# Supporting Information

# Development of novel aminoglycoside (NB54) with reduced toxicity and enhanced suppression of disease-causing premature stop mutations

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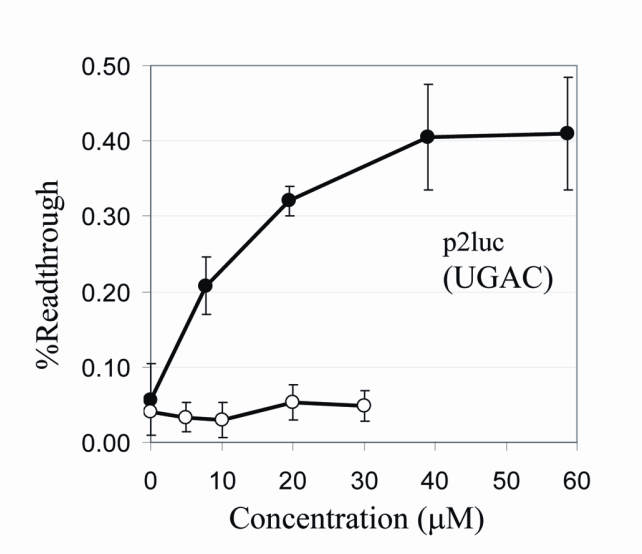



**Figure S1.** Comparative *in vitro* stop codon readthrough levels promoted by amikacin (●) and kanamycin A (○) in the p2luc vector. The p2luc vector was transcribed and translated using TNT quick coupled transcription/translation system. The amount of the translated products was evaluated using the dual luciferase reporter assay system and the suppression level was calculated as described into the manuscript. The results are averages of at least three independent experiments.

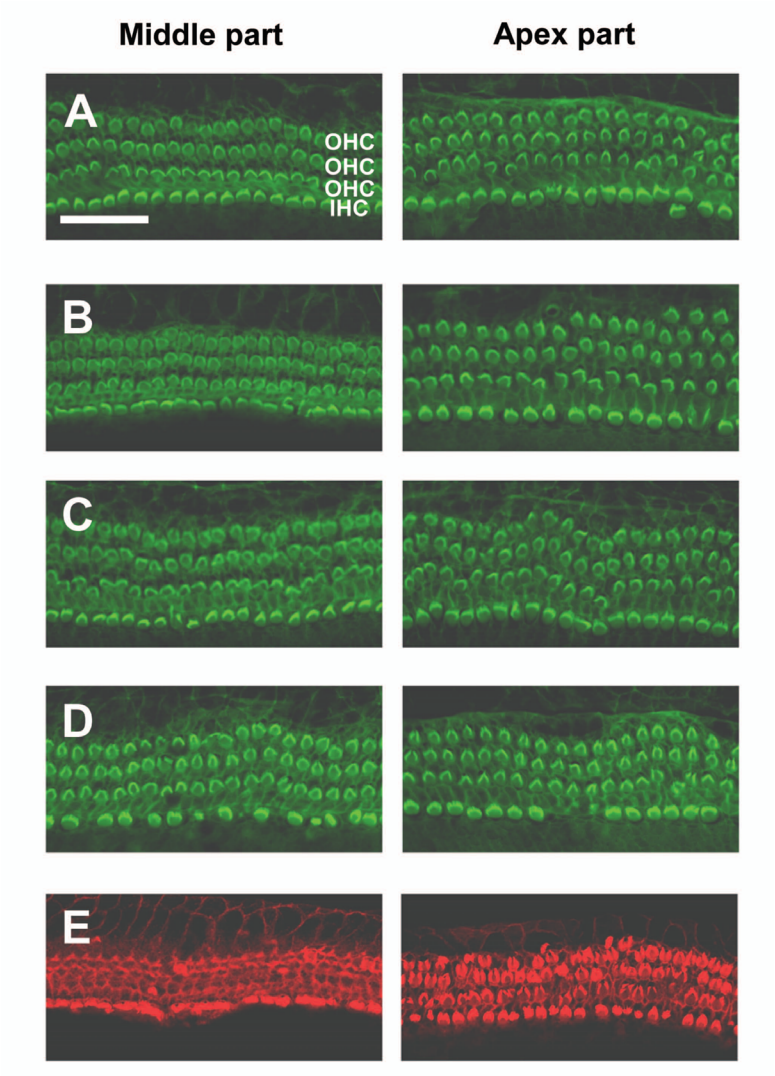



**Figure S2.** Drug-induced hair cell loss in cochlear explants. Sections of the middle and apex parts are shown. (A) 0.1 mM NB30, (B) 1 mM NB30, (C) 0.1 mM NB54, (D) 1 mM NB54 and (E) 0.2 mM gentamicin. A-D show essentially normal morphology, and cell loss is almost complete in E. "OHC" indicates outer, "IHC" inner hair cells. Size bar indicates the increment of 40 μm.

HPLC-ESI-MS analysis for the purity determination of compounds **1** and **2**.

The HPLC system consisted of an Acquity UPLC from Waters. The chromatographic separation was achieved using a Phenomenex Luna Hilic 3.0μ 2.0x150mm column. Sample aliquots of 1 μL were injected onto the HPLC column. The flow-rate of the eluent was 300 μL/min and the mobile phase gradient is shown in tables 1-2.
A LCT Premier mass spectrometer from Waters under ESI conditions was used for mass detection in positive and negative modes.

**Table 1S.** HPLC-ESI-MS conditions for separation of compound **2** (NB54).

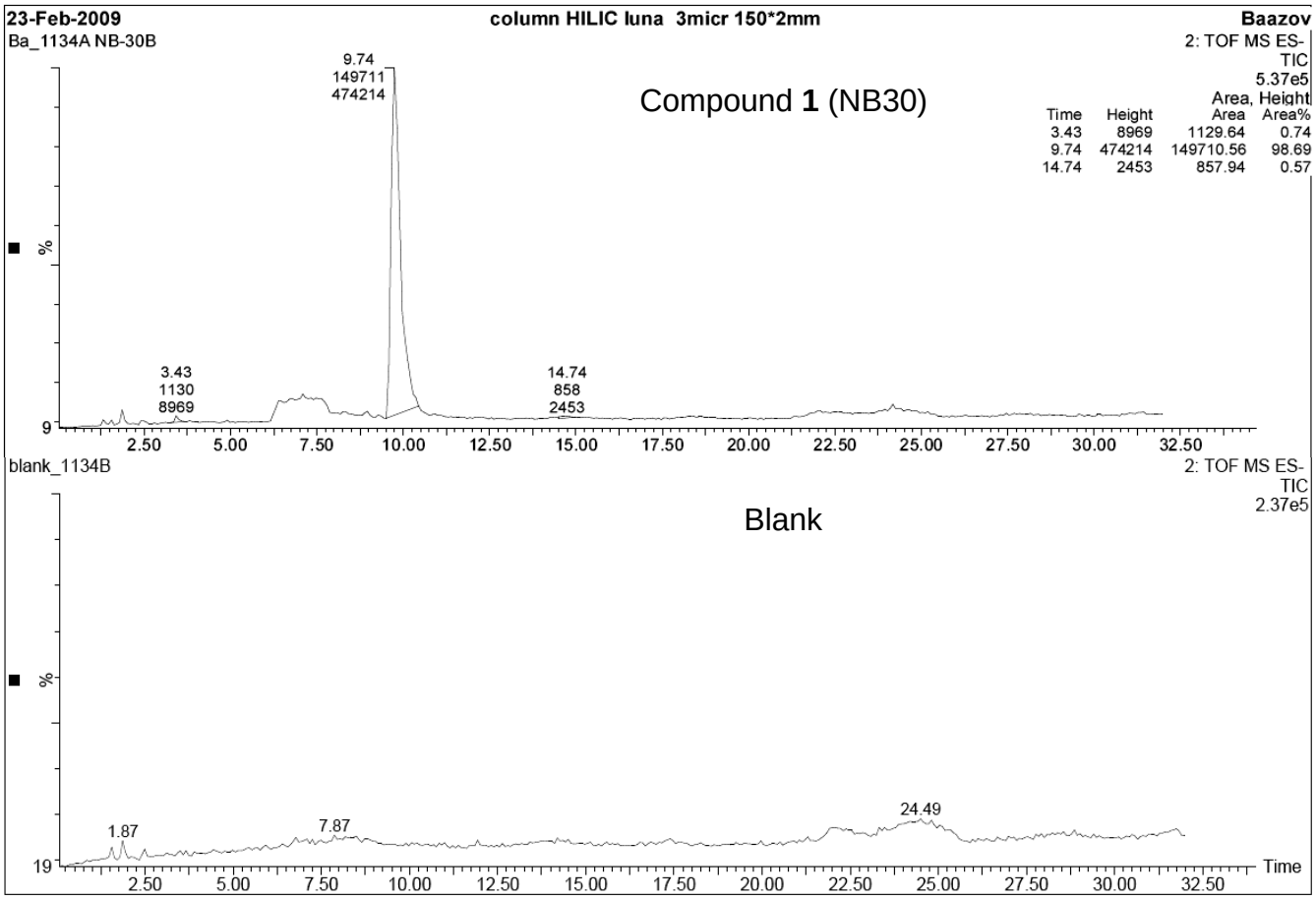
| Time Duration (min) | 0.1% Formic acid in Acetonitrile (%) | 30mM ammonium formate pH=3 in Water (%) |
|---|---|---|
| 0 | 75 | 25 |
| 10 | 75 | 25 |
| 20 | 50 | 50 |
| 23 | 50 | 50 |
| 24 | 75 | 25 |
| 32 | 75 | 25 |

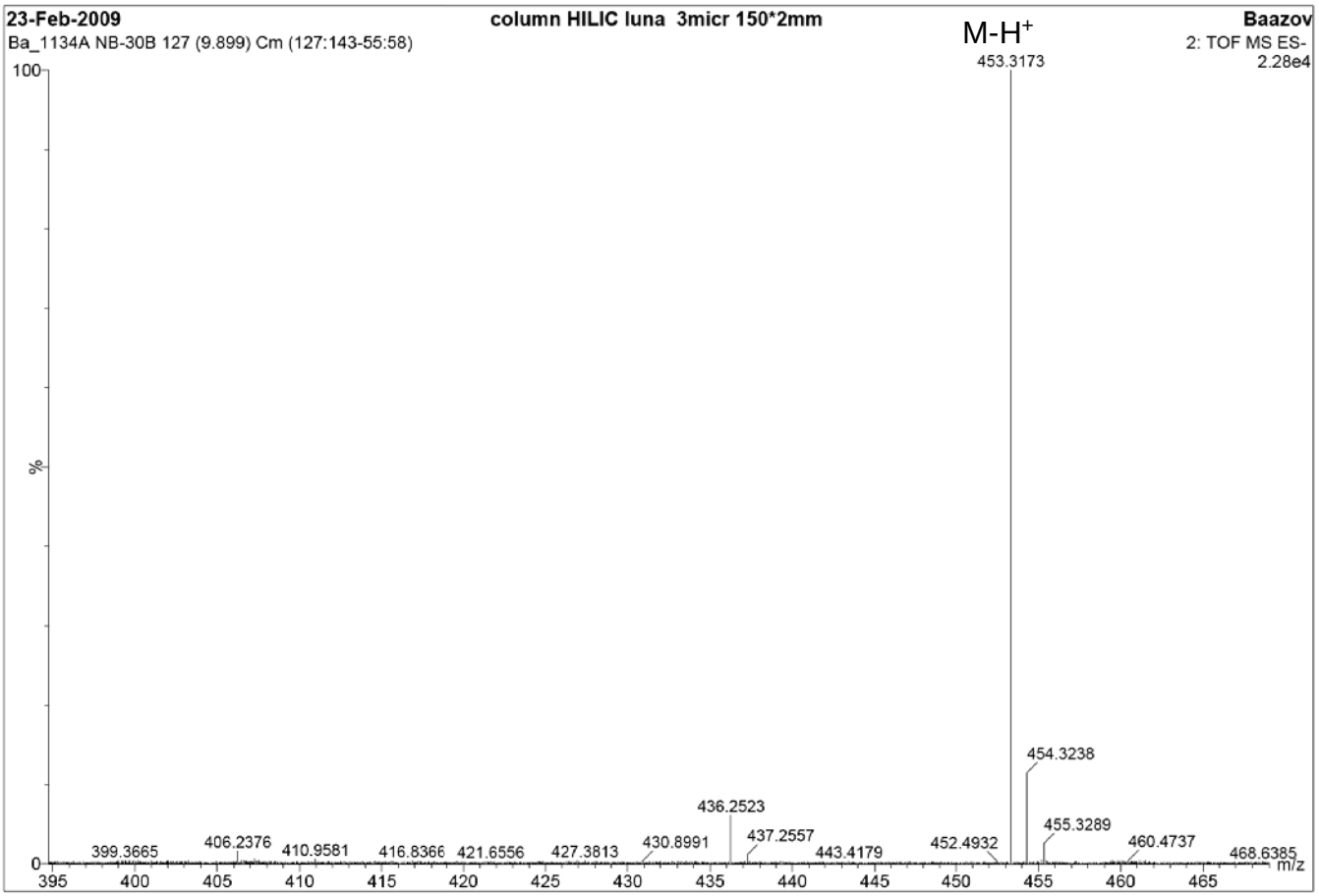
**Table 2S**. HPLC-ESI-MS conditions for separation of compound **1** (NB30).

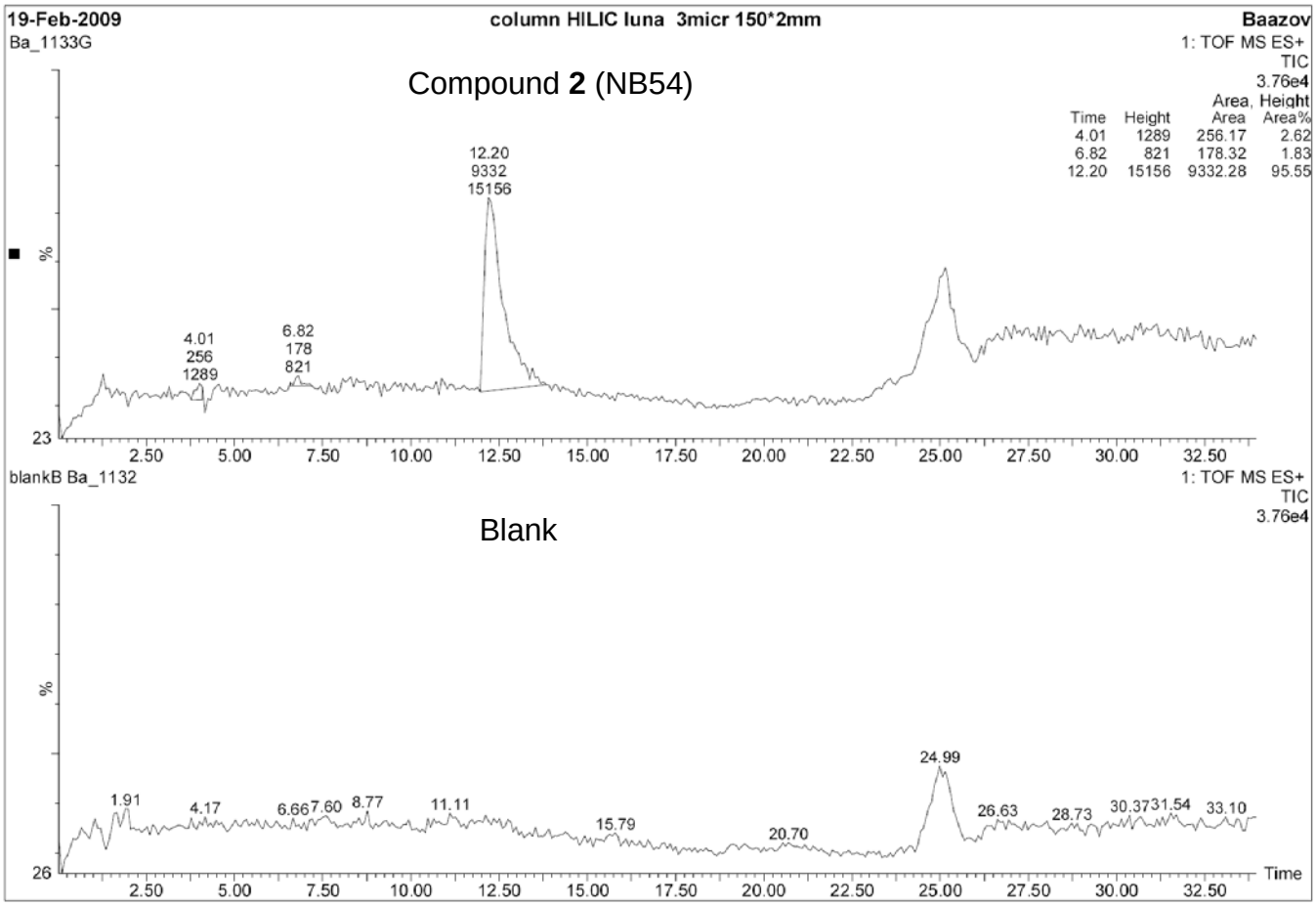
| Time Duration (min) | 0.1% Formic acid in Acetonitrile (%) | 30mM ammonium formate pH=3 in Water (%) |
|---|---|---|
| 0 | 85 | 15 |
| 15 | 50 | 50 |
| 20 | 50 | 50 |
| 21 | 85 | 15 |
| 31 | 85 | 15 |

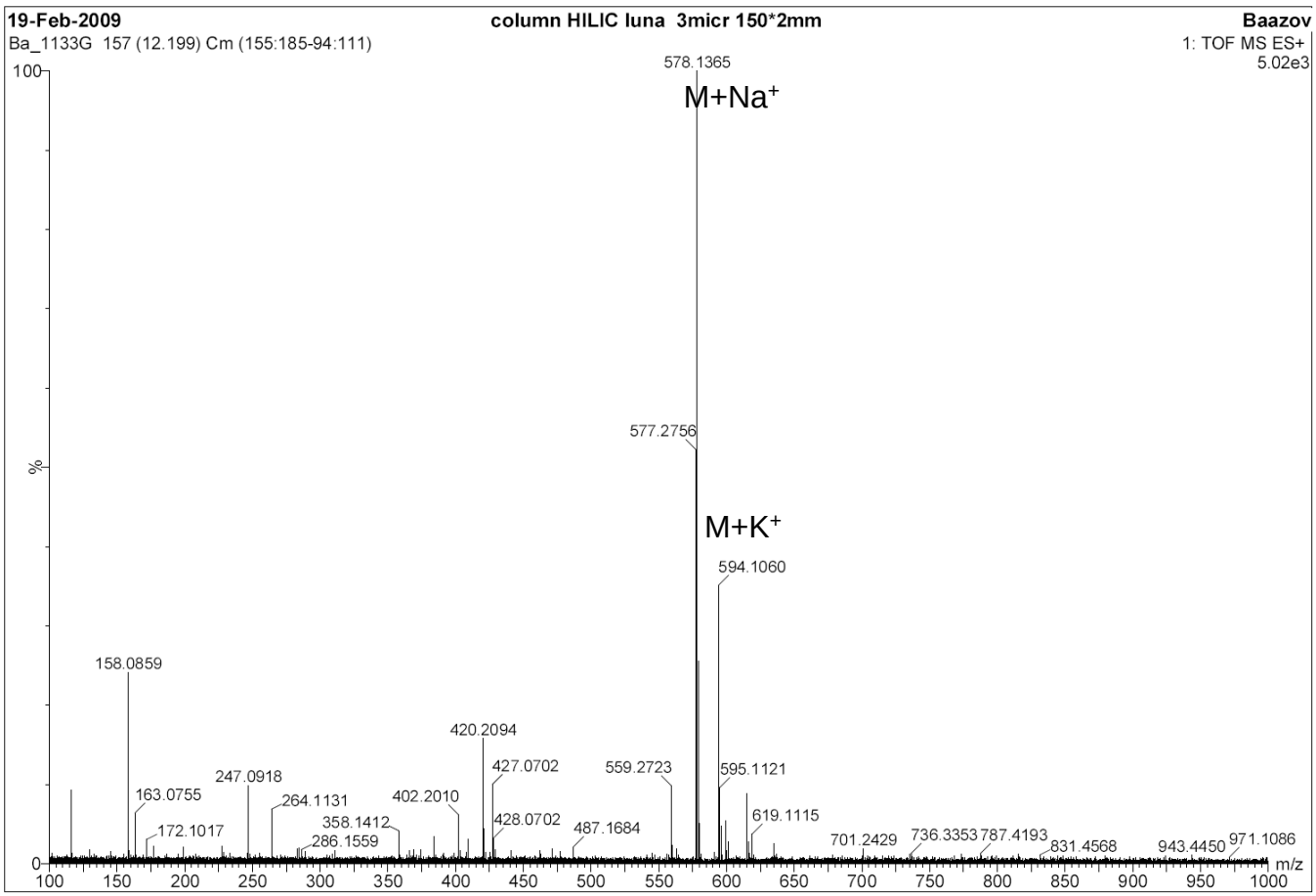
**Table 3S.** HPLC-ESI-MS retention times, purity and HRMS analysis of compounds **1-2.**

| Compound | Retention time (min) | Purity (%) | HRMS m/z |
|---|---|---|---|
| **1** (NB30) | 9.74 | 98.69 | 455.2360 |
| **2** (NB54) | 12.20 | 95.55 | 555.2842 |


23-Feb-2009
column HILIC luna 3micr 150*2mm
Baazov
Ba_1134A NB-30B
2: TOF MS ES-
TIC
5.37e5
Area, Height
Compound 1 (NB30)
Time Height Area Area%
3.43 8969 1129.64 0.74
9.74 474214 149710.56 98.69
14.74 2453 857.94 0.57
9.74
149711
474214
3.43
1130
8969
14.74
858
2453
%
9
2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50
blank_1134B
2: TOF MS ES-
TIC
2.37e5
Blank
%
1.87
7.87
24.49
19
Time
2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50


23-Feb-2009
column HILIC luna 3micr 150*2mm
Baazov
Ba_1134A NB-30B 127 (9.899) Cm (127:143-55:58)
M-H+
453.3173
2: TOF MS ES-
2.28e4
100
%
454.3238
436.2523
455.3289
437.2557
460.4737
399.3665
406.2376
410.9581
416.8366
421.6556
427.3813
430.8991
443.4179
452.4932
468.6385
0
m/z
395 400 405 410 415 420 425 430 435 440 445 450 455 460 465


19-Feb-2009
column HILIC luna 3micr 150*2mm
Baazov
Ba_1133G
1: TOF MS ES+
TIC
3.76e4
Compound 2 (NB54)
Area, Height
Time Height Area Area%
4.01 1289 256.17 2.62
6.82 821 178.32 1.83
12.20 15156 9332.28 95.55
12.20
9332
15156
4.01
256
1289
6.82
178
821
%
23
2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50
blankB Ba_1132
1: TOF MS ES+
TIC
3.76e4
Blank
%
1.91
4.17
6.66
7.60
8.77
11.11
15.79
20.70
24.99
26.63
28.73
30.37
31.54
33.10
26
Time
2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50


19-Feb-2009
column HILIC luna 3micr 150*2mm
Baazov
Ba_1133G 157 (12.199) Cm (155:185-94:111)
1: TOF MS ES+
5.02e3
100
%
0
578.1365
M+Na+
577.2756
M+K+
594.1060
158.0859
420.2094
427.0702
559.2723
595.1121
247.0918
163.0755
264.1131
402.2010
428.0702
619.1115
358.1412
172.1017
286.1559
487.1684
701.2429
736.3353
787.4193
831.4568
943.4450
971.1086
m/z
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000