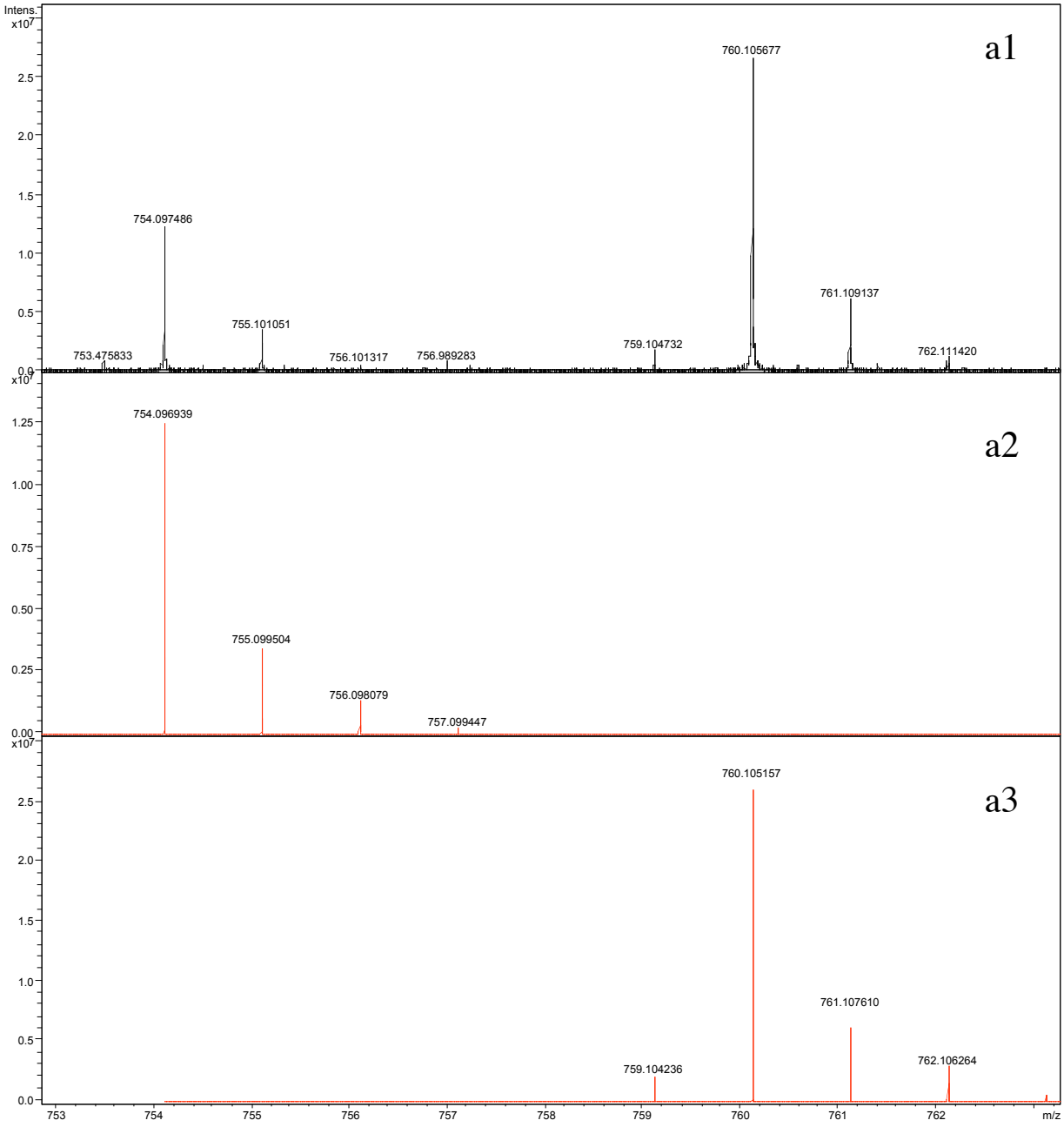
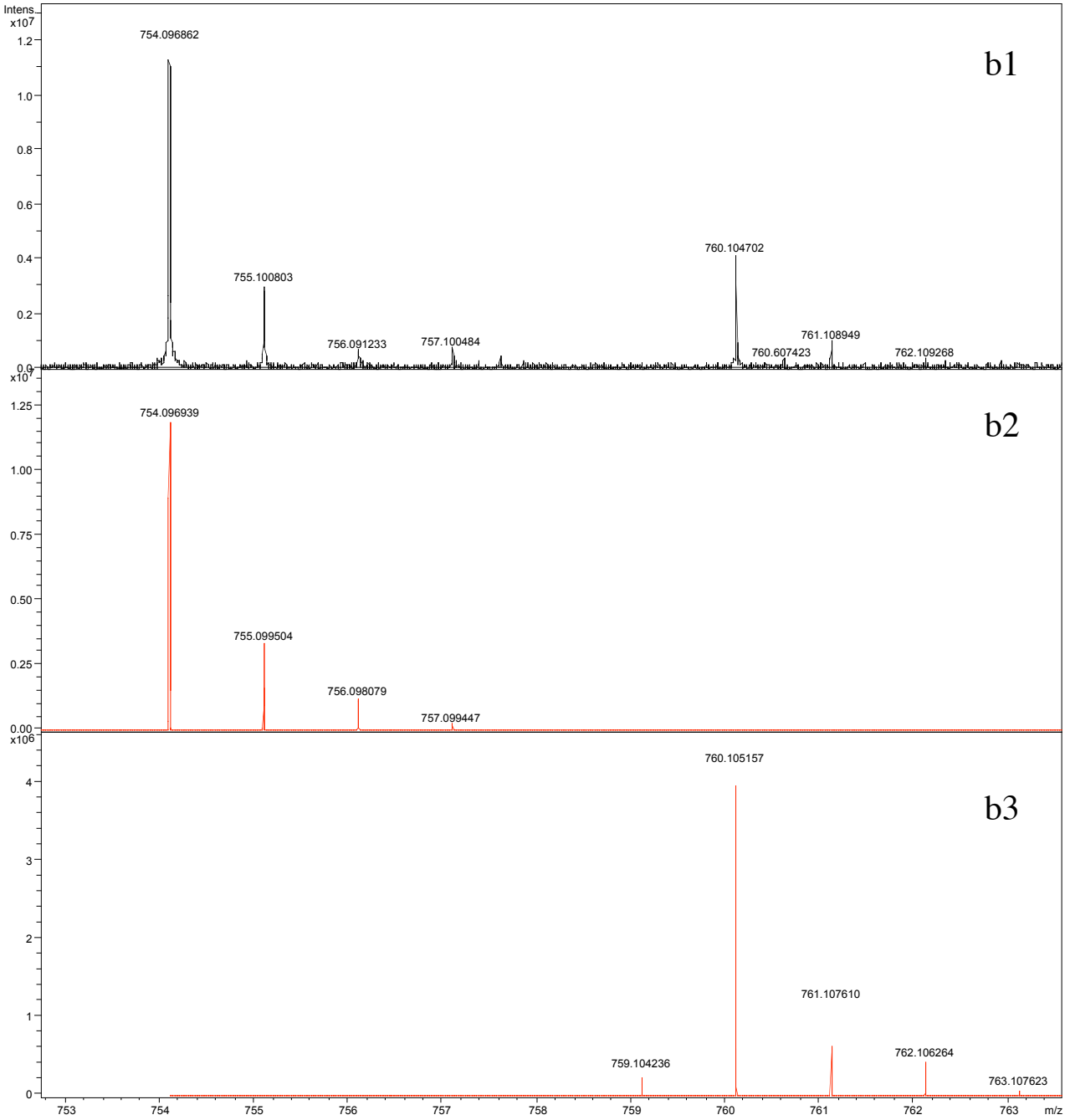
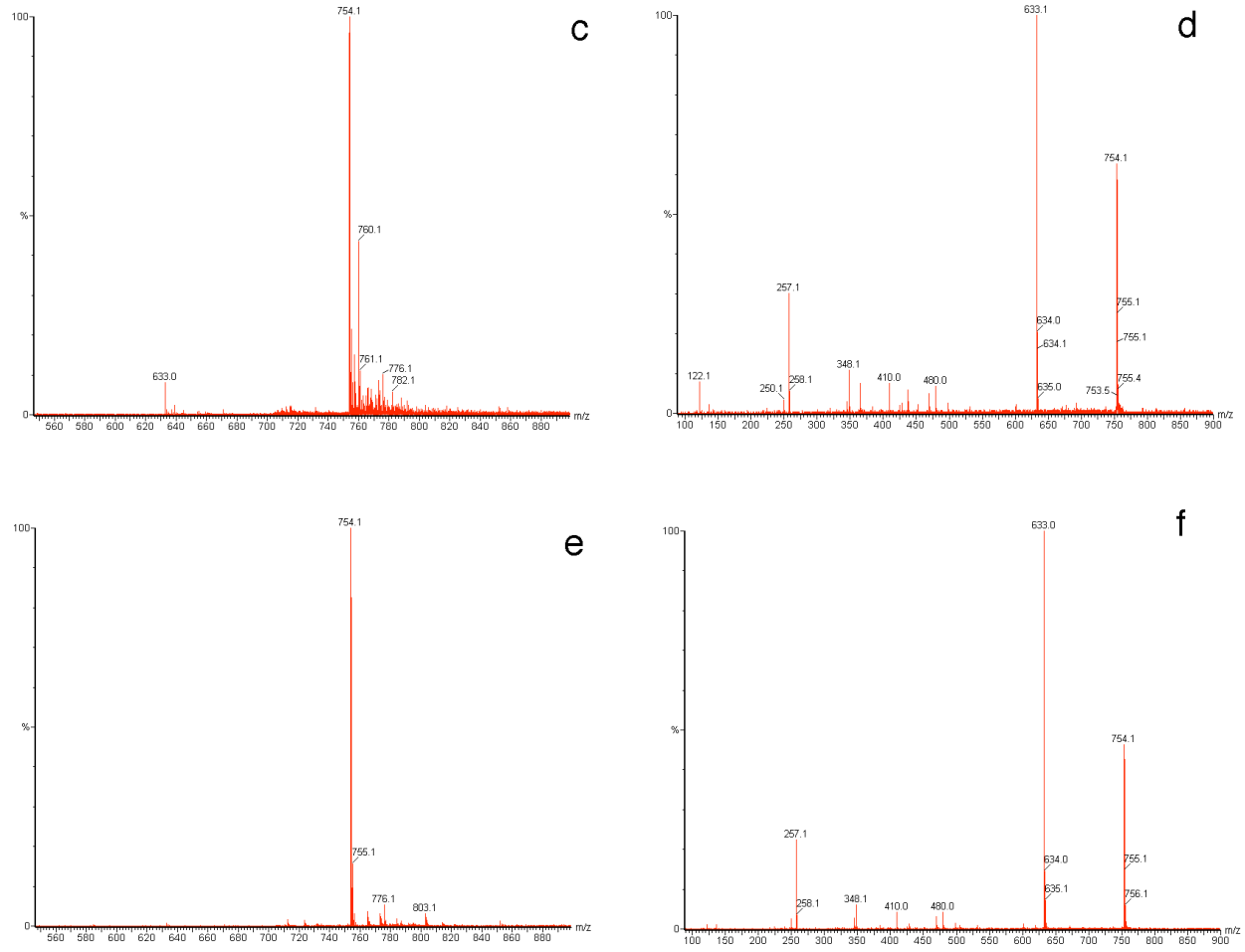
Supplementary Figure 2 High resolution positive ion ESI-FT-ICR mass spectrum of AThTP isolated from bacteria (**a1**). Theoretical isotopic distribution of $C_{22}H_{31}N_9O_{13}P_3S^+$ (**a2**) and $C_{22}H_{30}N_9O_{13}P_3SLi^+$ (**a3**). High resolution positive ion ESI-FT-ICR mass spectrum of chemically synthesized AThTP (**b1**). Theoretical isotopic distribution of $C_{22}H_{31}N_9O_{13}P_3S^+$ (**b2**) and $C_{22}H_{30}N_9O_{13}P_3SLi^+$ (**b3**). Positive ion ESI mass spectrum of AThTP isolated from bacteria (**c**). AThTP was diluted at a concentration of 150 μM in H_2O: acetonitrile (50% : 50%) containing 1 mM lithium iodide. The ESI-MS/MS fragmentation pattern for AThTP isolated from *E. coli* is shown in (**d**). Positive ion ESI mass spectrum of chemically synthesized AThTP (**e**), diluted at a concentration of 150 μM in H_2O : acetonitrile (50% : 50%) and the ESI-MS/MS fragmentation pattern (**f**).


Intens.
x10^7
a1
760.105677
754.097486
761.109137
755.101051
753.475833
756.101317
756.989283
759.104732
762.111420
x10^7
a2
754.096939
755.099504
756.098079
757.099447
x10^7
a3
760.105157
761.107610
762.106264
759.104236
753
754
755
756
757
758
759
760
761
762
m/z


Intens.
x10^7
1.2
1.0
0.8
0.6
0.4
0.2
0.0
754.096862
755.100803
756.091233
757.100484
760.104702
760.607423
761.108949
762.109268
b1
x10^7
1.25
1.00
0.75
0.50
0.25
0.00
754.096939
755.099504
756.098079
757.099447
b2
x10^6
4
3
2
1
0
760.105157
759.104236
761.107610
762.106264
763.107623
b3
753
754
755
756
757
758
759
760
761
762
763
m/z



AThTP analysis by electrospray tandem mass spectrometry. Experiments were performed on a Micromass Q-TOF Ultima Global apparatus (Micromass, now Waters Corporation, Manchester, UK) operated in nano-ESI positive ion mode. The synthesized AThTP was injected at a concentration of 200 µM in 50% water - 50% acetonitrile. Lithium iodide was added at a final concentration of 1 mM to the solution containing the purified AThTP from *E. coli* to suppress the multiple undesired salt adducts. The source parameters were: capillary voltage: 1.8 kV, cone voltage: 100 V, RF lens 1: 90 V, source temperature: 80 °C, collision energy: 6 eV. The fragmentation pattern of the mass 754.1 was obtained with 30 V acceleration voltage. The major product ions were of *m/z* 633.1, 348.1 and 257.1. The 633.1 peak might arise from fragmentation at the level of the quaternary nitrogen of the thiazole moiety of thiamine with loss of the pyrimidinium part (M^+ – 121 – pyrimidinium), while the 348.1 ion might correspond to AMP.

Determination of the exact mass of AThTP. Experiments were performed on an ESI-FT-ICR mass spectrometer (Apex Qe 9.4 T, Bruker Daltonik GmbH, Bremen Germany) operated in positive ion mode. The mass spectrometer was calibrated with 0.1% phosphoric acid – 50% water – 50% acetonitrile solution in the range of the mass to be measured and gave a standard deviation accuracy of 0.284 ppm over eight calibration points. The synthesized and natural AThTP were injected separately at a concentration of 20 µM in 50% water – 50% acetonitrile containing 20 µM lithium iodide. External calibration was applied. The source parameters were: capillary voltage: 4.48 kV, capillary exit voltage: 60 V, desolvatation gas temperature: 200 °C. Molecular formula were generated (see below) using the following parameters: relative mass tolerance: 1 ppm, minimum elements: H_{30} (based on ^{1}H-NMR), maximum element: $C_{\infty}H_{31}N_{\infty}O_{\infty}P_5S_5Li$, nitrogen rule applied.

Molecular formula generated for the natural compound (M^+, M-H Li^+).

M^+

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 22 H 31 N 9 O 13 P 3 S 1	0.029	754.096939	-0.73	-1.20	-0.55	13.50	ok	even
C 24 H 31 Li 1 N 1 O 22 P 2	0.038	754.096750	-0.98	-0.88	-0.74	10.50	ok	even
C 28 H 30 N 5 O 14 P 2 S 1	0.046	754.097971	0.64	0.29	0.49	17.50	ok	even
C 25 H 30 Li 1 N 13 O 1 P 5 S 2	0.061	754.098032	0.72	-0.05	0.55	19.50	ok	even
C 32 H 30 Li 1 N 7 O 3 P 5 S 1	0.064	754.097347	-0.18	-0.48	-0.14	23.50	ok	even
C 38 H 31 N 1 O 8 P 3 S 1	0.087	754.097774	0.38	0.19	0.29	25.50	ok	even
C 23 H 31 Li 1 N 11 O 6 P 2 S 4	0.090	754.097139	-0.46	-1.34	-0.35	14.50	ok	even
C 36 H 30 Li 1 N 1 O 9 P 1 S 3	0.105	754.097486	-0.00	-0.44	-0.00	22.50	ok	even
C 29 H 30 Li 1 N 7 O 7 P 1 S 4	0.105	754.098171	0.91	0.06	0.69	18.50	ok	even
C 34 H 30 N 9 P 2 S 4	0.125	754.097674	0.25	-0.58	0.19	25.50	ok	even
C 41 H 30 N 3 O 2 P 2 S 3	0.130	754.096989	-0.66	-1.11	-0.50	29.50	ok	even
C 39 H 31 Li 1 N 3 O 1 P 2 S 4	0.135	754.097974	0.65	-1.17	0.49	26.50	ok	even

M-H Li^+

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 17 H 31 N 15 O 10 P 5	0.007	760.106091	0.54	0.09	0.41	12.50	ok	even
C 25 H 30 N 1 O 26	0.035	760.105057	-0.82	-0.86	-0.62	11.50	ok	even
C 18 H 30 N 7 O 24 S 1	0.038	760.105742	0.09	-0.46	0.06	7.50	ok	even
C 22 H 30 Li 1 N 9 O 13 P 3 S 1	0.038	760.105118	-0.74	-1.23	-0.56	13.50	ok	even
C 23 H 31 Li 1 N 1 O 25 S 1	0.043	760.106042	0.48	0.17	0.36	8.50	ok	even
C 15 H 30 Li 1 N 15 O 11 P 3 S 2	0.048	760.105803	0.17	-0.02	0.13	9.50	ok	even
C 21 H 31 N 9 O 16 P 1 S 2	0.058	760.106230	0.73	-0.05	0.55	11.50	ok	even
C 20 H 30 N 17 O 4 P 4 S 2	0.064	760.105306	-0.49	-0.53	-0.37	16.50	ok	even
C 25 H 31 Li 1 N 11 O 5 P 4 S 2	0.065	760.105606	-0.09	0.01	-0.07	17.50	ok	even
C 18 H 31 Li 1 N 17 O 3 P 4 S 3	0.066	760.106291	0.81	0.53	0.61	13.50	ok	even
C 28 H 31 N 3 O 18 P 1 S 1	0.067	760.105545	-0.17	-0.61	-0.13	15.50	ok	even
C 32 H 31 Li 1 N 5 O 7 P 4 S 1	0.074	760.104921	-1.00	-1.32	-0.76	21.50	ok	even
C 38 H 30 Li 1 N 1 O 8 P 3 S 1	0.098	760.105953	0.36	0.11	0.28	25.50	ok	even
C 22 H 31 Li 1 N 11 O 9 S 5	0.112	760.106431	0.99	0.01	0.75	12.50	ok	even
C 24 H 30 N 11 O 10 S 4	0.114	760.105445	-0.30	-0.21	-0.23	15.50	ok	even
C 29 H 31 Li 1 N 5 O 11 S 4	0.118	760.105745	0.09	0.27	0.07	16.50	ok	even
C 17 H 30 N 17 O 8 S 5	0.119	760.106131	0.60	0.31	0.45	11.50	ok	even
C 27 H 31 N 13 O 2 P 1 S 5	0.132	760.105934	0.34	-0.71	0.26	19.50	ok	even
C 34 H 31 N 7 O 4 P 1 S 4	0.134	760.105248	-0.56	-1.13	-0.43	23.50	ok	even
C 43 H 30 N 3 O 1 P 4 S 1	0.140	760.105456	-0.29	-0.48	-0.22	32.50	ok	even
C 40 H 30 N 3 O 5 S 4	0.152	760.106280	0.79	-0.75	0.60	27.50	ok	even

Molecular formula generated for the synthesized compound (M^+, M-H Li^+).

M^+

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e^-
C 19 H 30 N 7 O 21 P 2	0.030	754.096451	-0.55	-0.73	-0.41	9.50	ok	even
C 22 H 31 N 9 O 13 P 3 S 1	0.037	754.096939	0.10	-0.66	0.08	13.50	ok	even
C 24 H 31 Li 1 N 1 O 22 P 2	0.045	754.096750	-0.15	-0.02	-0.11	10.50	ok	even
C 29 H 31 N 3 O 15 P 3	0.046	754.096253	-0.81	-0.70	-0.61	17.50	ok	even
C 20 H 30 Li 1 N 9 O 14 P 1 S 3	0.068	754.096651	-0.28	-0.65	-0.21	10.50	ok	even
C 32 H 30 Li 1 N 7 O 3 P 5 S 1	0.073	754.097347	0.64	-0.02	0.48	23.50	ok	even
C 25 H 30 N 11 O 7 P 2 S 3	0.074	754.096154	-0.94	-1.25	-0.71	17.50	ok	even
C 18 H 30 N 17 O 5 P 2 S 4	0.079	754.096839	-0.03	-0.84	-0.02	13.50	ok	even
C 39 H 30 Li 1 N 1 O 5 P 5	0.086	754.096661	-0.27	0.17	-0.20	27.50	ok	even
C 30 H 31 Li 1 N 5 O 8 P 2 S 3	0.087	754.096454	-0.54	-0.60	-0.41	18.50	ok	even
C 23 H 31 Li 1 N 11 O 6 P 2 S 4	0.088	754.097139	0.37	-0.21	0.28	14.50	ok	even
C 36 H 30 Li 1 N 1 O 9 P 1 S 3	0.106	754.097486	0.83	-0.21	0.62	22.50	ok	even
C 41 H 30 N 3 O 2 P 2 S 3	0.133	754.096989	0.17	-0.52	0.13	29.50	ok	even

M-H Li^+

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e^-
C 19 H 31 N 5 O 25 P 1	0.017	760.104025	-0.89	-1.20	-0.68	7.50	ok	even
C 25 H 30 N 1 O 26	0.019	760.105057	0.47	0.31	0.35	11.50	ok	even
C 20 H 30 N 17 O 4 P 4 S 2	0.034	760.105306	0.79	-0.51	0.60	16.50	ok	even
C 22 H 30 Li 1 N 9 O 13 P 3 S 1	0.041	760.105118	0.55	-0.22	0.42	13.50	ok	even
C 27 H 30 N 11 O 6 P 4 S 1	0.047	760.104621	-0.11	-0.91	-0.08	20.50	ok	even
C 29 H 30 Li 1 N 3 O 15 P 3	0.050	760.104432	-0.36	-0.44	-0.27	17.50	ok	even
C 20 H 31 Li 1 N 7 O 18 S 3	0.060	760.104225	-0.63	-1.69	-0.48	8.50	ok	even
C 32 H 31 Li 1 N 5 O 7 P 4 S 1	0.067	760.104921	0.29	-0.26	0.22	21.50	ok	even
C 18 H 31 N 15 O 9 P 1 S 4	0.070	760.104413	-0.38	-1.81	-0.29	11.50	ok	even
C 24 H 30 N 11 O 10 S 4	0.087	760.105445	0.98	-0.39	0.74	15.50	ok	even
C 31 H 30 N 5 O 12 S 3	0.089	760.104760	0.08	-0.90	0.06	19.50	ok	even
C 37 H 31 N 7 P 5 S 1	0.094	760.104424	-0.37	-0.97	-0.28	28.50	ok	even
C 35 H 30 Li 1 N 7 O 1 P 3 S 3	0.100	760.104136	-0.75	-1.60	-0.57	25.50	ok	even
C 34 H 31 N 7 O 4 P 1 S 4	0.109	760.105248	0.72	-0.41	0.55	23.50	ok	even
C 43 H 30 N 3 O 1 P 4 S 1	0.122	760.105456	0.99	0.47	0.75	32.50	ok	even
C 41 H 31 N 1 O 6 P 1 S 3	0.128	760.104563	-0.18	-0.99	-0.14	27.50	ok	even