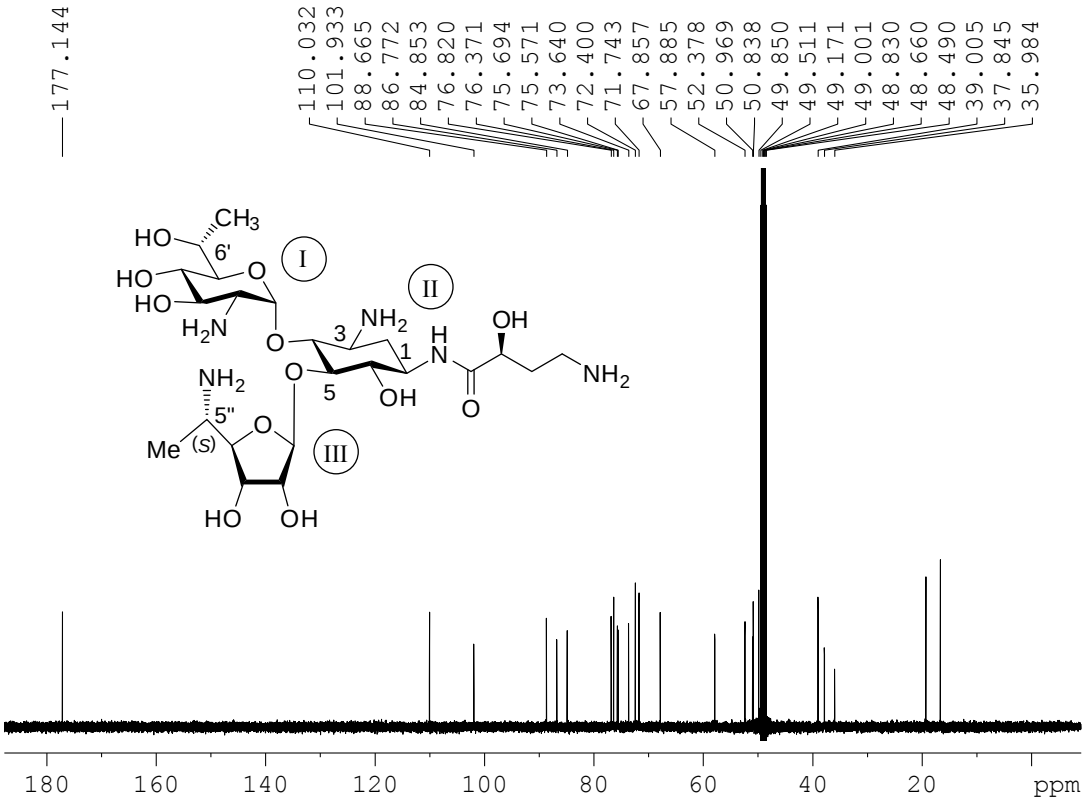
^{1}H and ^{13}C NMR spectra of (*R*)-**10** in CD_3OD



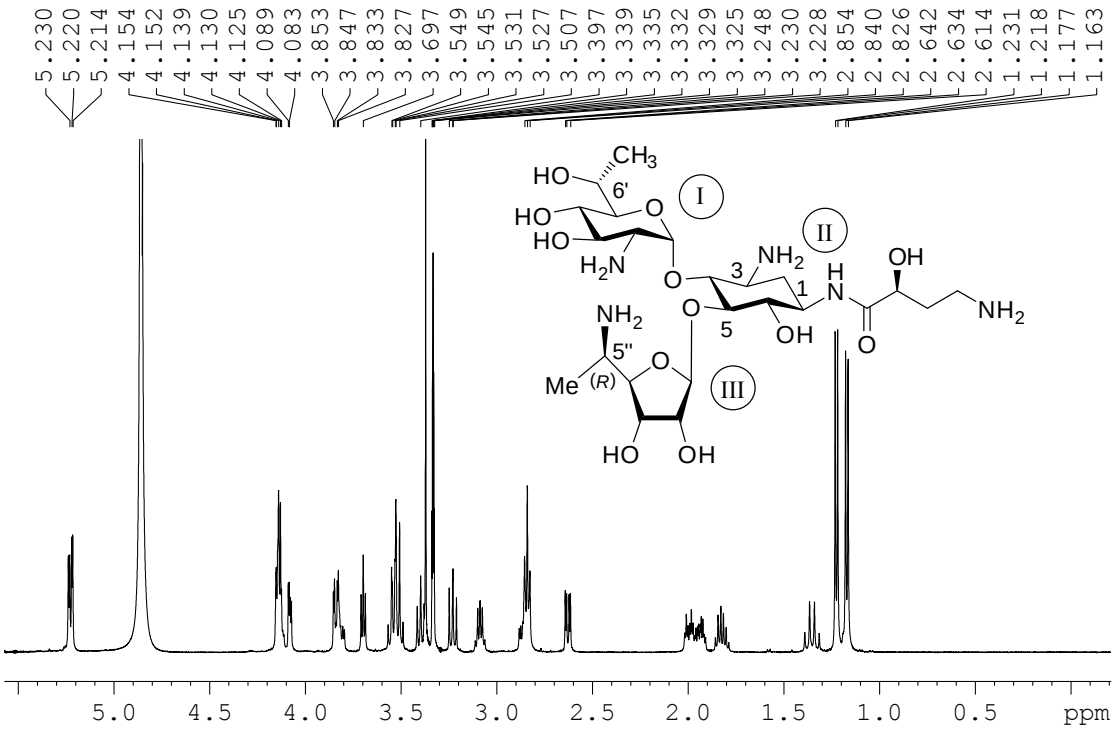


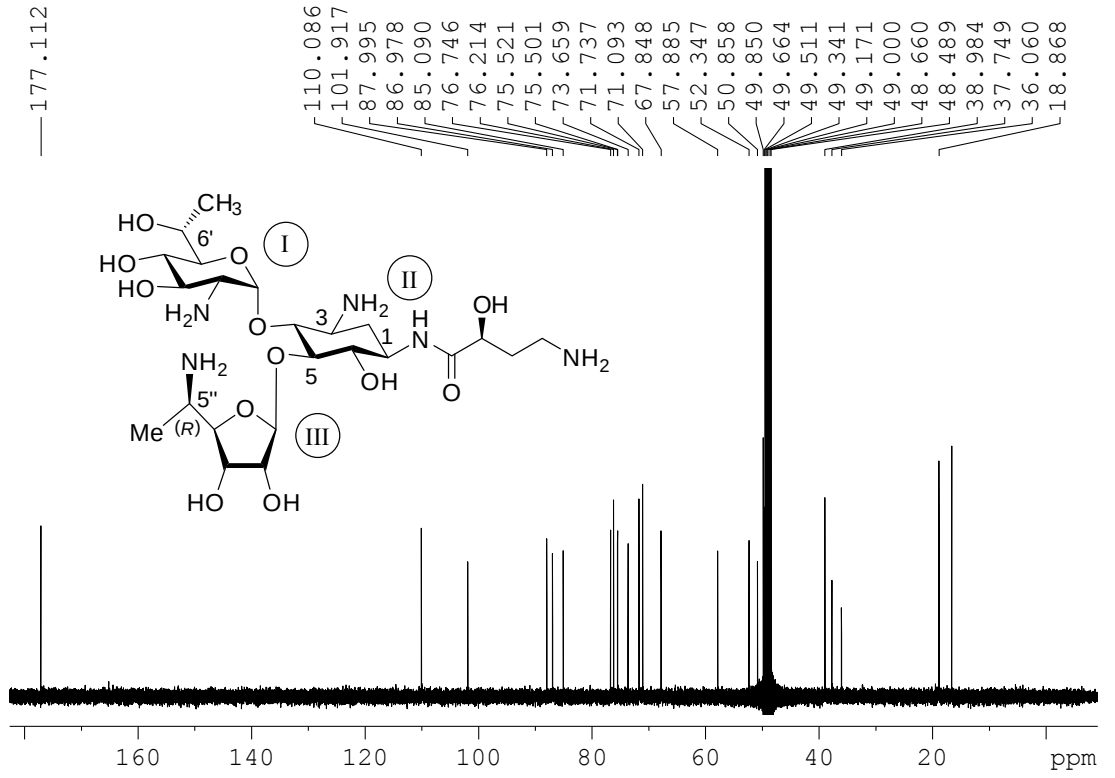
^{1}H and ^{13}C NMR spectra of (*S*)-**11** in CD_3OD

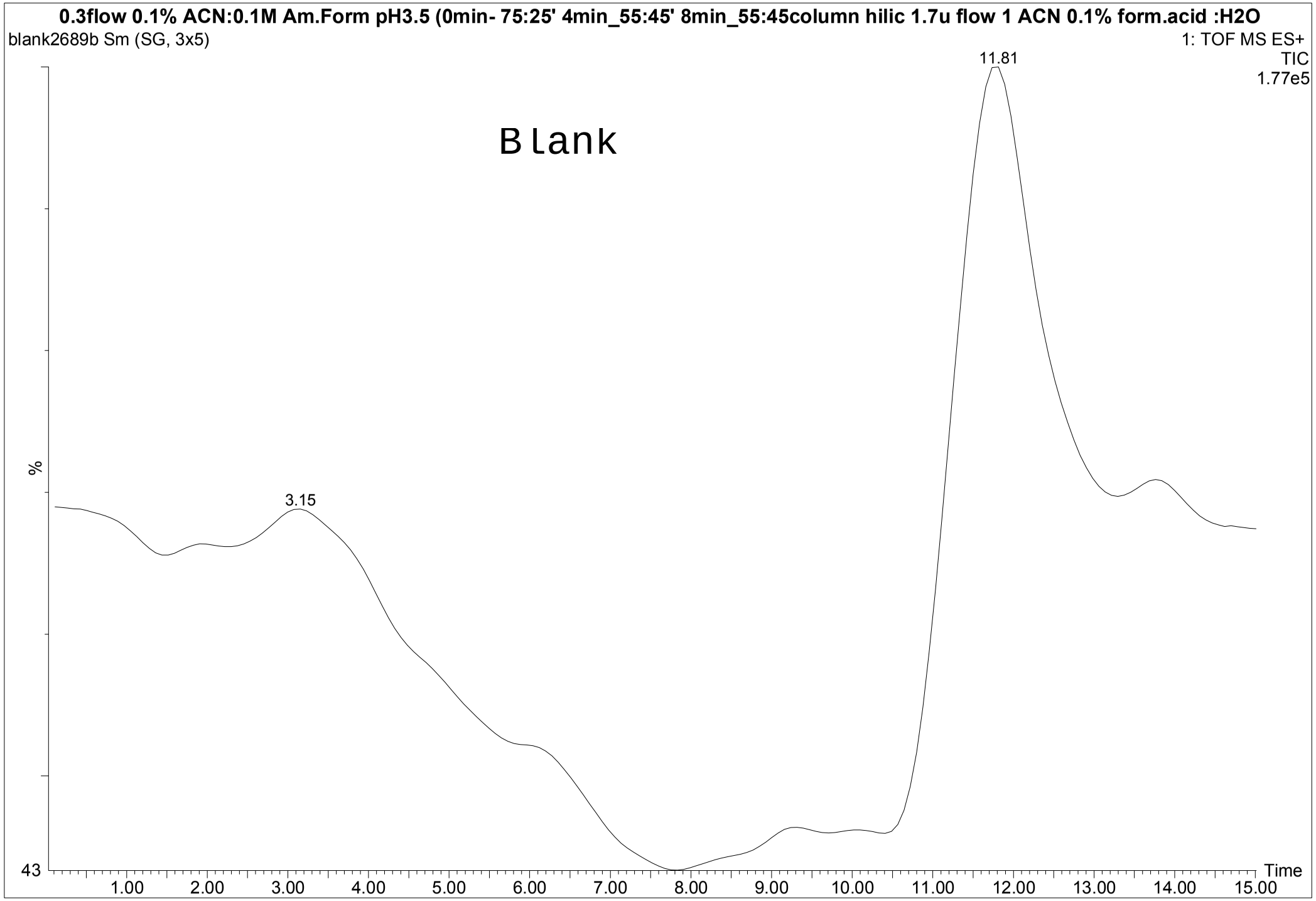




^{1}H and ^{13}C NMR spectra of (*R*)-**12** in CD_3OD



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blank2689b Sm (SG, 3x5)
1: TOF MS ES+
TIC
1.77e5
11.81
Blank
3.15
S10
%
43
Time
1.00
2.00
3.00
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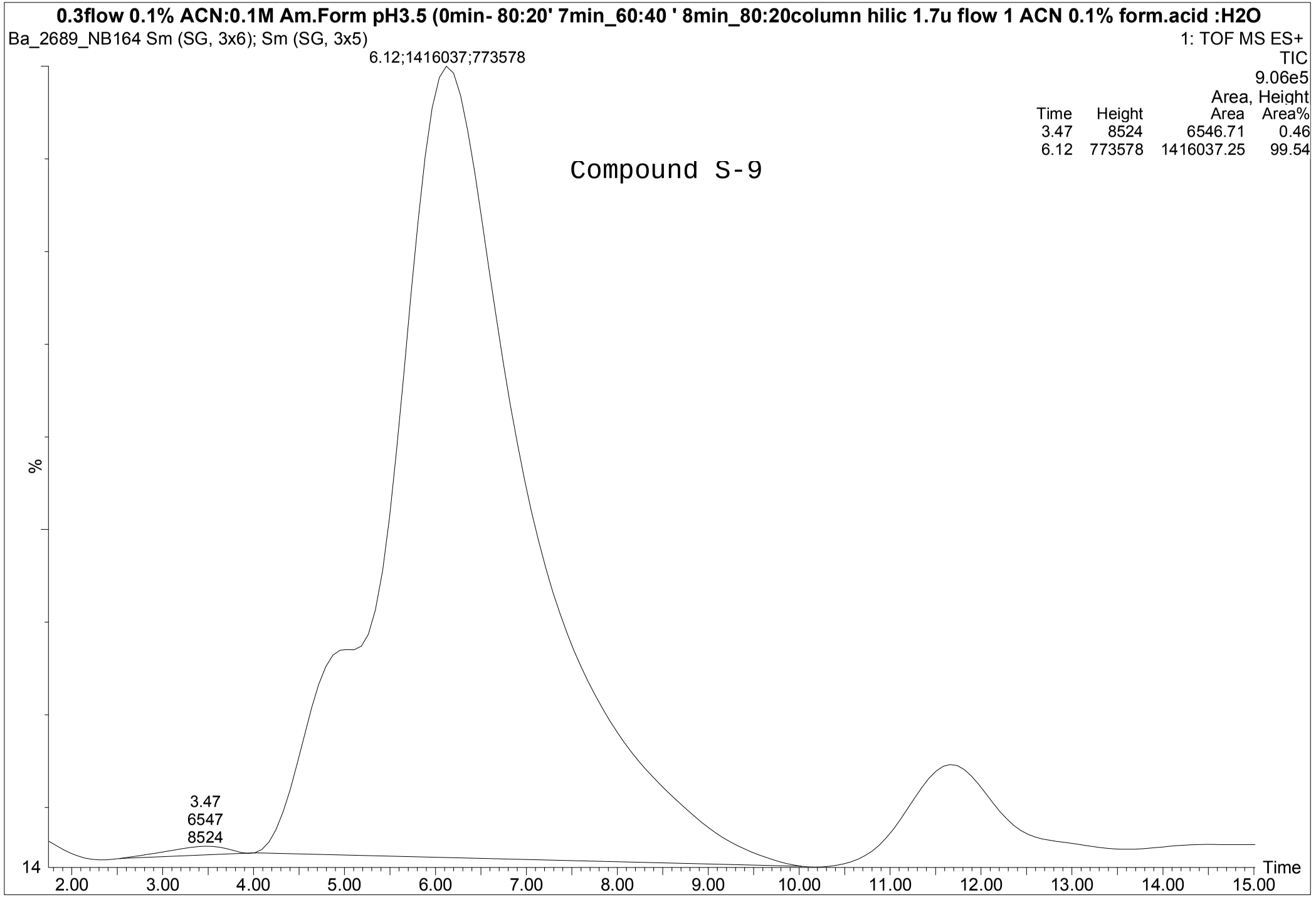
10.00
11.00
12.00
13.00
14.00
15.00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Ba_2689_NB164 Sm (SG, 3x6); Sm (SG, 3x5)
1: TOF MS ES+
TIC
9.06e5
Area, Height
Time Height Area Area%
3.47 8524 6546.71 0.46
6.12 773578 1416037.25 99.54
6.12;1416037;773578
Compound S-9
3.47
6547
8524
SII
%
14
Time
2.00
3.00
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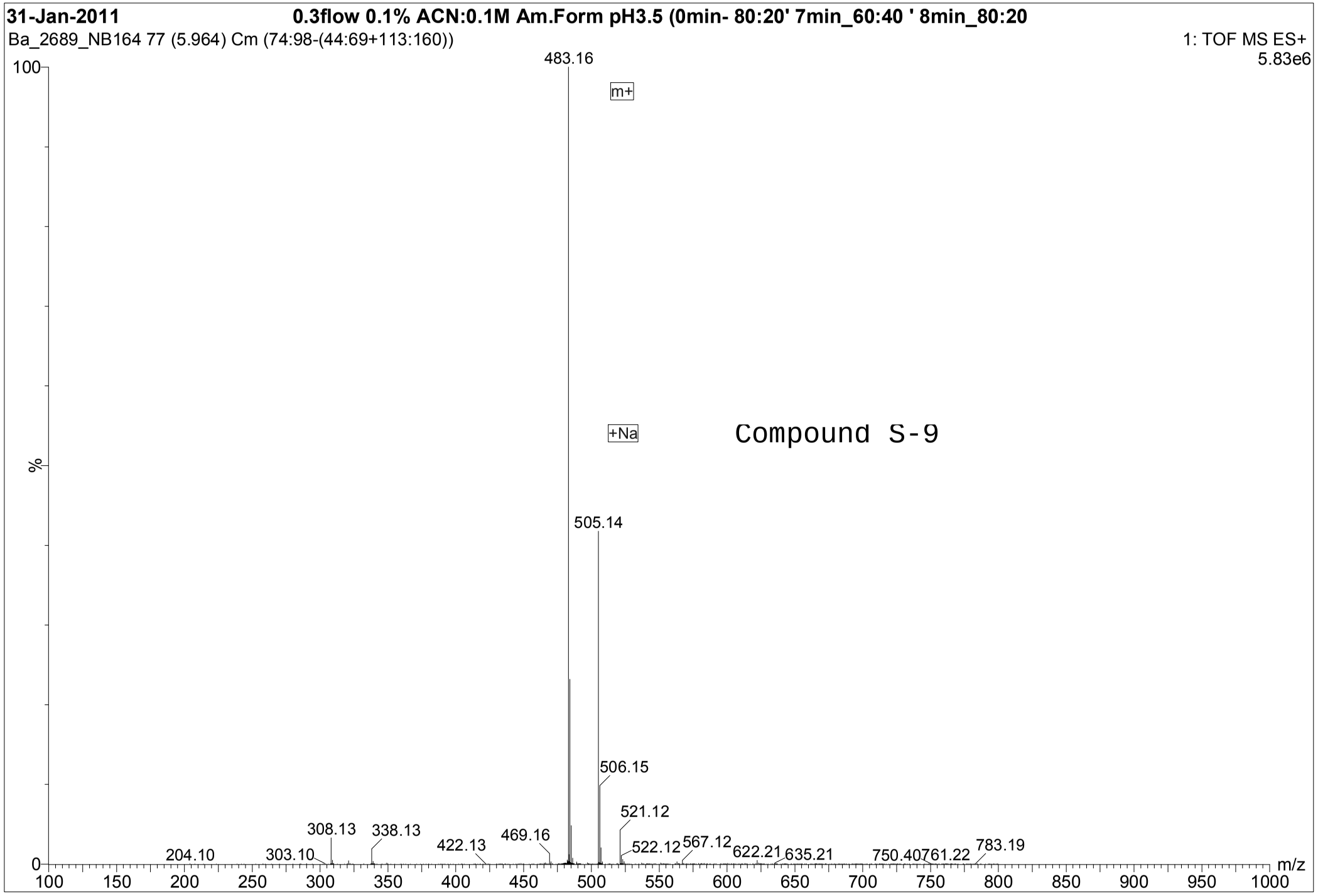
10.00
11.00
12.00
13.00
14.00
15.00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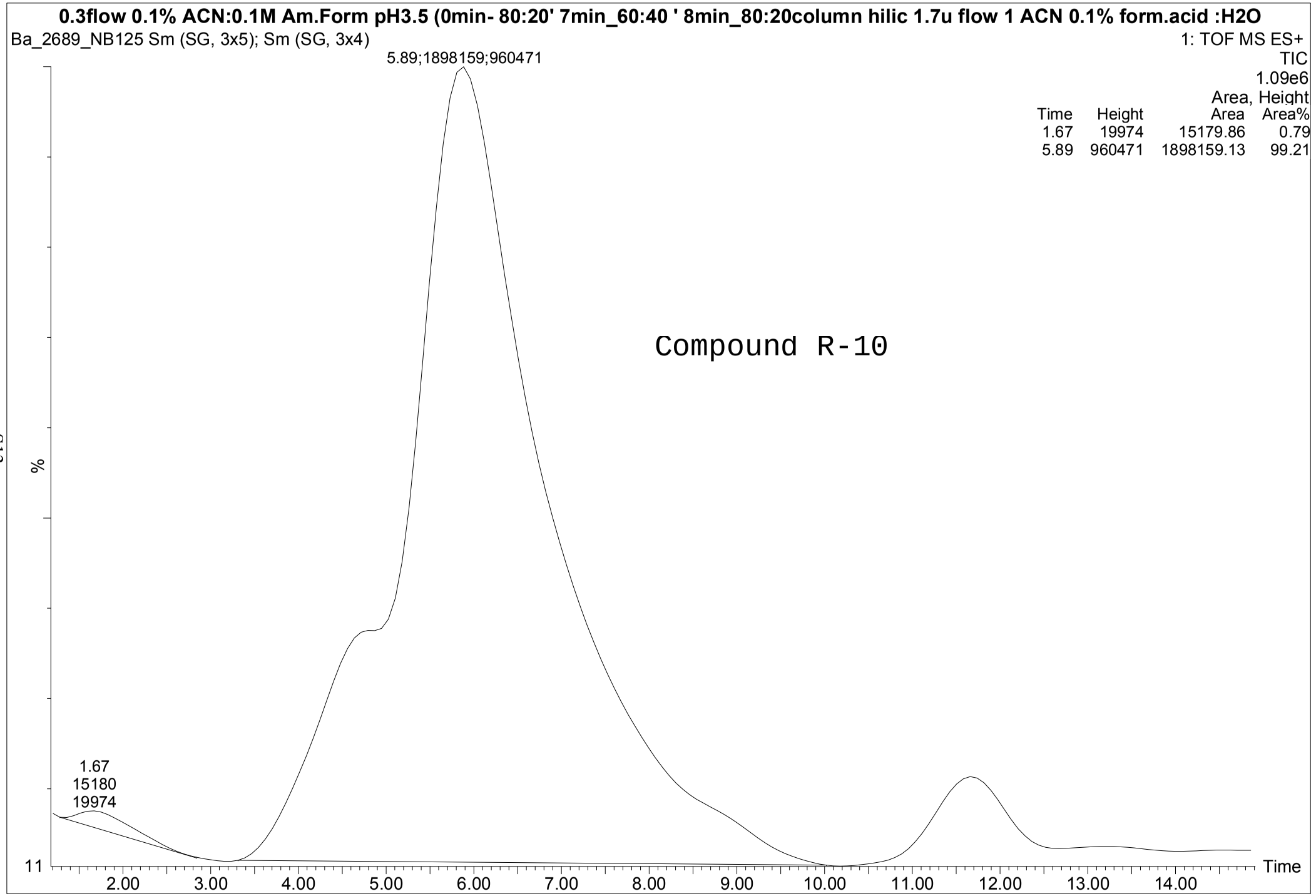
1: TOF MS ES+
5.83e6
100
%
0
483.16
m+
+Na
505.14
506.15
521.12
308.13
338.13
422.13
469.16
204.10
303.10
522.12
567.12
622.21
635.21
750.40
761.22
783.19
m/z
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000

Compound S-9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Ba_2689_NB125 Sm (SG, 3x5); Sm (SG, 3x4)
1: TOF MS ES+
TIC
1.09e6
Area, Height
Time Height Area Area%
1.67 19974 15179.86 0.79
5.89 960471 1898159.13 99.21
5.89;1898159;960471
Compound R-10
1.67
15180
19974
%
11
Time
2.00
3.00
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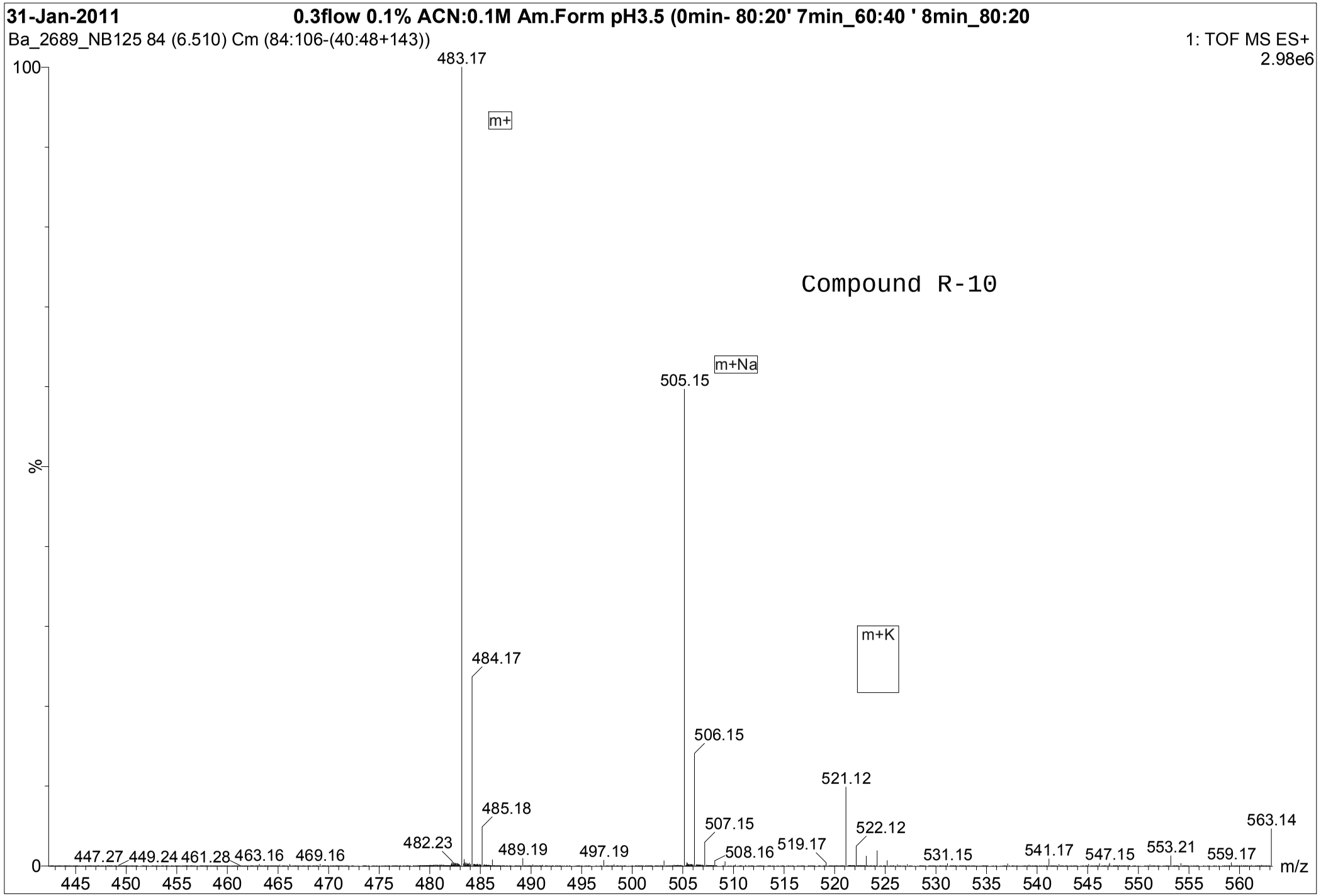
9.00
10.00
11.00
12.00
13.00
14.00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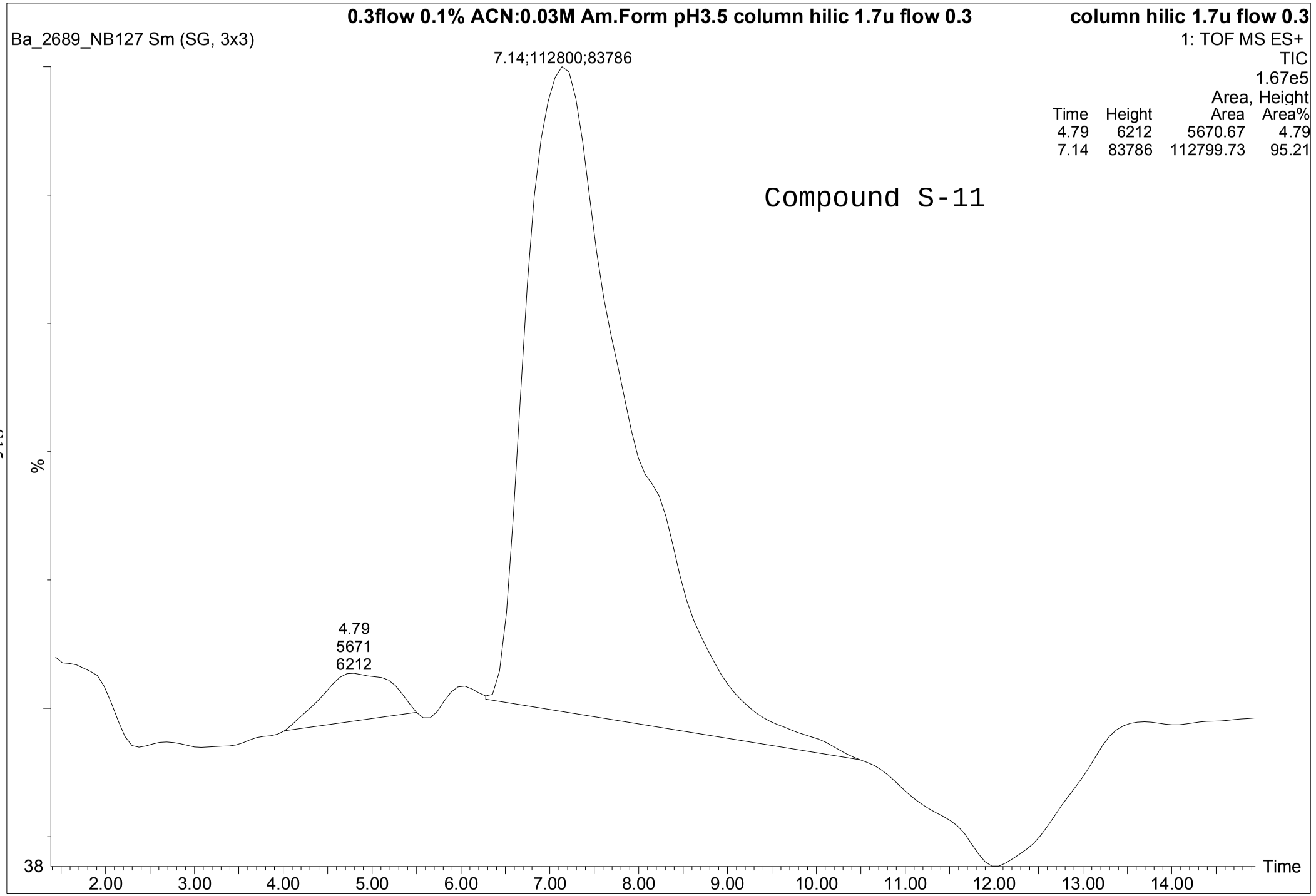
1: TOF MS ES+
2.98e6
100
%
0
483.17
m+
505.15
m+Na
m+K
484.17
506.15
521.12
485.18
507.15
522.12
563.14
482.23
489.19
497.19
508.16
519.17
531.15
541.17
547.15
553.21
559.17
447.27
449.24
461.28
463.16
469.16
445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560
m/z
Compound R-10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column hilic 1.7u flow 0.3
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1: TOF MS ES+
TIC
1.67e5
Area, Height
Time Height Area Area%
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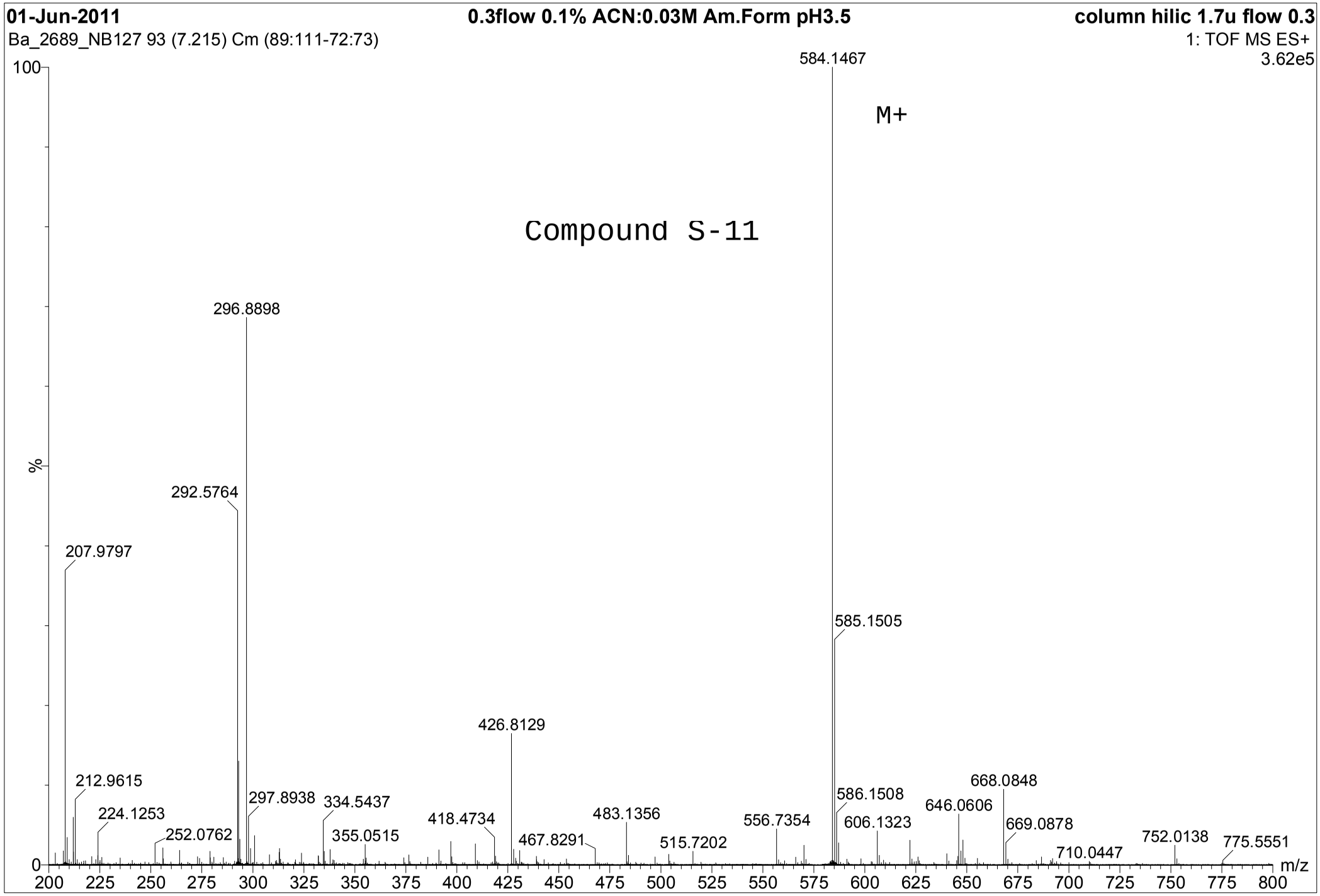
7.14 83786 112799.73 95.21
7.14;112800;83786
Compound S-11
4.79
5671
6212
S15
%
38
Time
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00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3.62e5
Compound S-11
M+
584.1467
296.8898
292.5764
207.9797
585.1505
426.8129
212.9615
297.8938
334.5437
224.1253
252.0762
355.0515
418.4734
467.8291
483.1356
515.7202
556.7354
586.1508
606.1323
646.0606
668.0848
669.0878
710.0447
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775.5551
100
%
0
m/z
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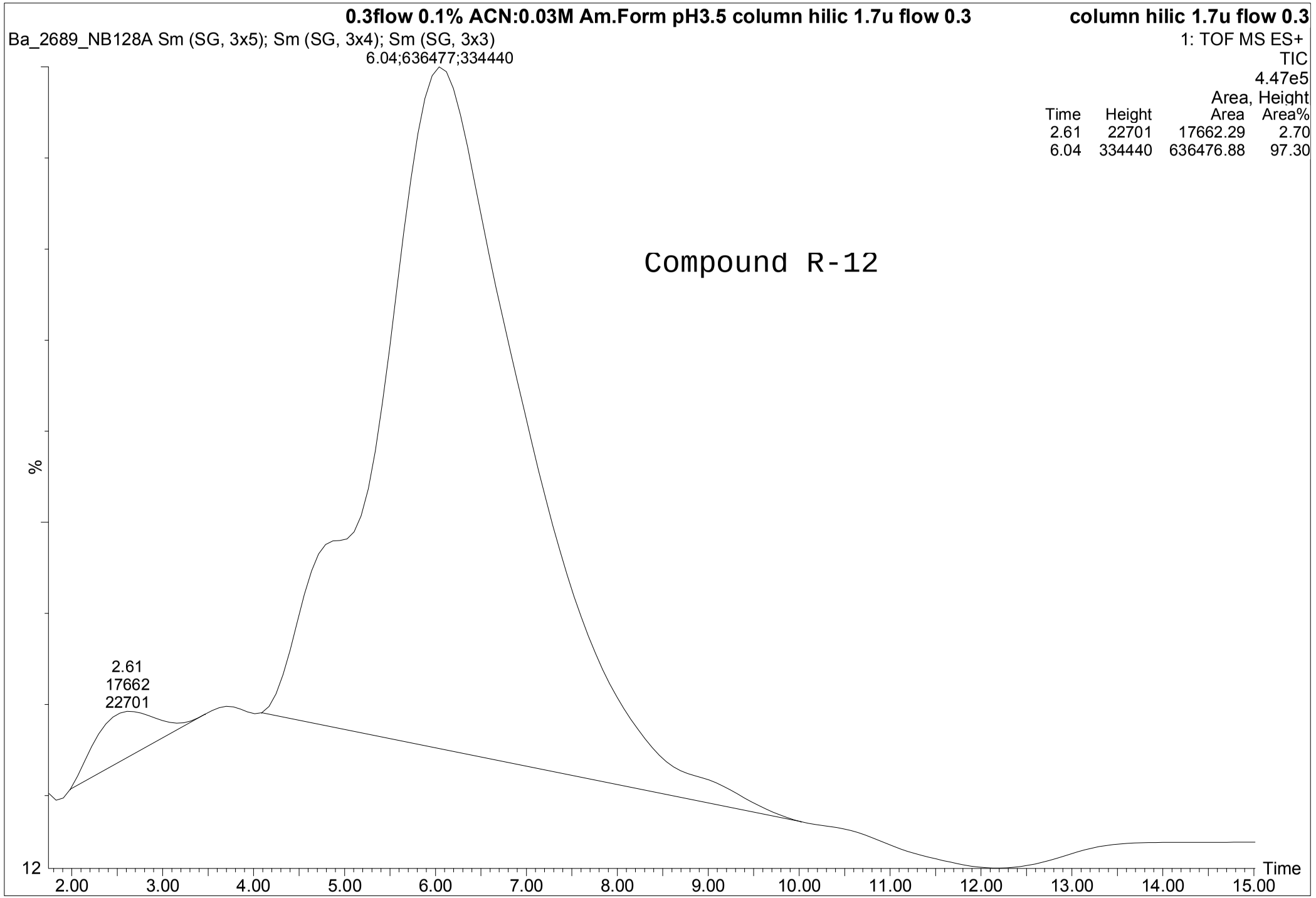
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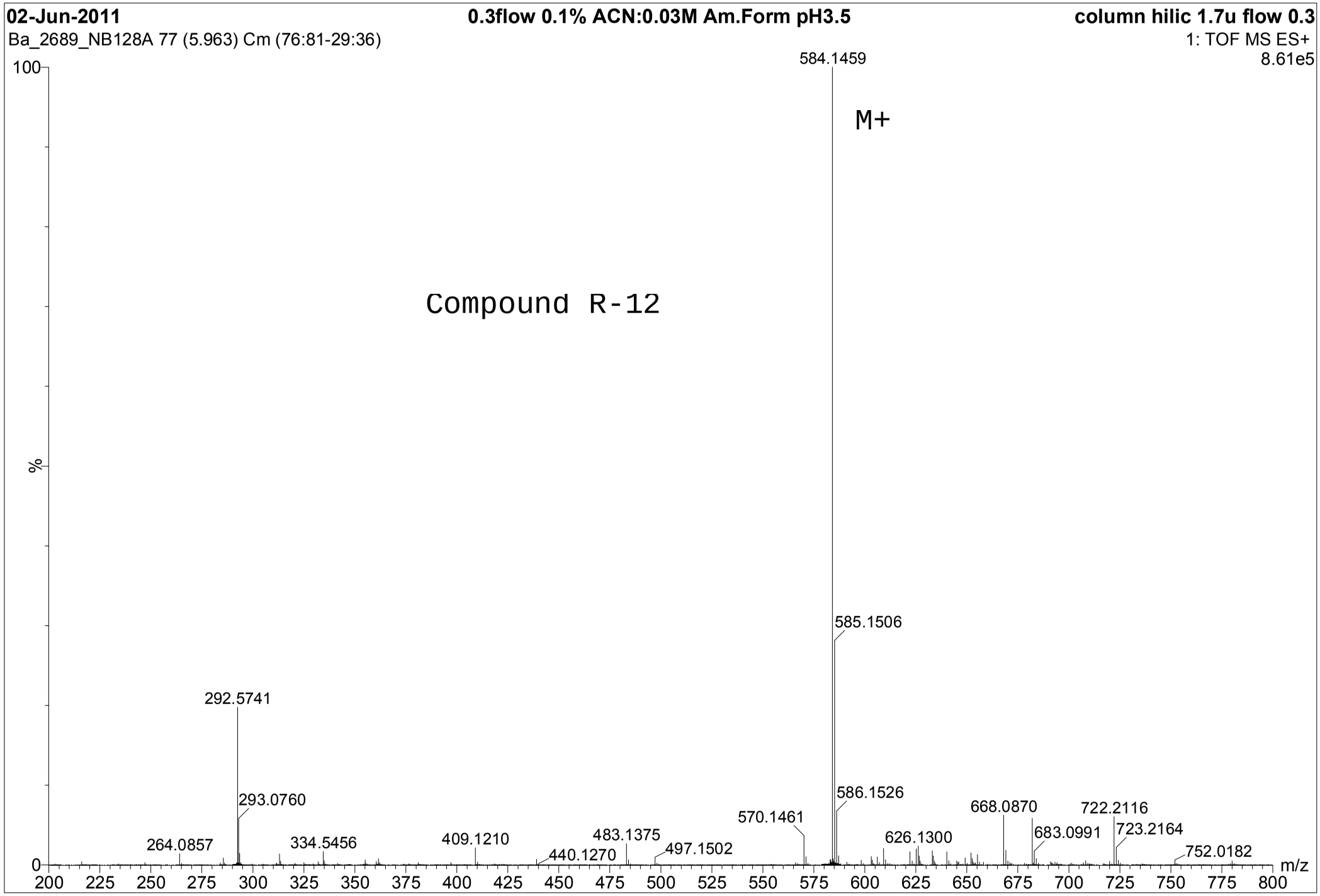
column hilic 1.7u flow 0.3
Ba_2689_NB128A Sm (SG, 3x5); Sm (SG, 3x4); Sm (SG, 3x3)
1: TOF MS ES+
TIC
4.47e5
Area, Height
Time Height Area Area%
2.61 22701 17662.29 2.70
6.04 334440 636476.88 97.30
6.04;636477;334440
Compound R-12
2.61
17662
22701
S17
%
12
Time
2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00


02-Jun-2011
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1: TOF MS ES+
8.61e5
100
%
0
584.1459
M+
Compound R-12
585.1506
292.5741
293.0760
586.1526
570.1461
668.0870
722.2116
683.0991
723.2164
626.1300
264.0857
334.5456
409.1210
483.1375
440.1270
497.1502
752.0182
m/z
200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725 750 775 800
SI8