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Supplementary Information for

2 **Stable isotope labeling nitrogen metabolism in microcystin biosynthesis**

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9 1000.5, 1001.5, 1002.5, 1003.5, 1004.5 and 1005.5

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1 **Table S1:** Components of BG11 medium

Component	Concentration(g/L)
NaNO ₃	1.5
K ₂ HPO ₄	0.04
MgSO ₄	0.075
CaCl ₂ ·2H ₂ O	0.036
Citric	0.006
Ferric Citrate	0.006
Na ₂ -EDTA	0.001
Na ₂ CO ₃	0.02
H ₃ BO ₃	0.00286
MnCl ₂ ·4H ₂ O	0.00181
ZnSO ₄ ·7H ₂ O	0.000222
Na ₂ MoO ₄ ·2H ₂ O	0.00039
CuSO ₄ ·5H ₂ O	0.000079
Co(NO ₃) ₂ ·6H ₂ O	0.0000494

1 **Table S2:** Gradient elution of HPLC (A: %5 acetonitrile containing 0.1% formic acid

2 B: acetonitrile containing 0.1% formic acid)

Retention time (min)	% A	% B
0	70	30
20	60	40
25	10	90
28	70	30
28.1	70	30
35	70	30

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1 **Table S3:** Gradient elution of HPLC (A: 0.1% formic acid B: methanol)

Retention time (min)	% A	% B
0	99	1
5	99	1
10	85	15
20	0	100
30	0	100

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1 **Table S4:** Major Raman spectral shifts in wavenumber shifts detected due to ^{15}N
2 incorporation

Unlabeled (cm^{-1})	^{15}N (Δcm^{-1})	Assignment
1259	-10	Amide III
1452	-12	Arg N-C-N asymmetric stretch, Arg C-N-H side chain vibrations, Arg C-H vibrations
1644	-24	Amide I (C=O), water in aqueous peptide sample

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1 **Table S5:** MS-MS parent ion and daughter ions for m/z 996.5, 997.5, 998.5, 999.5,
 2 1000.5, 1001.5, 1002.5, 1003.5, 1004.5 and 1005.5

Parent ions	Daughter ions	Identity
996.5 (M+H+1 ¹⁵ N)	600	Arg-Adda-Glu+H+1 ¹⁵ N/MeAsp-Arg-Adda+H+1 ¹⁵ N
	470	Ala-Leu-MeAsp-Arg+H
	553	Mdha-Ala-Leu-MeAsp-Arg+H
	711	Adda-Glu-Mdha-Ala-Leu+H+1 ¹⁵ N
	865	Mdha-Ala-Leu-MeAsp-Arg-Adda+H
	867	Arg-Adda-Glu-Mdha-Ala-Leu+H+1 ¹⁵
997.5 (M+H+2 ¹⁵ N)	600	Arg-Adda-Glu+H+1 ¹⁵ N/MeAsp-Arg-Adda+H+1 ¹⁵ N
	471	Ala-Leu-MeAsp-Arg+H+1 ¹⁵ N
	554	Mdha-Ala-Leu-MeAsp-Arg+H+1 ¹⁵ N
	712	Adda-Glu-Mdha-Ala-Leu+H+2 ¹⁵ N
	868	Arg-Adda-Glu-Mdha-Ala-Leu+H+2 ¹⁵ N
	925	Leu-MeAsp-Arg-Adda-Glu-Mdha+H+1 ¹⁵ N
998.5 (M+H+3 ¹⁵ N)	601	Arg-Adda-Glu+H+2 ¹⁵ N/MeAsp-Arg-Adda+H+2 ¹⁵ N
	472	Ala-Leu-MeAsp-Arg+H+2 ¹⁵ N
	555	Mdha-Ala-Leu-MeAsp-Arg+H+2 ¹⁵ N
	868	Mdha-Ala-Leu-MeAsp-Arg-Adda+H+3 ¹⁵ N
	868	Arg-Adda-Glu-Mdha-Ala-Leu+H+2 ¹⁵ N
	926	Leu-MeAsp-Arg-Adda-Glu-Mdha+H+2 ¹⁵ N
999.5 (M+H+4 ¹⁵ N)	602	Arg-Adda-Glu+H+3 ¹⁵ N/MeAsp-Arg-Adda+H+3 ¹⁵ N
	473	Ala-Leu-MeAsp-Arg+H+3 ¹⁵ N
	870	Arg-Adda-Glu-Mdha-Ala-Leu+H+4 ¹⁵ N
1000.5 (M+H+5 ¹⁵ N)	602	Arg-Adda-Glu+H+3 ¹⁵ N/MeAsp-Arg-Adda+H+3 ¹⁵ N
	473	Ala-Leu-MeAsp-Arg+H+3 ¹⁵ N
	557	Mdha-Ala-Leu-MeAsp-Arg+H+4 ¹⁵ N
	870	Arg-Adda-Glu-Mdha-Ala-Leu+H+4 ¹⁵ N
	928	Leu-MeAsp-Arg-Adda-Glu-Mdha+H+4 ¹⁵ N
1001.5 (M+H+6 ¹⁵ N)	602	Arg-Adda-Glu+H+3 ¹⁵ N/MeAsp-Arg-Adda+H+3 ¹⁵ N
	474	Ala-Leu-MeAsp-Arg+H+4 ¹⁵ N
	558	Mdha-Ala-Leu-MeAsp-Arg+H+5 ¹⁵ N
	714	Adda-Glu-Mdha-Ala-Leu+H+4 ¹⁵ N
	871	Mdha-Ala-Leu-MeAsp-Arg-Adda+H+6 ¹⁵ N

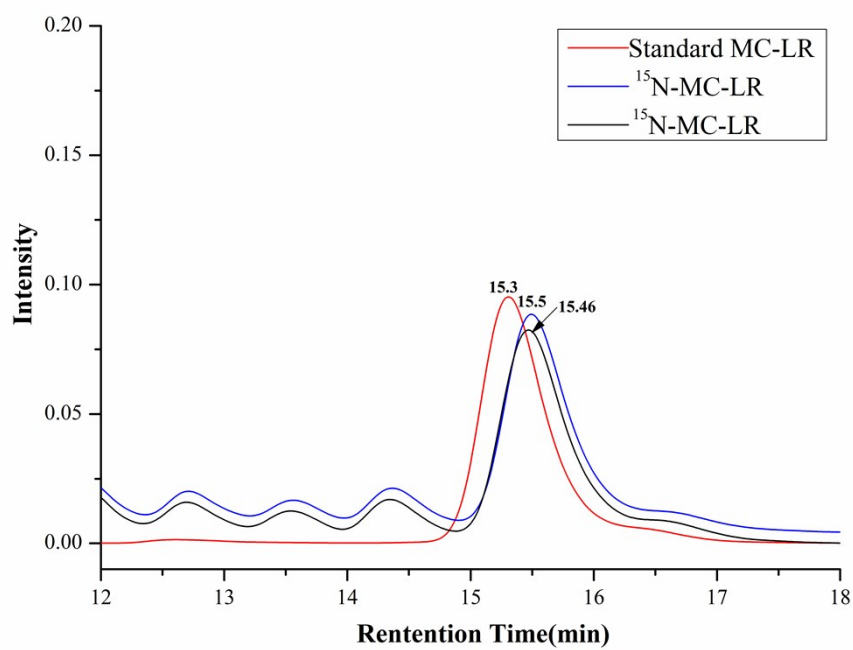
	929	Leu-MeAsp-Arg-Adda-Glu-Mdha+H+5 ¹⁵ N
1002.5 (M+H+7 ¹⁵ N)	603	Arg-Adda-Glu+H+4 ¹⁵ N/MeAsp-Arg-Adda+H+4 ¹⁵ N
	474	Ala-Leu-MeAsp-Arg+H+4 ¹⁵ N
	558	Mdha-Ala-Leu-MeAsp-Arg+H+5 ¹⁵ N
	872	Mdha-Ala-Leu-MeAsp-Arg-Adda+H+7 ¹⁵ N
	930	Leu-MeAsp-Arg-Adda-Glu-Mdha+H+6 ¹⁵ N
1003.5 (M+H+8 ¹⁵ N)	604	Arg-Adda-Glu+H+5 ¹⁵ N/MeAsp-Arg-Adda+H+5 ¹⁵ N
	475	Ala-Leu-MeAsp-Arg+H+5 ¹⁵ N
	559	Mdha-Ala-Leu-MeAsp-Arg+H+6 ¹⁵ N
1004.5 (M+H+9 ¹⁵ N)	560	Mdha-Ala-Leu-MeAsp-Arg+H+7 ¹⁵ N
	476	Ala-Leu-MeAsp-Arg+H+6 ¹⁵ N
	932	Leu-MeAsp-Arg-Adda-Glu-Mdha+H+8 ¹⁵ N
1005.5 (M+H+10 ¹⁵ N)	561	Mdha-Ala-Leu-MeAsp-Arg+H+8 ¹⁵ N
	605	Arg-Adda-Glu+H+6 ¹⁵ N/MeAsp-Arg-Adda+H+6 ¹⁵ N
	477	Ala-Leu-MeAsp-Arg+H+7 ¹⁵ N
	875	Mdha-Ala-Leu-MeAsp-Arg-Adda+H+10 ¹⁵ N

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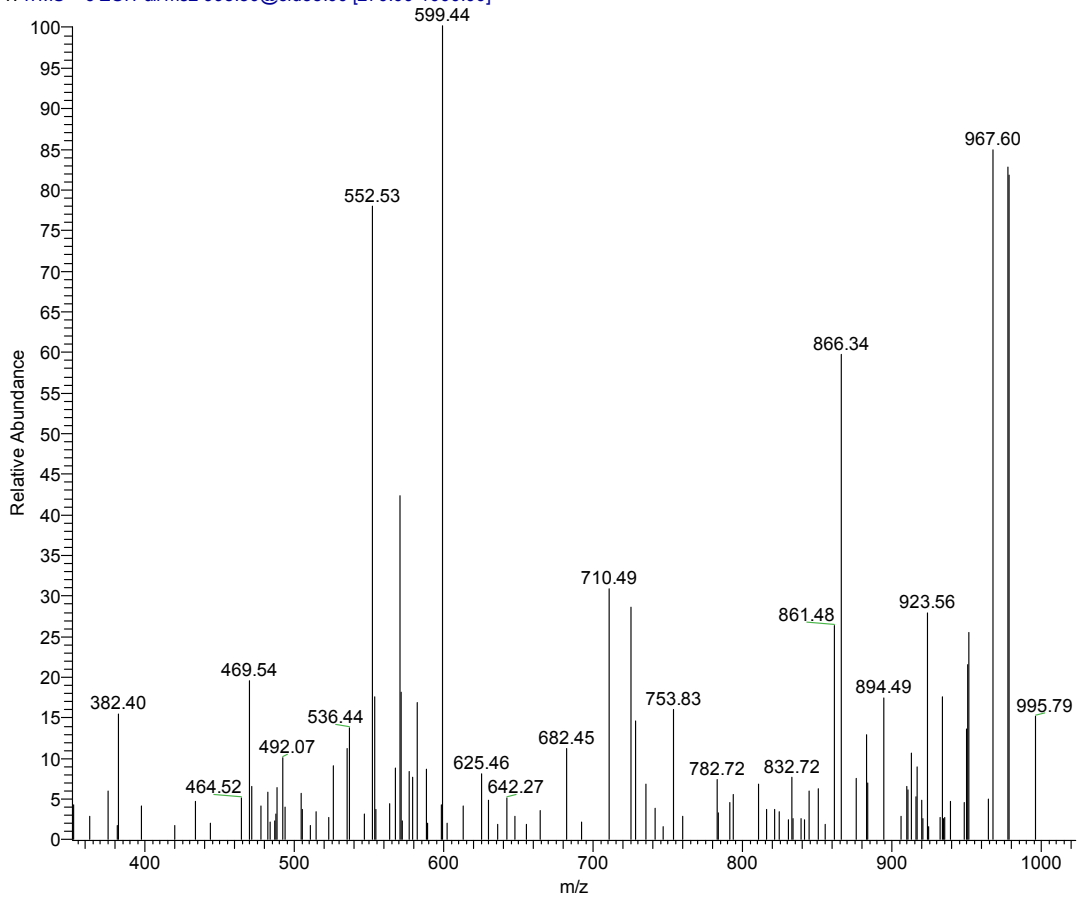


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Fig.S1: HPLC chromatograms of MC-LR and purified ¹⁵N-MC-LR

LR-STD-995MS2-20130330 #294 RT: 3.79 AV: 1 NL: 7.25E1
T: ITMS + c ESI Full ms2 995.50@cid35.00 [270.00-1000.00]



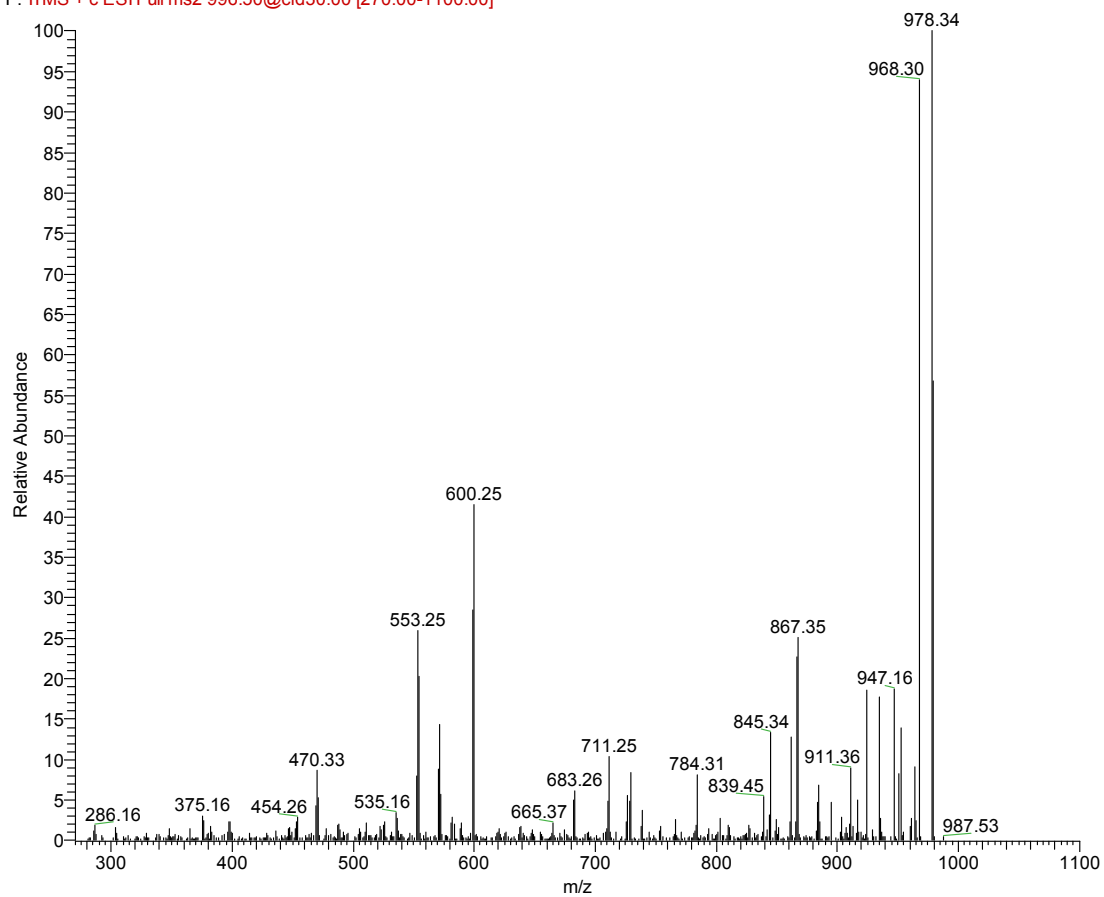
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Fig. S2: MS/MS spectra of MC-LR standard (m/z=995.5)

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15N-1 #260 RT: 3.82 AV: 1 NL: 1.93E3
F: ITMS + c ESI Full ms2 996.50@cid30.00 [270.00-1100.00]

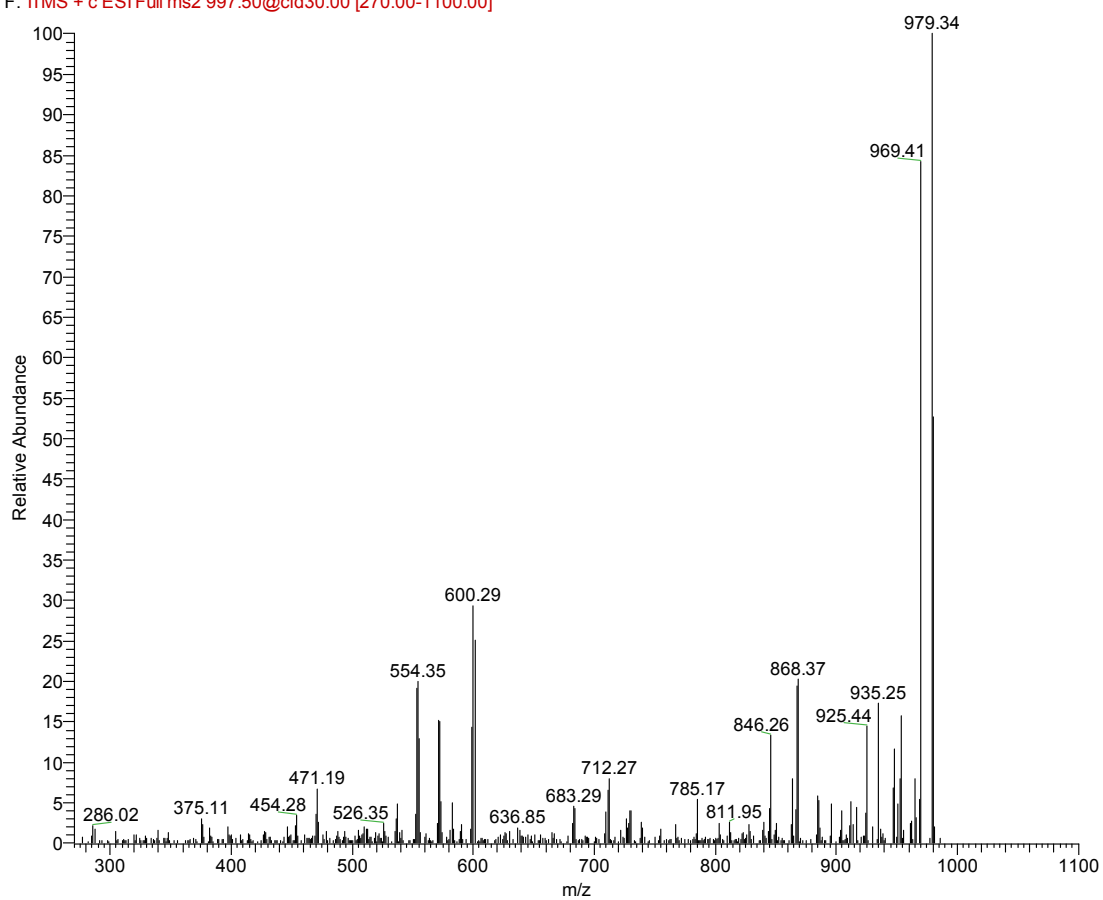


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Fig. S3: MS/MS spectra of $^{15}\text{N}_1\text{-MC}$ ($m/z=996.5$)

15N-1 #261 RT: 3.84 AV: 1 NL: 9.74E2
F: ITMS + c.ESI Full ms2 997.50@cid30.00 [270.00-1100.00]

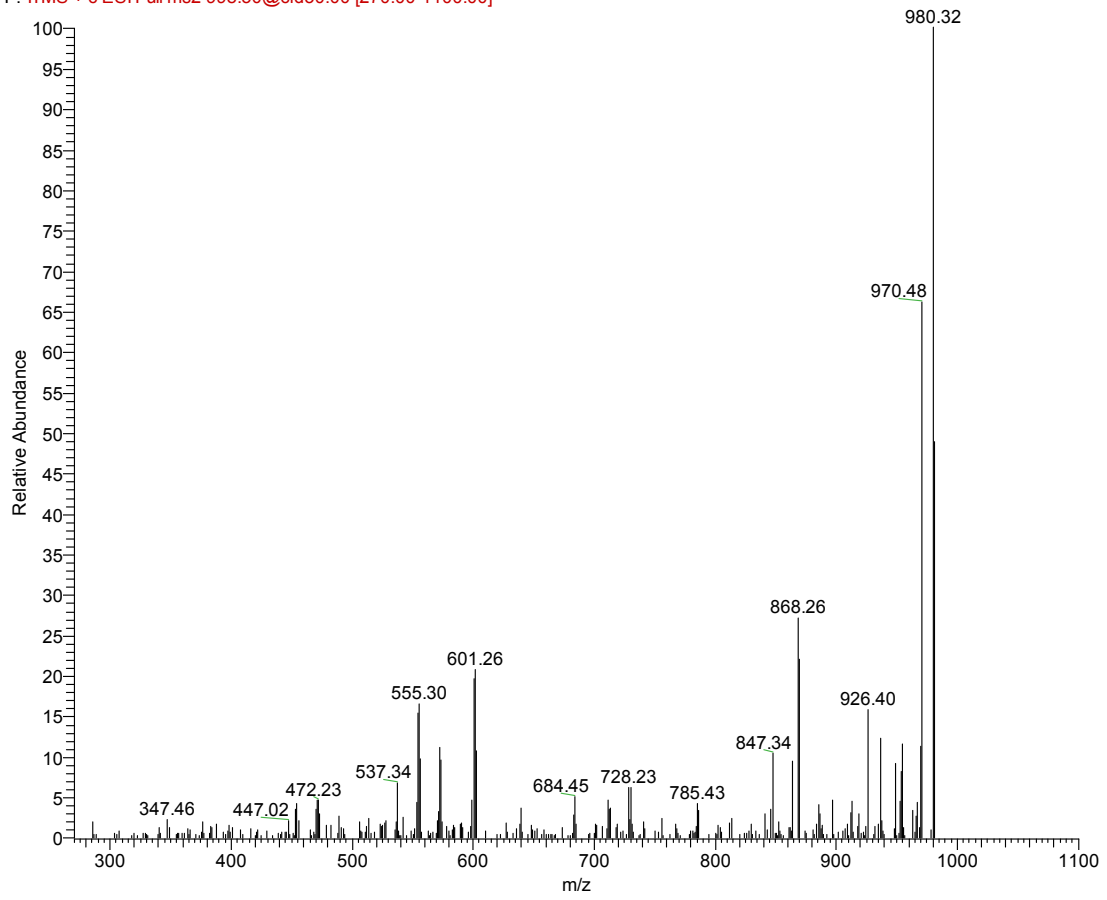


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Fig. S4: MS/MS spectra of $^{15}\text{N}_2\text{-MC}$ ($m/z=997.5$)

15N-1 #262 RT: 3.86 AV: 1 NL: 4.94E2
F: ITMS + c.ESI Full ms2 998.50@cid30.00 [270.00-1100.00]

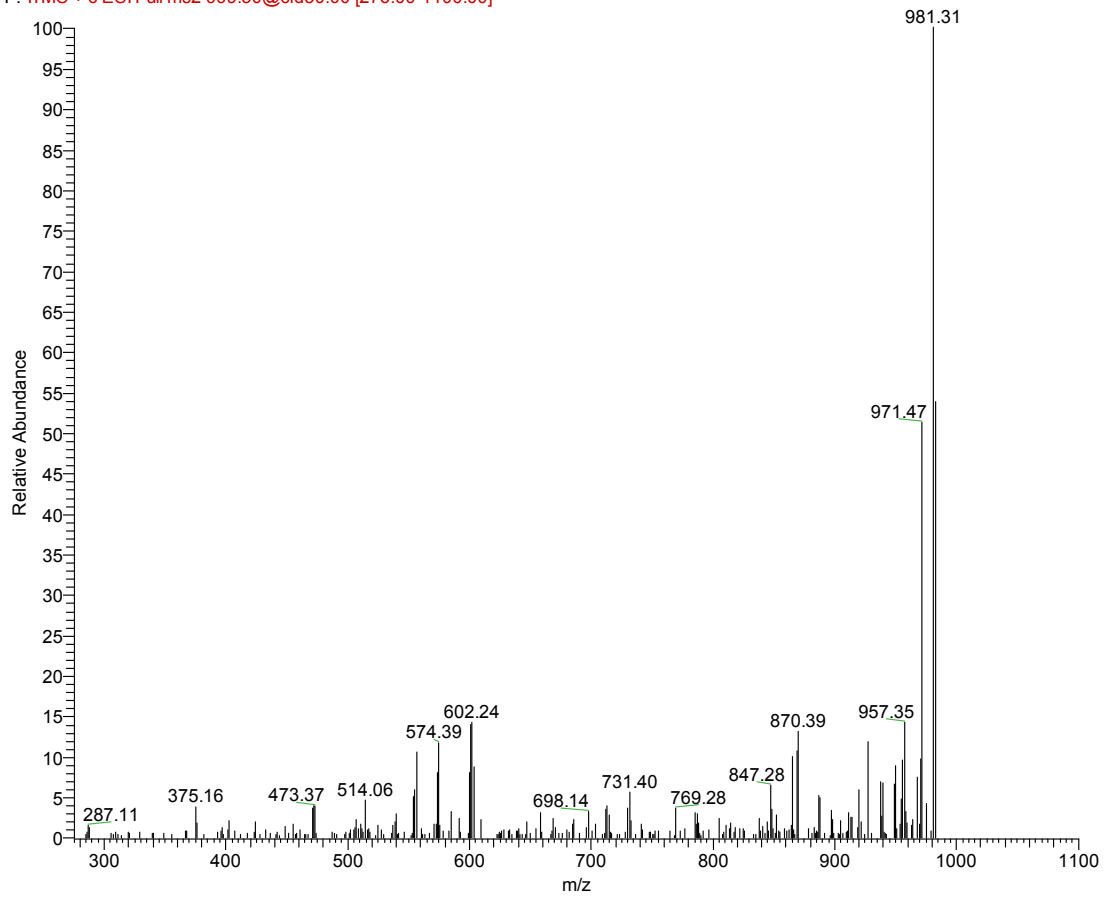


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Fig. S5: MS/MS spectra of $^{15}\text{N}_3\text{-MC}$ ($m/z=998.5$)

15N-1 #257 RT: 3.78 AV: 1 NL: 4.43E2
F: ITMS + c.ESI Full ms2 999.50@cid30.00 [275.00-1100.00]

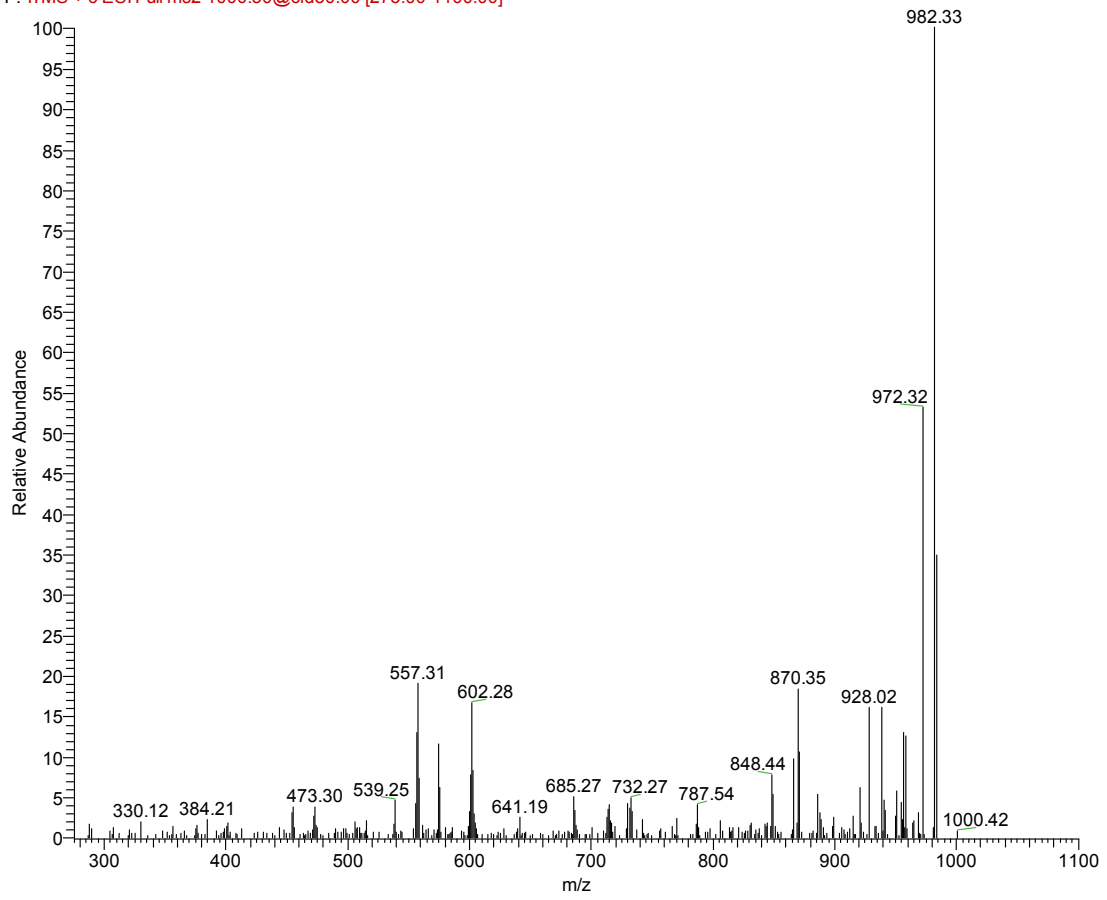


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Fig. S6: MS/MS spectra of $^{15}\text{N}_4\text{-MC}$ ($m/z=999.5$)

15N-1 #258 RT: 3.80 AV: 1 NL: 5.74E2
F: ITMS + c.ESI Full ms2 1000.50@cid30.00 [275.00-1100.00]

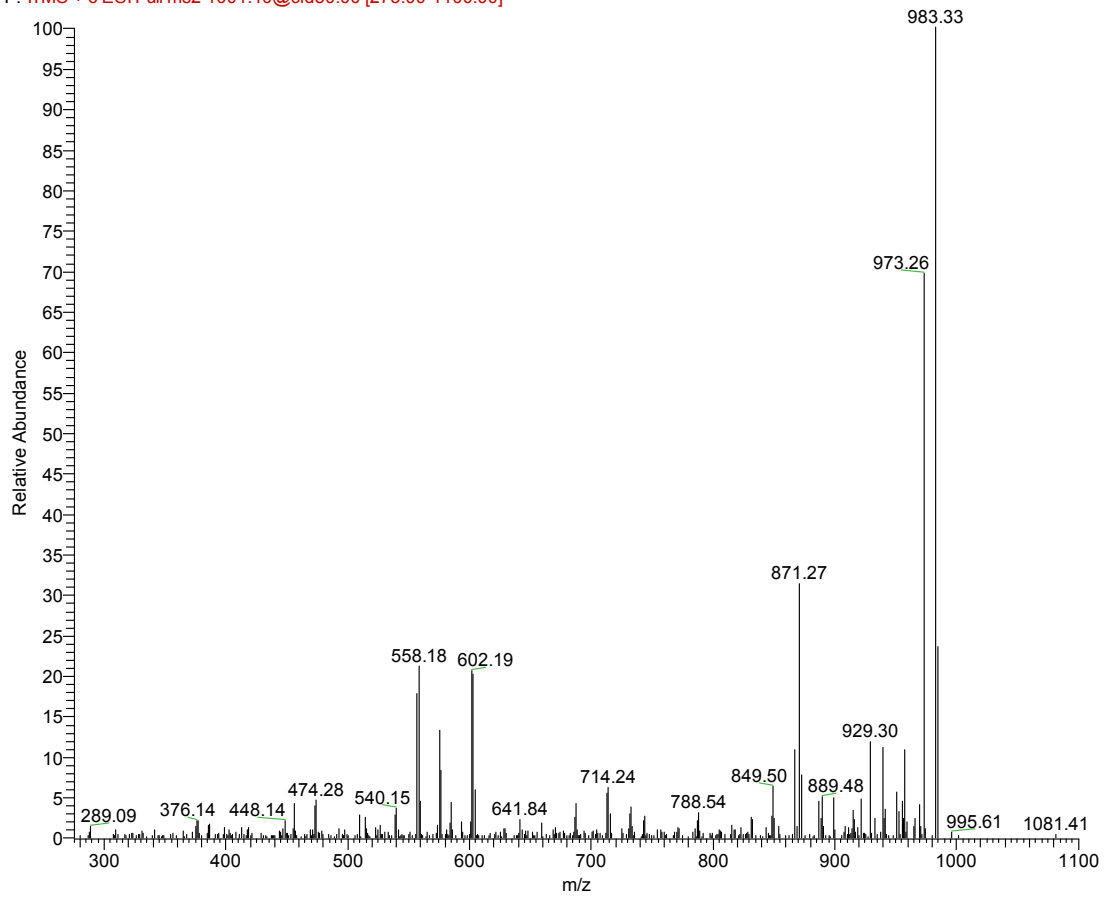


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Fig. S7: MS/MS spectra of $^{15}\text{N}_5\text{-MC}$ ($m/z=1000.5$)

15N-2 #258 RT: 3.84 AV: 1 NL: 1.02E3
F: ITMS + c.ESI Full ms2 1001.40@cid30.00 [275.00-1100.00]

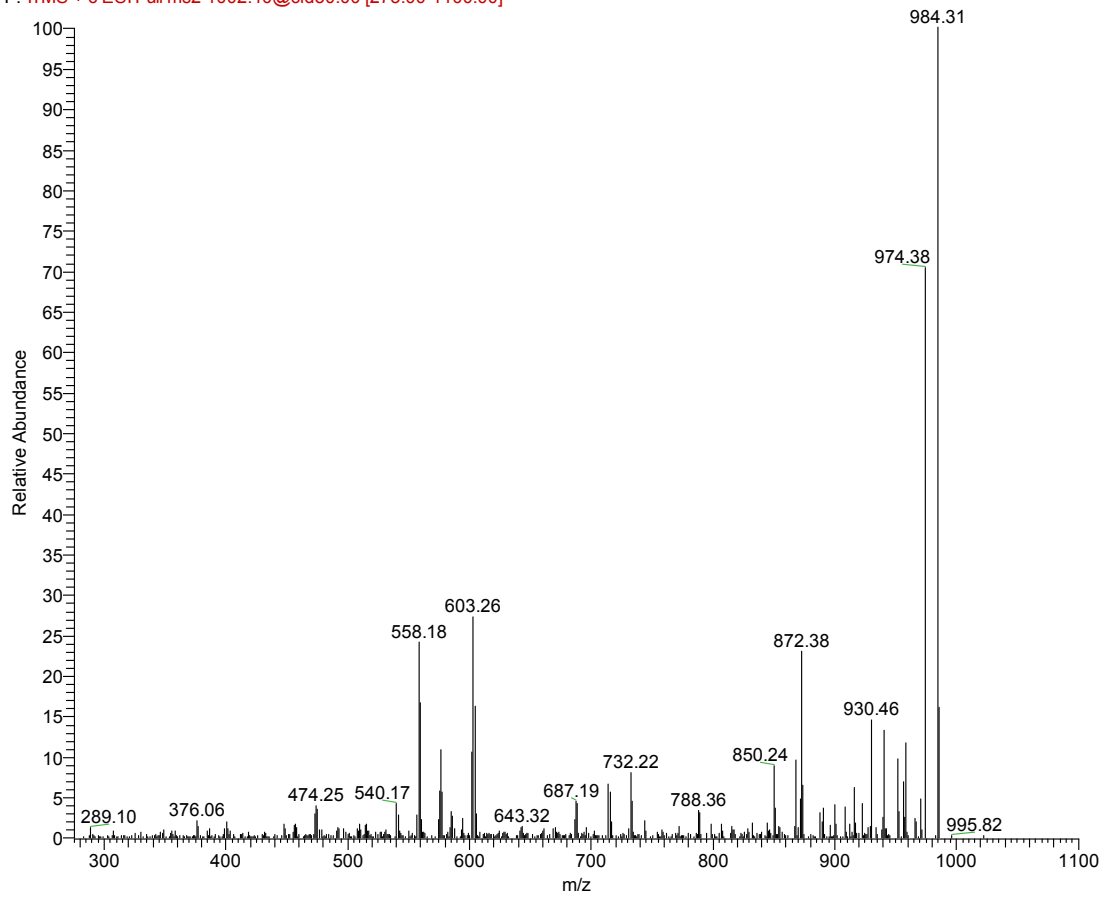


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Fig. S8: MS/MS spectra of $^{15}\text{N}_6\text{-MC}$ ($m/z=1001.5$)

15N-2 #259 RT: 3.86 AV: 1 NL: 2.06E3
F: ITMS + c.ESI Full ms2 1002.40@cid30.00 [275.00-1100.00]



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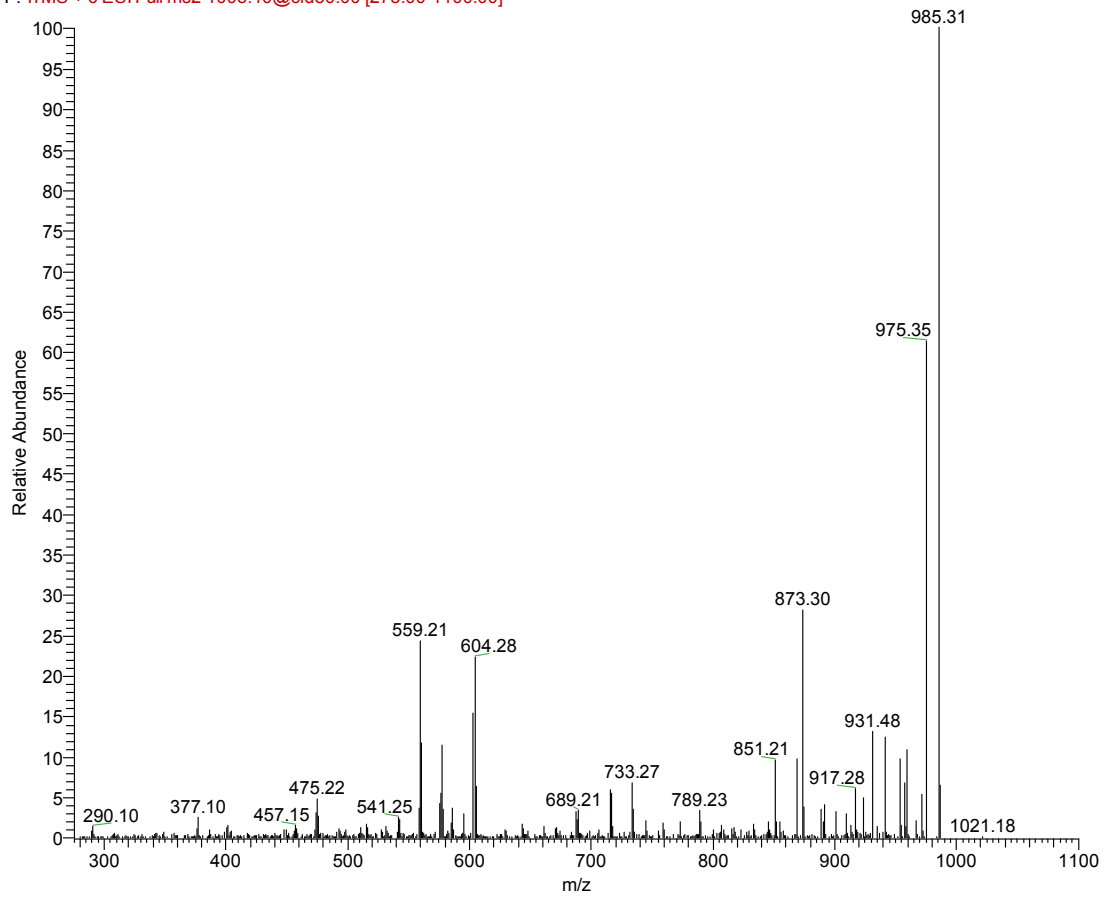
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Fig. S9: MS/MS spectra of $^{15}\text{N}_7\text{-MC}$ ($m/z=1002.5$)

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15N-2 #260 RT: 3.88 AV: 1 NL: 3.74E3
F: ITMS + c.ESI Full ms2 1003.40@cid30.00 [275.00-1100.00]

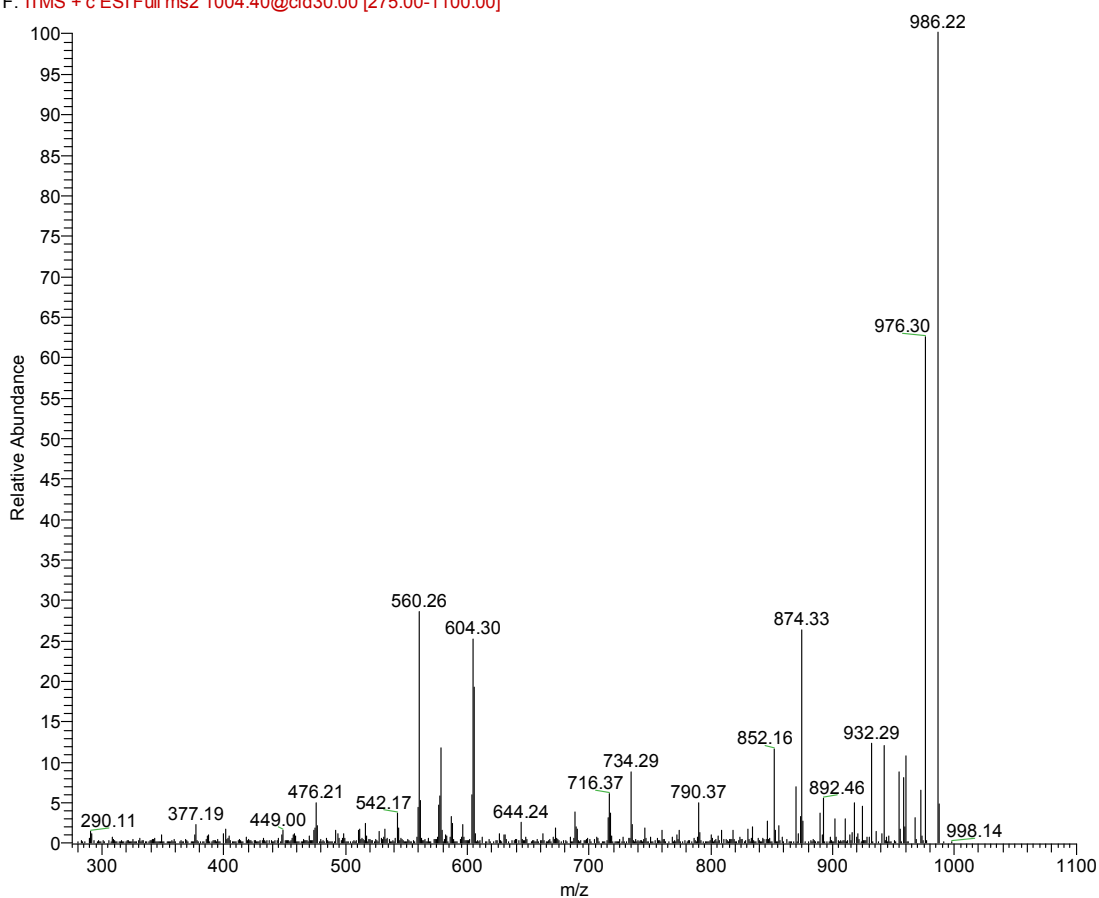


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Fig. S10: MS/MS spectra of $^{15}\text{N}_8\text{-MC}$ ($m/z=1003.5$)

15N-2 #253 RT: 3.78 AV: 1 NL: 6.33E3
F: ITMS + c ESI Full ms2 1004.40@cid30.00 [275.00-1100.00]



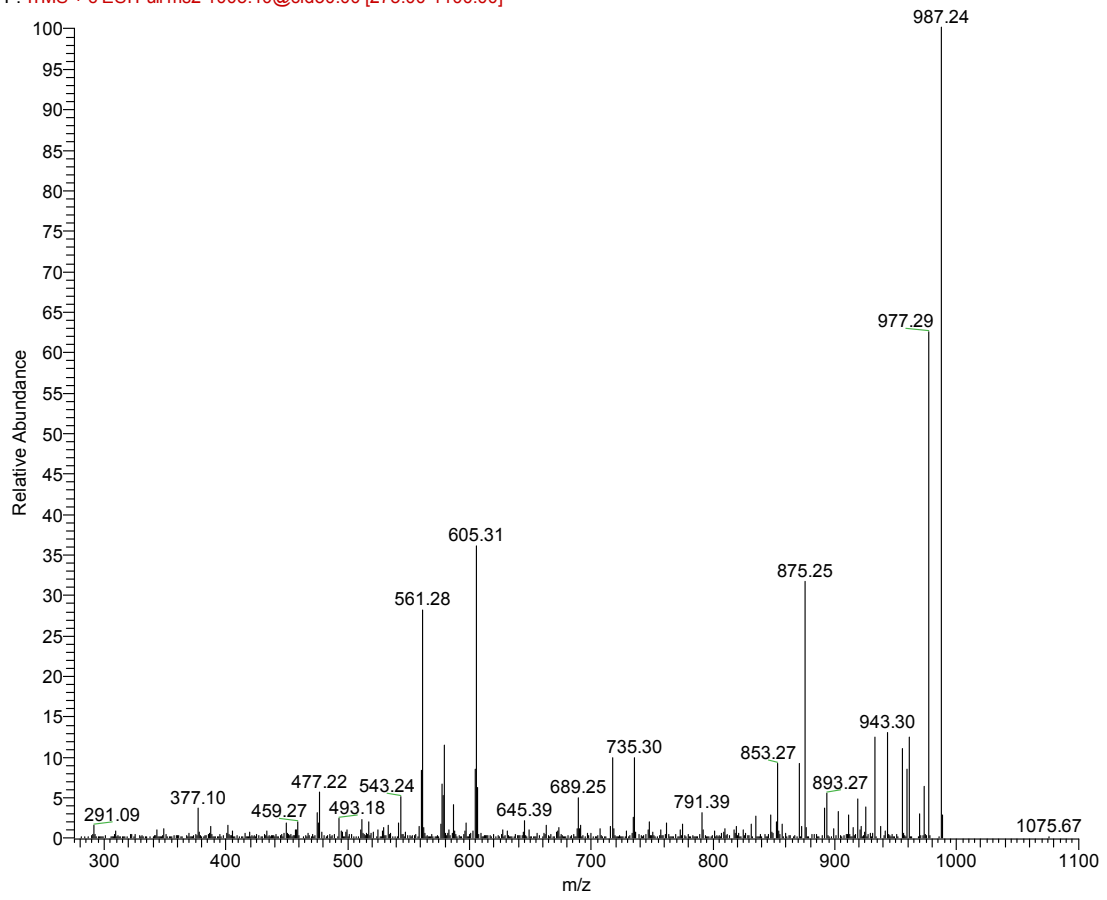
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Fig. S11: MS/MS spectra of $^{15}\text{N}_9\text{-MC}$ ($m/z=1004.5$)

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15N-2 #254 RT: 3.79 AV: 1 NL: 1.01E4
F: ITMS + c.ESI Full ms2 1005.40@cid30.00 [275.00-1100.00]



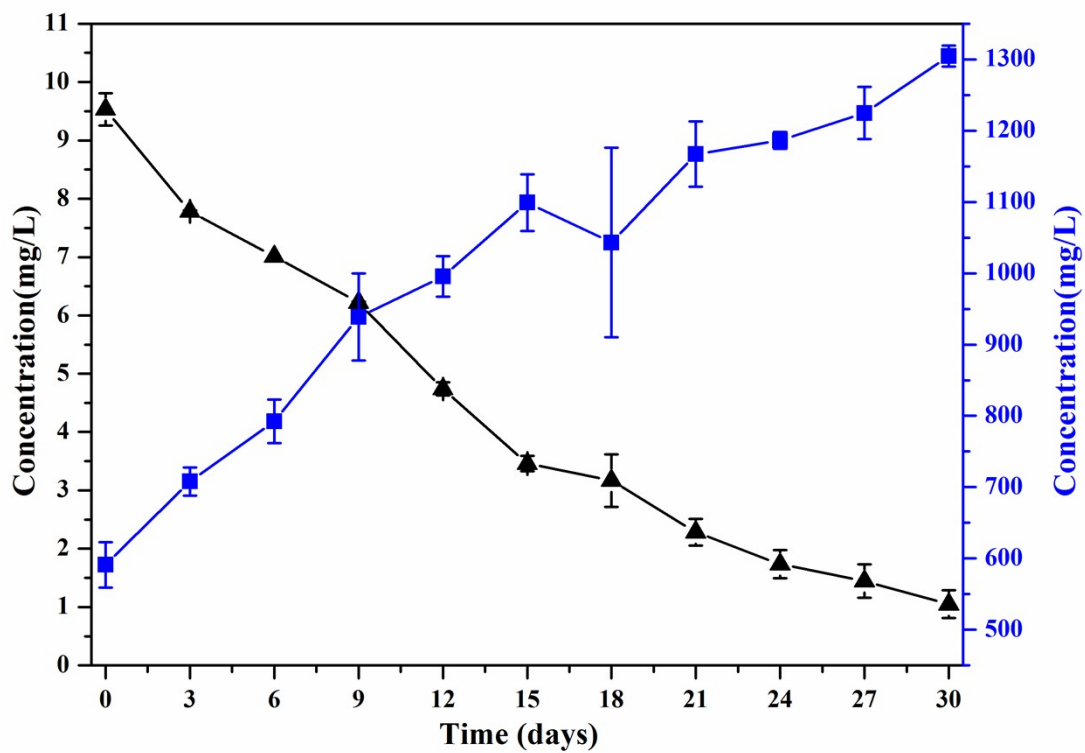
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Fig. S12: MS/MS spectra of $^{15}\text{N}_{10}$ -MC ($m/z=1005.5$)

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Fig. S13: Growth curve of ^{15}N -labeled MA and variants of ammonia in the

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cultivation of the 5th generation ■: MA, ▲: ammonia

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