Supplementary Information for

Stable isotope labeling nitrogen metabolism in microcystin biosynthesis

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Table S1: Components of BG11medium

Component	Concentration(g/L)
$NaNO_3$	1.5
K_2HPO_4	0.04
$MgSO_4$	0.075
$CaCl_2$·2H2O	0.036
Citric	0.006
Ferric Citrate	0.006
Na_2-EDTA	0.001
Na_2CO_3	0.02
H_3BO_3	0.00286
$MnCl_2·4H_2O$	0.00181
$ZnSO_4·7H_2O$	0.000222
$Na_2MoO_4·2H_2O$	0.00039
$CuSO_4·5H_2O$	0.000079
$Co(NO_3)_2$·6H2O	0.0000494

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

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

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

Unlabeled (cm^{-1})	^{15}N (Δ cm^{-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

Table S5: MS-MS parent ion and daughter ions for m/z 996.5, 997.5, 998.5, 999.5, 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

1002.5 (M+H+7^{15}N)	929	Leu-MeAsp-Arg-Adda-Glu-Mdha+H+5^{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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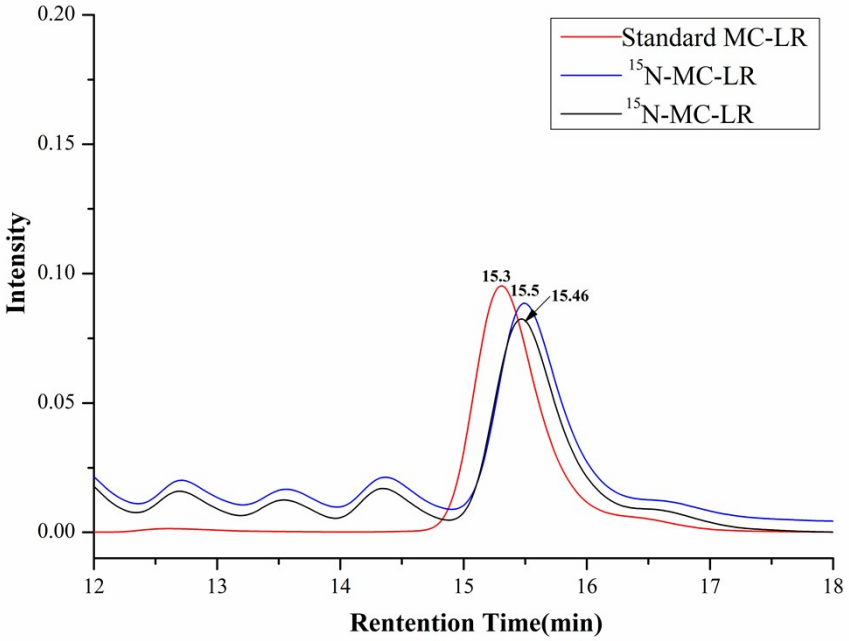

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2 **Fig.S1:** HPLC chromatograms of MC-LR and purified ^{15}N-MC-LR

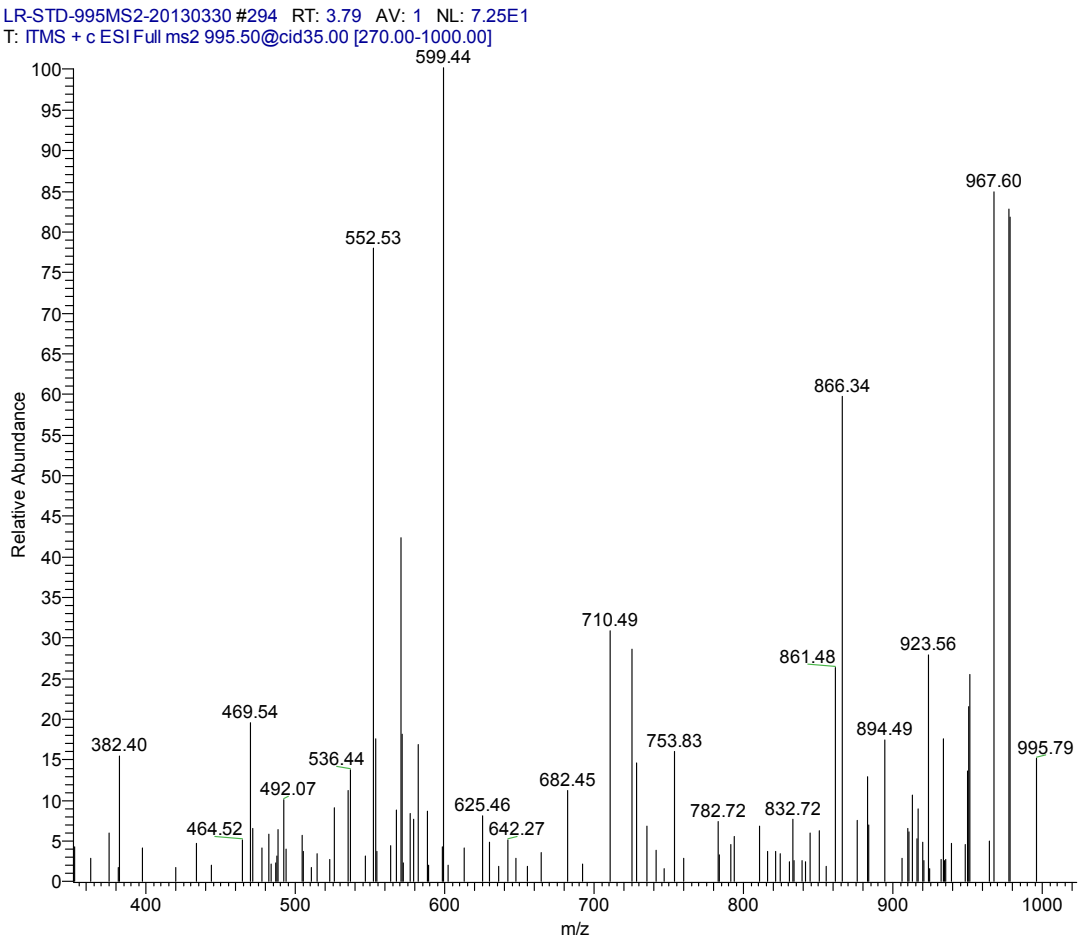

Fig. S2: MS/MS spectra of MC-LR standard (m/z=995.5)

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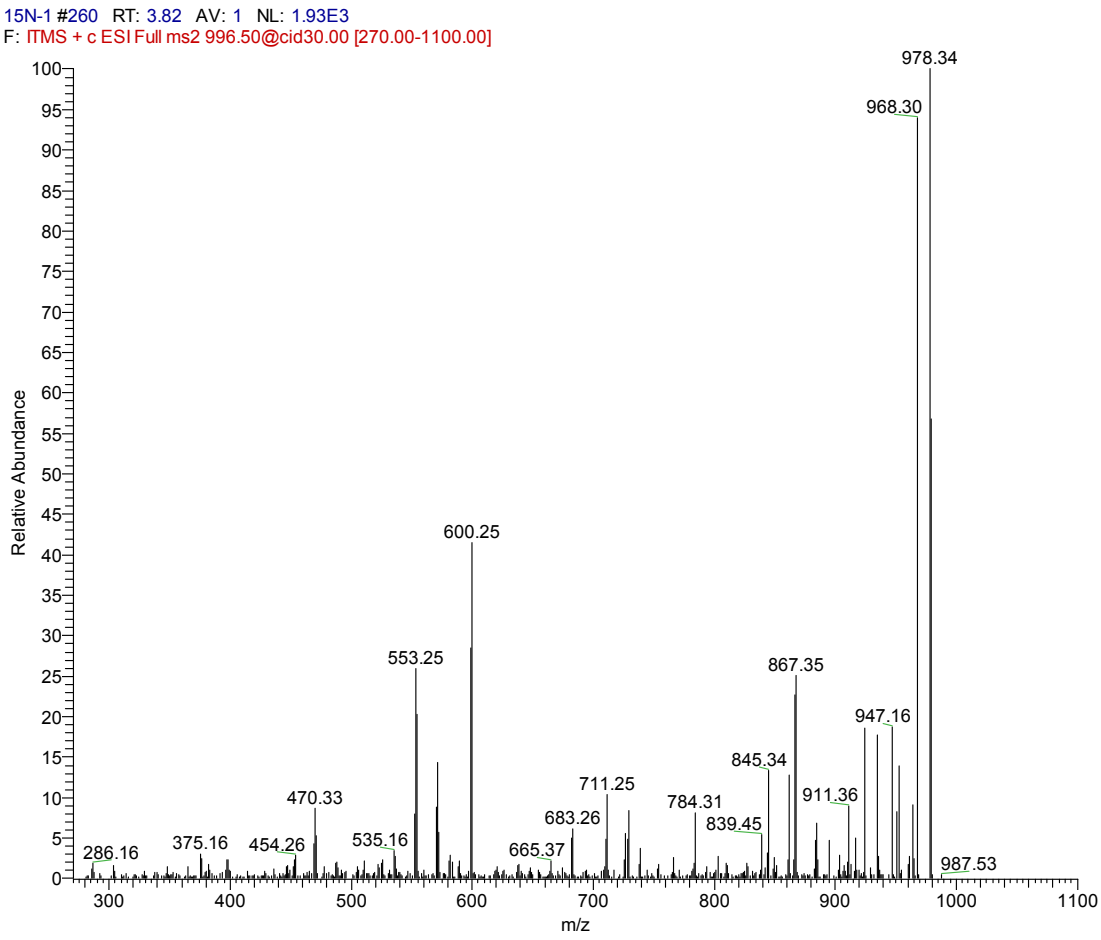

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3 **Fig. S3**: MS/MS spectra of $^{15}N_1$-MC (m/z=996.5)

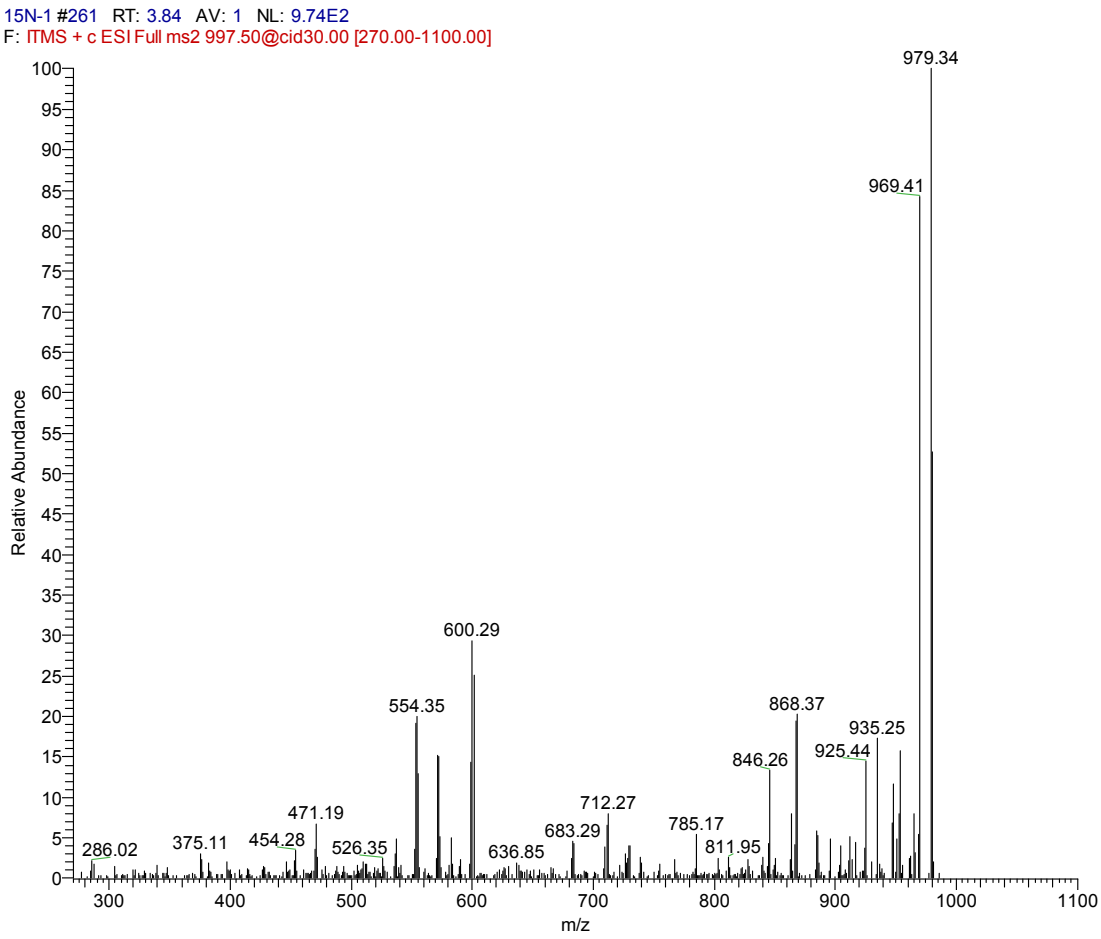

Fig. S4: MS/MS spectra of $^{15}N_2$-MC (m/z=997.5)

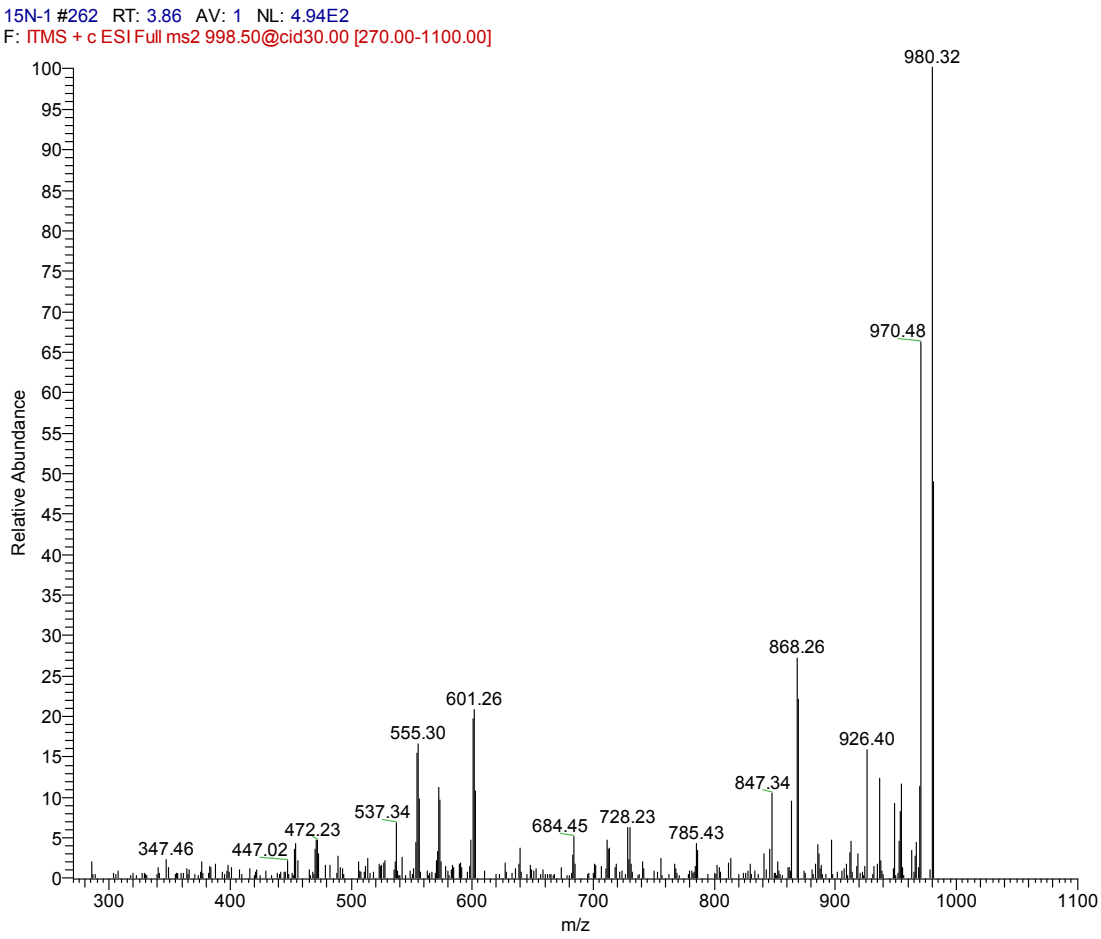

Fig. S5: MS/MS spectra of $^{15}N_3$-MC (m/z=998.5)

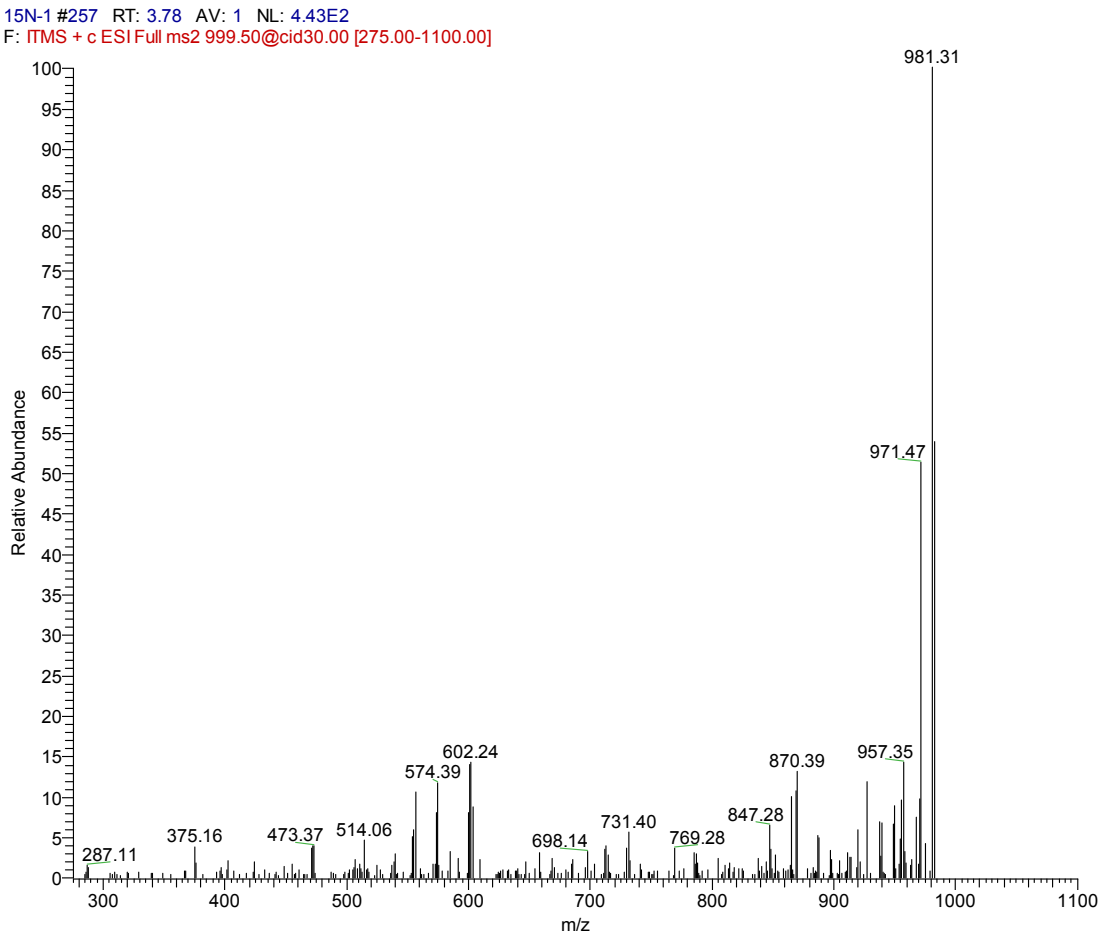

Fig. S6: MS/MS spectra of $^{15}N_4$-MC (m/z=999.5)

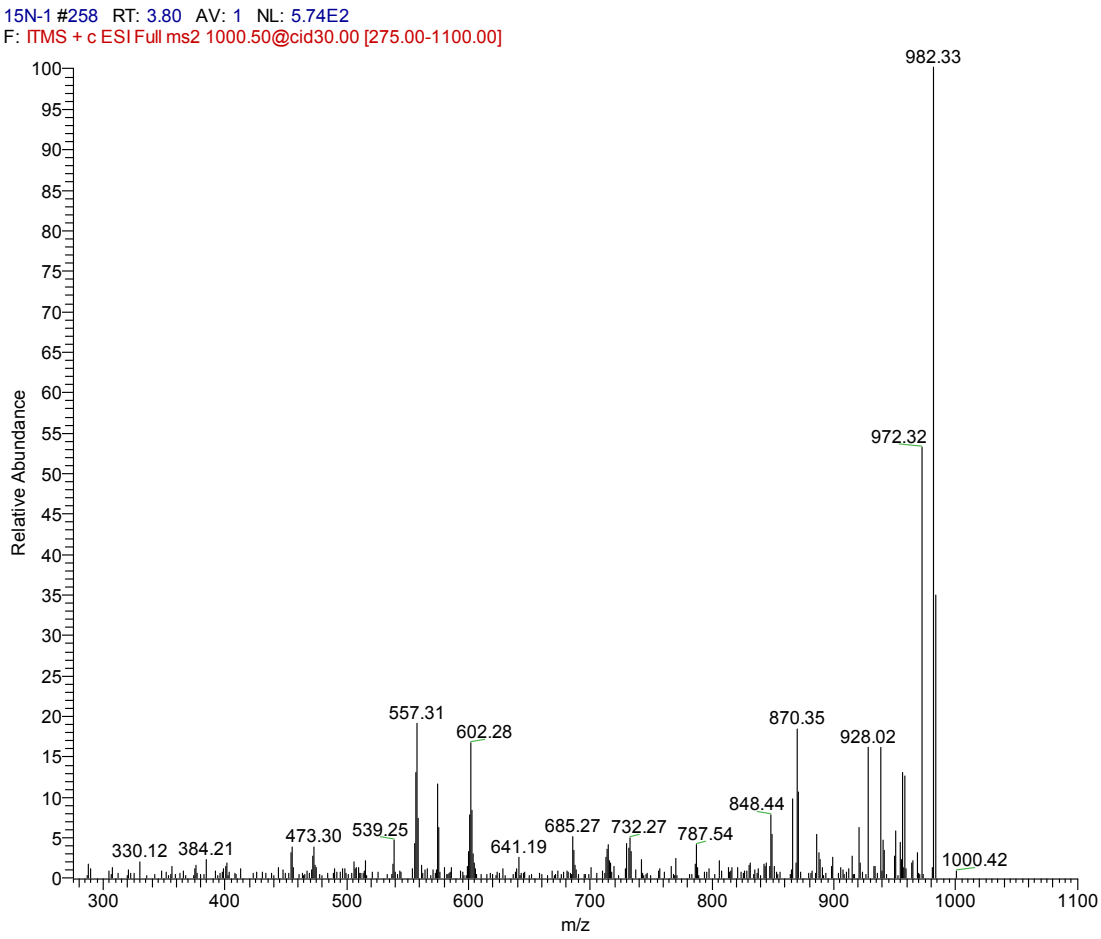

Fig. S7: MS/MS spectra of $^{15}N_5$-MC (m/z=1000.5)

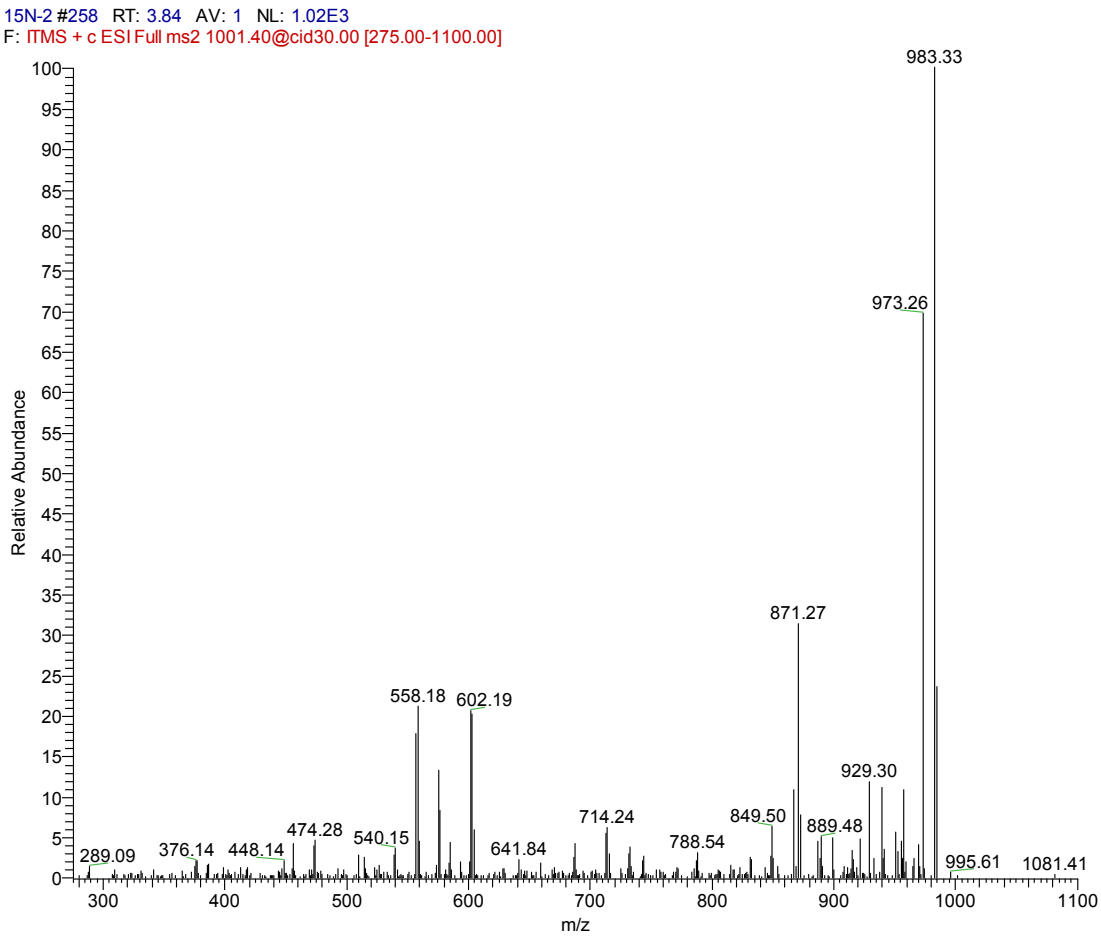

Fig. S8: MS/MS spectra of $^{15}N_6$-MC (m/z=1001.5)

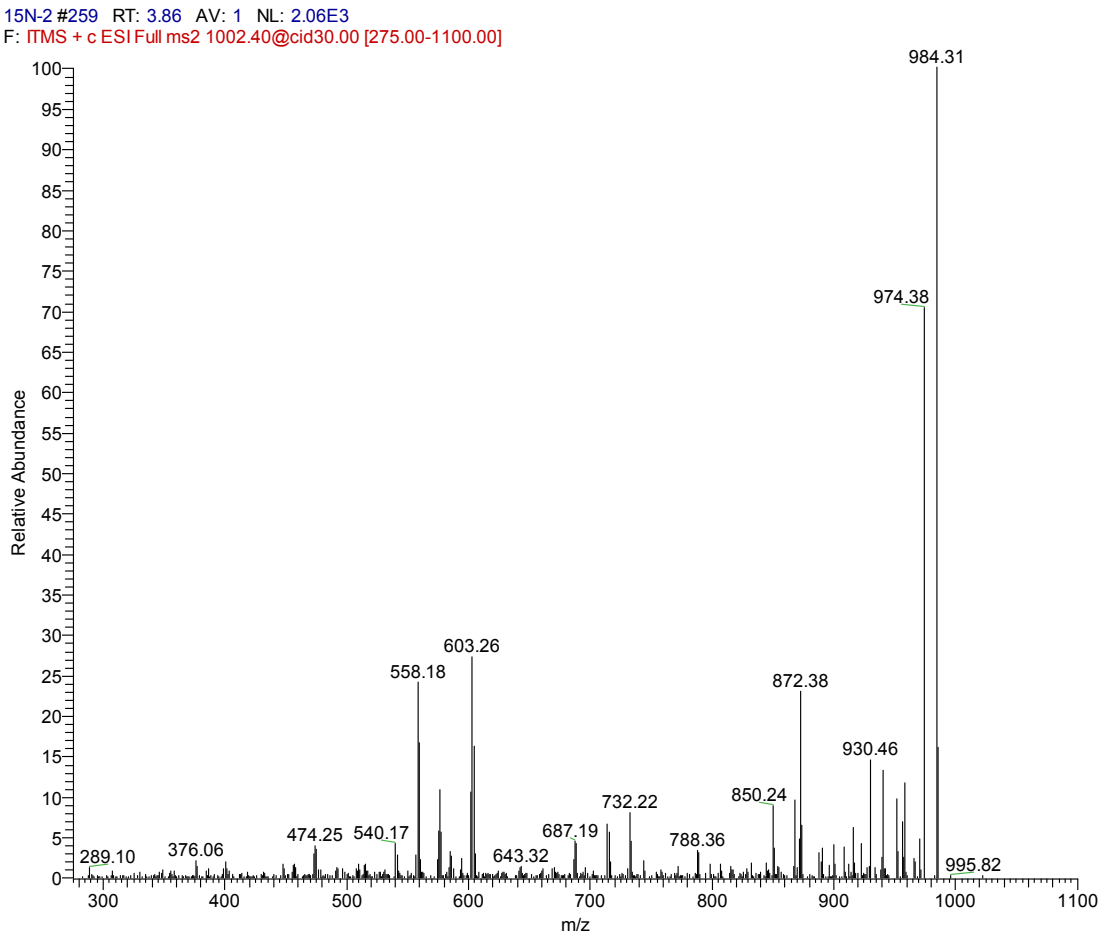

Fig. S9: MS/MS spectra of $^{15}N_7$-MC (m/z=1002.5)

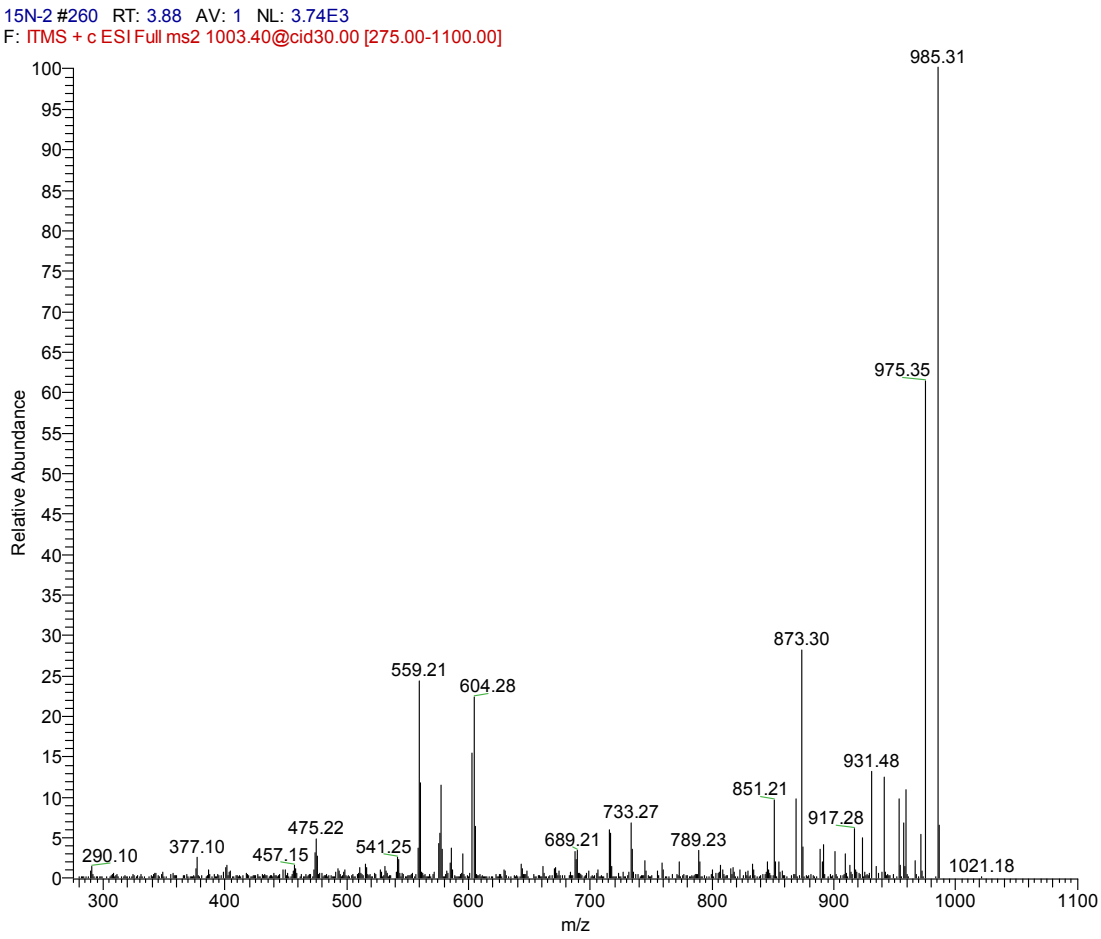

Fig. S10: MS/MS spectra of $^{15}N_8$-MC (m/z=1003.5)

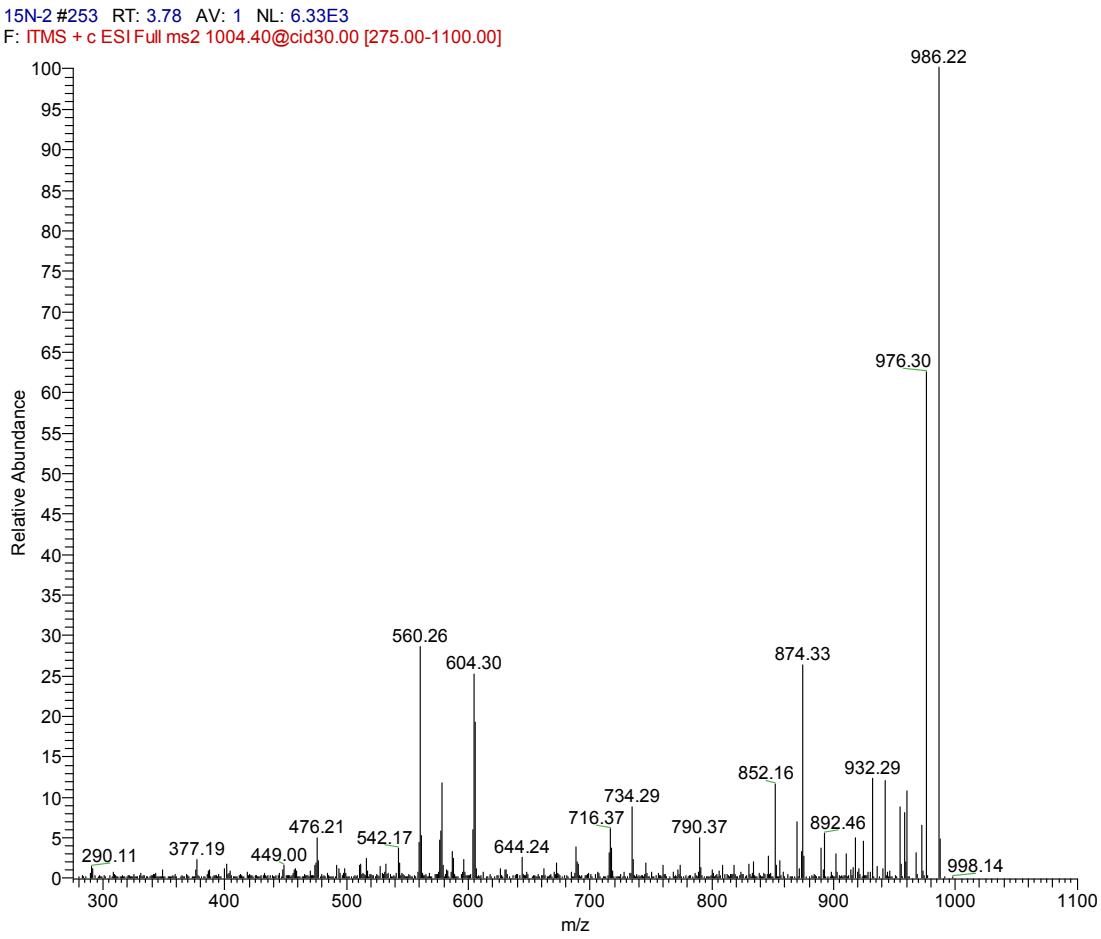

Fig. S11: MS/MS spectra of $^{15}N_9$-MC (m/z=1004.5)

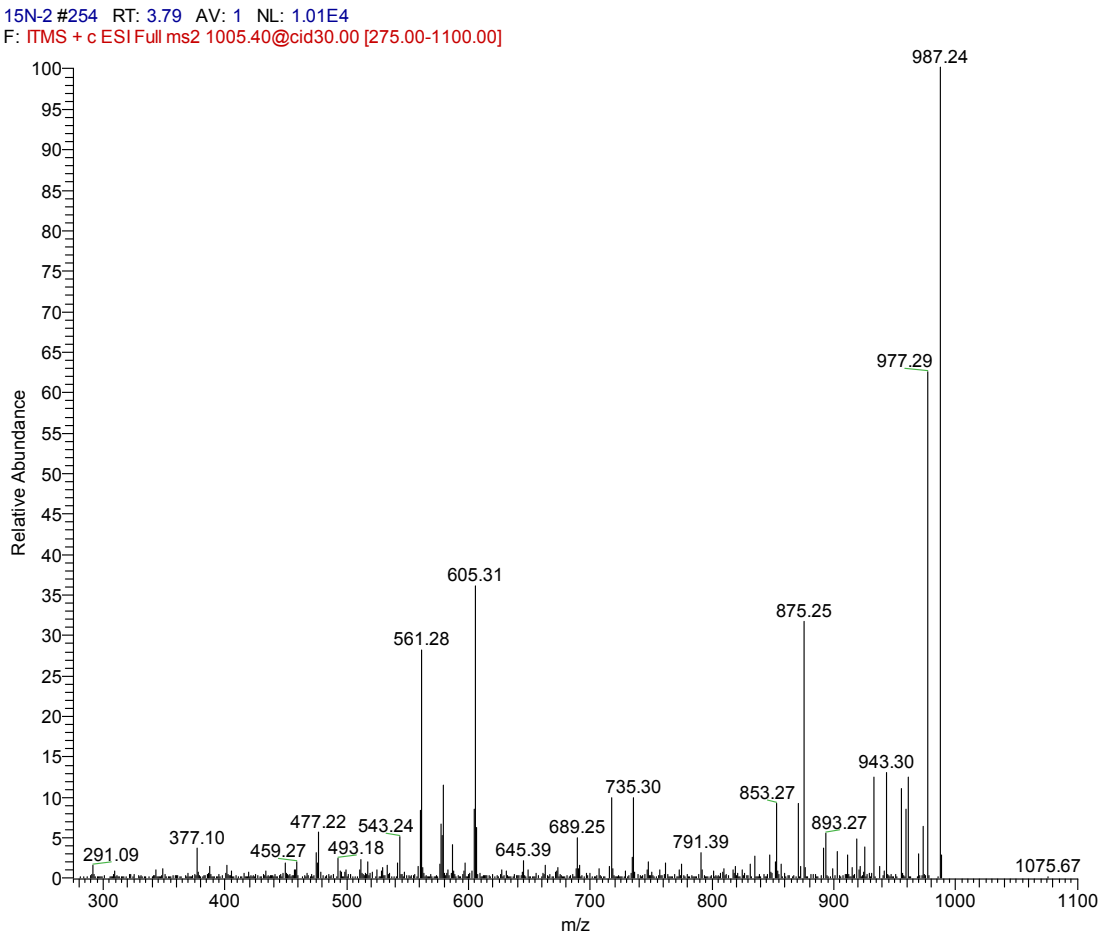

Fig. S12: MS/MS spectra of $^{15}N_{10}$-MC (m/z=1005.5)

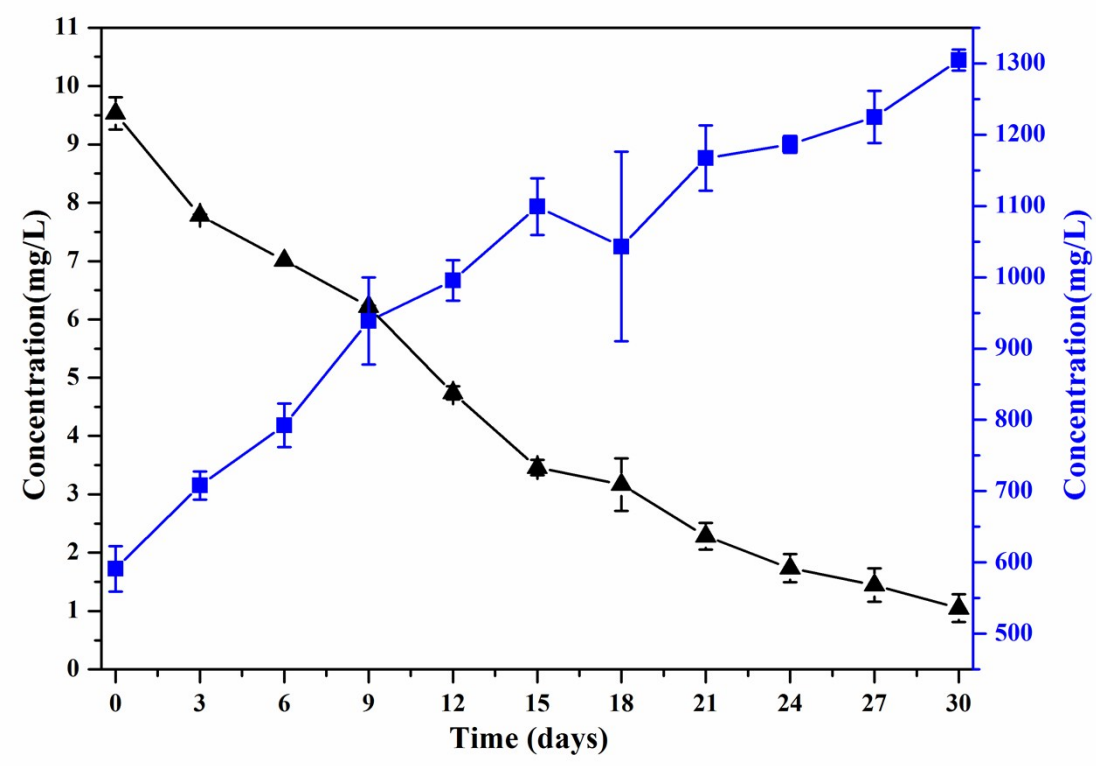

Fig. S13: Growth curve of ^{15}N-labeled MA and variants of ammonia in the cultivation of the 5th generation ■: MA, ▲: ammonia