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RESEARCH ARTICLE

# PRODUCTION OF NEW WAP-8294A CYCLODEPSIPEPTIDES BY THE BIOLOGICAL CONTROL AGENT *LYSOBACTER ENZYMOGENES* OH11

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## SUPPLEMENTARY MATERIALS

**Table S1** Primers used in this study

| Primers | Sequences (5‘–3‘) |
|---|---|
| dWAPorf4F1(Pst) | AACTGCAGTAGTCGCGGTCGTCGTCCAA |
| dWAPorf4R1(BamH) | CGCGGATCCATCGCCGTCACCCTCAGCA |
| dWAPorf4F2(BamH) | CGCGGATCCCGTGACCAGCAAAGGAGCG |
| dWAPorf4R2(Xba) | TGCTCTAGATGATCGAACAAGGCAACAACCT |
| InWAPorf4F | TTCCTCATCGAGCCATTCGTG |
| InWAPorf4R | TGCCCATTGCGATCTCCC |
| OutWAPorf4F | GGCCACCGGGAAATCGA |
| OutWAPorf4R | CACGCTCACCGCCTTCATC |
| ORF4-epF(BamH) | CGGGATCCGACATGAATAGAAAGCGGCT |
| ORF4-epR(Hind) | CCCAAGCTTCTAGTCCGGCAGGTGGCCGG |
| orf4 H104A-F | TCGCAGGAAATTCCCCTGGCATGCGAGAACG |
| orf4 H104A-R | TGCCAGGGGAATTTCCTGCGATTGCGGGAAT |
| orf4-site-vF | ACAGCGAGACCGAACACCAGG |

| | |
|---|---|
| orf4-site-vR | GGCGAACCACATTTCCGACT |
| orf4 H298A-F | GACAACATGCAGTTCGCGGCCGGGCGCCGC |
| orf4 H298A-R | GCCGCGAACTGCATGTTGTCCAGCAGCAGG |
| dWAPorf5F1(ApaI) | TGCGGGCCCCTTGTCGTCGGTCTGGAACAC |
| dWAPorf5R1(XbaI) | TGCTCTAGAATGGAGGCCGGCTGAGC |
| dWAPorf5F2(XbaI) | TGCTCTAGAGCCACGCAACATCGATTGTG |
| dWAPorf5R2(SacI) | TGCGAGCTCGATCTGGGACAGCCTGTCGG |
| InWAPorf5F | CTGGAAGATGTGGGTGAGGATG |
| InWAPorf5R | GCCAAACAGTGAGCGAACAAAA |
| OutWAPorf5F | CCAGGGACGAGACATGGAACA |
| OutWAPorf5R | CGGACGCCAACAACGACA |
| dWAPorf6F1(Pst) | AACTGCAGCCAAATGCCGTTGCGAGGAT |
| dWAPorf6R1(BamH) | CGCGGATCCCAAGGCCGGATCTGGGACA |
| dWAPorf6F2(BamH) | CGCGGATCCCGCGAGGTATCGGGAAGAAGAA |
| dWAPorf6R2(Xba) | TGCTCTAGACGCTTGCGGCATGGTCAA |
| InWAPorf6F | GGACCGCTGTCGTTGTTGGC |
| InWAPorf6R | GCAAGGCAGGATCGTCGAAGTG |
| OutWAPorf6F | AGCAAGGCGACGGACGATG |
| OutWAPorf6R | GGCCACGACCAGGCGATAA |
| dWAPorf7F1(Pst) | AACTGCAGGAACGCGAGGTATCGGGAAGAA |
| dWAPorf7R1(BamH) | CGCGGATCCCGCTTGCGGCATGGTCAA |
| dWAPorf7F2(BamH) | CGCGGATCCGATCCGCTCAATTCGATCCTCG |
| dWAPorf7R2(Xba) | TGCTCTAGAGGTCAGGCTGCCCTTGATGC |
| InWAPorf7F | GCGGTTATCGCCTGGTCGTG |
| InWAPorf7R | GCTGGAATTGCCCTGGTTGC |
| OutWAPorf7F | CGAACCGGAATGCTGTCGC |
| OutWAPorf7R | TGAGCAGGACCATGTTGAAGTCG |

| | |
|---|---|
| dWAPorf9F1(Pst) | AACTGCAGCATCCGCAACTACATCCTCAATACC |
| dWAPorf9R1(BamH) | CGCGGATCCGAGCAATACGGTTCGGGACATC |
| dWAPorf9F2(BamH) | CGCGGATCCCGATCTGATCGAACGCTTGAGCC |
| dWAPorf9R2(Xba) | TGCTCTAGACCACATGCTCGGGATCAGCAC |
| InWAPorf9F | GACCTTCTTCGGCGAGTTCC |
| InWAPorf9R | ACGGCTCCAGCAGTTCGTG |
| OutWAPorf9F | CGAACTGGAGCAACCTCAACCC |
| OutWAPorf9R | CGTTCATCGGCGTATCCATCC |
| dWAPorf10Fcom1(ApaI) | TGCGGGCCCCTCGTTGACGAGGTCGTAGCC |
| dWAPorf10Rcom1(XbaI) | TGCTCTAGACGTTGAGGAGCGCCATGG |
| dWAPorf10Fcom2(XbaI) | TGCTCTAGACATGGCGGCGGCTCAAG |
| dWAPorf10Rcom2(SacI) | TGCGAGCTCCGATGTCCCGAACCGTATTGC |
| InWAPorf10F | GCTTCGGCTTCGCCCAACA |
| InWAPorf10R | CGTTCATCGGCGTATCCATCC |
| OutWAPorf10F | TGTTCGGGGTGCGCTACAA |
| OutWAPorf10R | GTCGGCGAAGCCCCAGTT |
| dWAPorf10F2(BsaBamH) | CCGGGTCTCGGATCCCGTTGAGGAGCGCCATGG |
| dWAPorf10R2(BsaXba) | CCGGGTCTCTCTAGACTCGTTGACGAGGTCGTAGCC |

**Table S2** Fragment list of WAP-8294A compounds in the ESI MS/MS. The blue highlights indicate the fragment variations from WAP-8294A2

| Position | WAP-8294A2 | A1 | A4 | AZ1 | AZ2 | AZ3 | AZ4 | Ax8 (AZ5) | AZ6 |
|---|---|---|---|---|---|---|---|---|---|
| 6 | 134.0961 | 134.0961 | 134.0961 | 134.0960 | 134.0962 | 134.0961 | 134.0961 | 134.0961 | 134.0961 |
| 11 | 159.0908 | 159.0916 | 159.0911 | 159.0916 | 159.0914 | 159.0914 | 159.0915 | 159.0909 | 191.1172 |
| 5-6 | 219.1120 | 219.1124 | 219.1112 | 219.1127 | 219.1125 | 219.1120 | 219.1124 | 219.1125 | 219.1123 |
| 1-2 | 226.1431 | 212.1277 | 240.1588 | 226.1437 | 196.1332 | 226.1432 | 198.1119 | 226.1431 | 226.1432 |
| 7-8/12-13 | 228.1702 | 228.1698 | 228.1698 | 228.1704 | 228.1702 | 228.1705 | 228.1702 | 228.1700 | 228.1703 |
| 9-10 | 244.0906 | 244.0925 | 244.0930 | 244.0927 | 244.0926 | 244.0919 | 244.0924 | 244.0928 | ---------- |
| 11-12 | 301.1656 | 301.1653 | 301.1657 | 301.1660 | 301.1664 | 301.1665 | 301.1660 | 301.1658 | 333.1563 |
| 4-6 | 306.1443 | 306.1447 | 306.1450 | 276.1344 | 306.1451 | 276.1335 | 306.1445 | 306.1441 | 306.1442 |
| 1-3 | 338.1702 | 324.1550 | 352.1869 | 338.1676 | 308.1603 | 339.1548 | 310.1393 | 338.1714 | 338.1698 |
| | 356.1819 | 342.1646 | 370.1964 | 356.1819 | 326.1708 | 357.1649 | 328.1499 | 356.1813 | 356.1809 |
| 6-8 | 389.2573 | 389.2539 | 389.2539 | 389.2550 | 389.2545 | 389.2547 | 389.2538 | 389.2557 | 389.2538 |
| 9-11 | 430.1721 | 430.1714 | 430.1743 | 430.1715 | 430.1733 | 430.1720 | 430.1698 | 430.1724 | 462.1626 |
| 3-6 | 436.1865 | 436.1823 | 436.1837 | 406.1707 | 436.1826 | 407.1557 | 436.1833 | 436.1823 | 436.1813 |
| 1-5 | 482.2257 | 468.2086 | 496.2414 | 452.2143 | 452.2137 | 453.1986 | 454.1908 | 482.2234 | 482.2237 |
| | 500.2330 | 486.2180 | 514.2527 | 470.2230 | 470.2225 | 471.2026 | 472.2036 | 500.2345 | 500.2323 |
| 8-11/9-12 | 544.2510 | 544.2488 | 544.2523 | 544.2502 | 544.2530 | 544.2509 | 544.2493 | 544.2501 | 576.2379 |
| 7-11/9-13 | 639.3236 | 639.3194 | 639.3250 | 639.3239 | 639.3254 | 639.3233 | 639.3240 | 639.3246 | 671.3107 |
| | 657.3335 | 657.3347 | 657.3339 | 657.3336 | 657.3339 | 657.3334 | 657.3365 | 657.3321 | 689.3230 |
| | | | | | | | | 643.3184 | |
| 1-6 | 661.3179 | 647.3032 | 675.3350 | 631.3071 | 631.3101 | 632.2837 | 633.2844 | 661.3188 | 661.3154 |
| 7-12 | 771.4117 | 771.4144 | 771.4116 | 771.4115 | 771.4126 | 771.4119 | 771.4144 | 771.4160 | 803.4042 |
| 6-11 | 818.4190 | 818.4116 | 818.4144 | 818.4136 | 818.4169 | 818.4172 | 818.4128 | 818.4127 | 850.4067 |
| 7-13 | 866.4812 | 866.4871 | 866.4794 | 866.4839 | 866.4854 | 866.4842 | 866.48907 | 852.4720 | 898.4758 |
| 6-12 | 932.4989 | 932.4725 | 932.4999 | 932.4934 | 932.4990 | 932.4862 | 932.4961 | 932.4894 | 964.4868 |
| 5-12 | 989.5142 | 989.5215 | 989.5135 | 989.5536 | 989.5486 | 989.5083 | 989.5061 | 989.5168 | 1021.5046 |
| 1-9 | 1017.5274 | 1003.5081 | 1031.5354 | 987.5200 | 987.5056 | 988.5030 | 989.5061 | ---------- | 1017.5114 |
| 4-12 | 1076.5460 | 1076.5463 | 1076.5472 | 1046.5394 | 1076.5498 | 1046.5341 | 1076.5331 | ---------- | 1108.5352 |
| 3-12 | 1206.5858 | 1206.5827 | 1206.5811 | 1176.5806 | 1206.5948 | 1177.5624 | 1206.5876 | 1206.5924 | 1238.5714 |

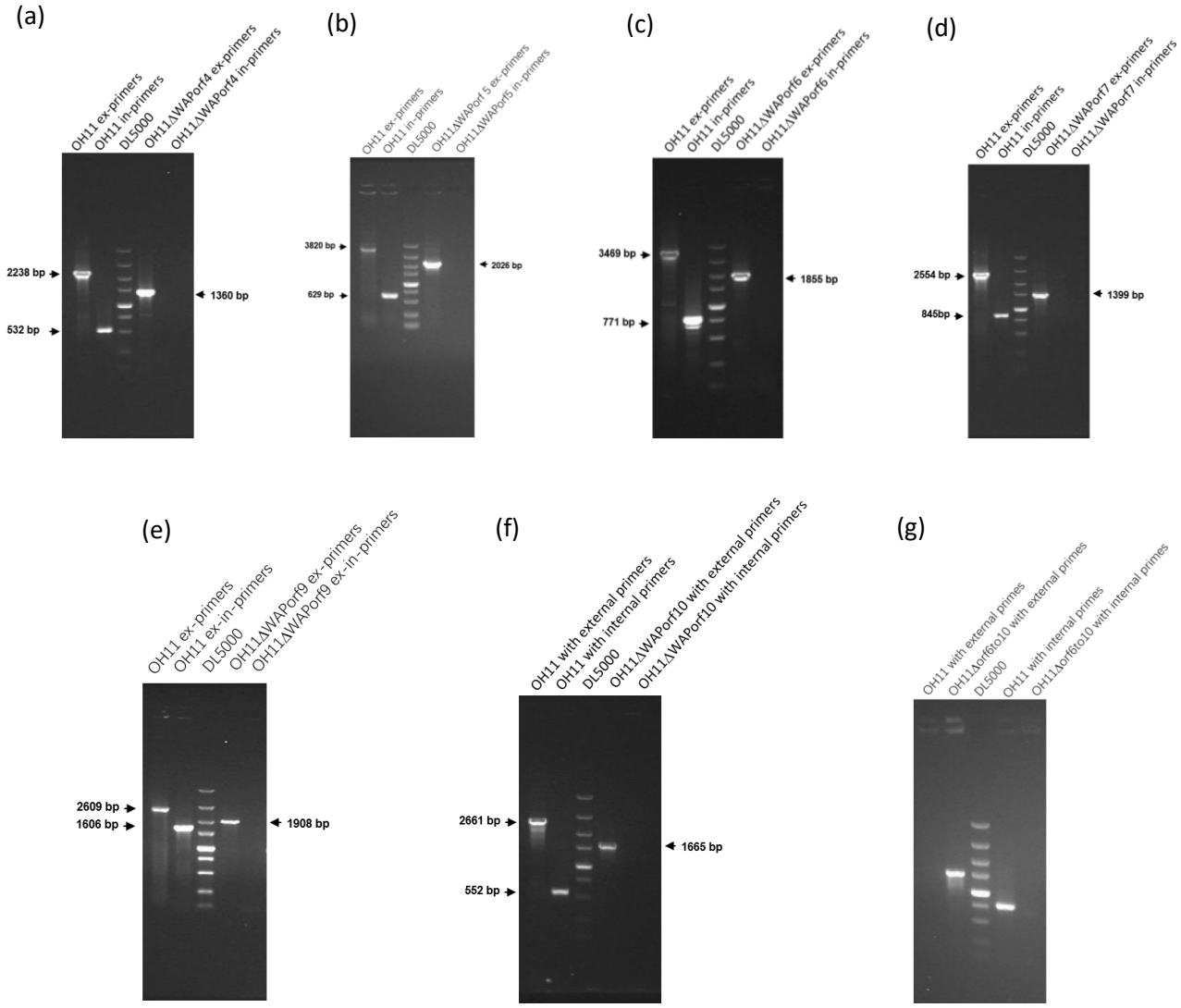



**Fig. S1** Diagnostic PCR for the confirmation of mutants with in-frame deletion of the WAP-8294A biosynthetic genes, ORF4 (a), ORF5 (b), ORF6 (c), ORF7 (d), ORF9 (e), ORF10 (f), and ORFs 6–10 quintet (g), in *L. enzymogenes* OH11.

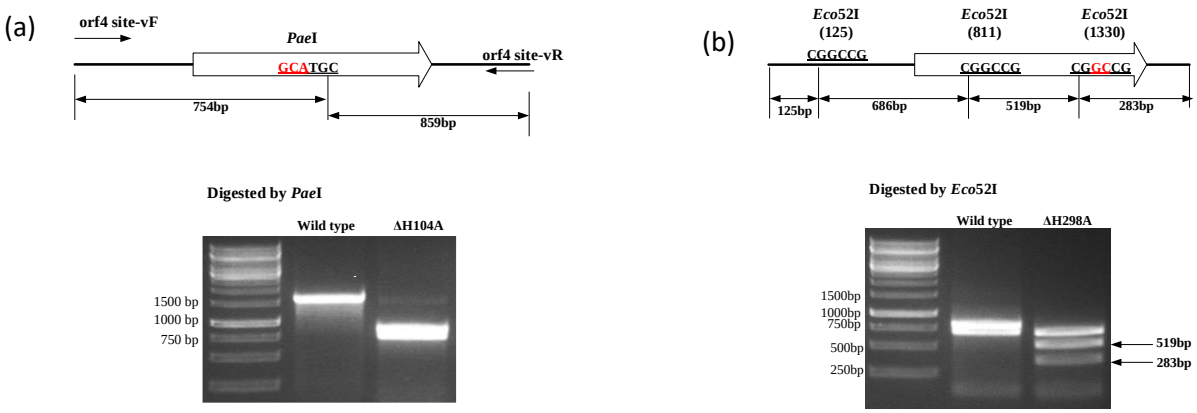



**Fig. S2** Diagnostic PCR for confirmation of the mutants with a single point mutagenesis of the WAP-8294A biosynthetic gene ORF4 in *L. enzymogenes* OH11, H104A and H298A. Mutant H104A was introduced with a unique *Pae*I at the point mutation site for the convenience of confirmation (a). The PCR product from mutant H104A gave the expected two fragments, 754 and 859 bp, after the *Pae*I digestion, while the PCR product from the wild type was not cut by this enzyme. Mutant H298A was introduced an extra *Eco*52I at the point mutation site for the convenience of confirmation (b). The PCR product from H298A gave the four expected fragments, 686, 519, 283, and 125 bp, after the *Pae*I digestion, while the PCR product from the wild type only gave the three expected fragments, 686, 802, and 125 bp.

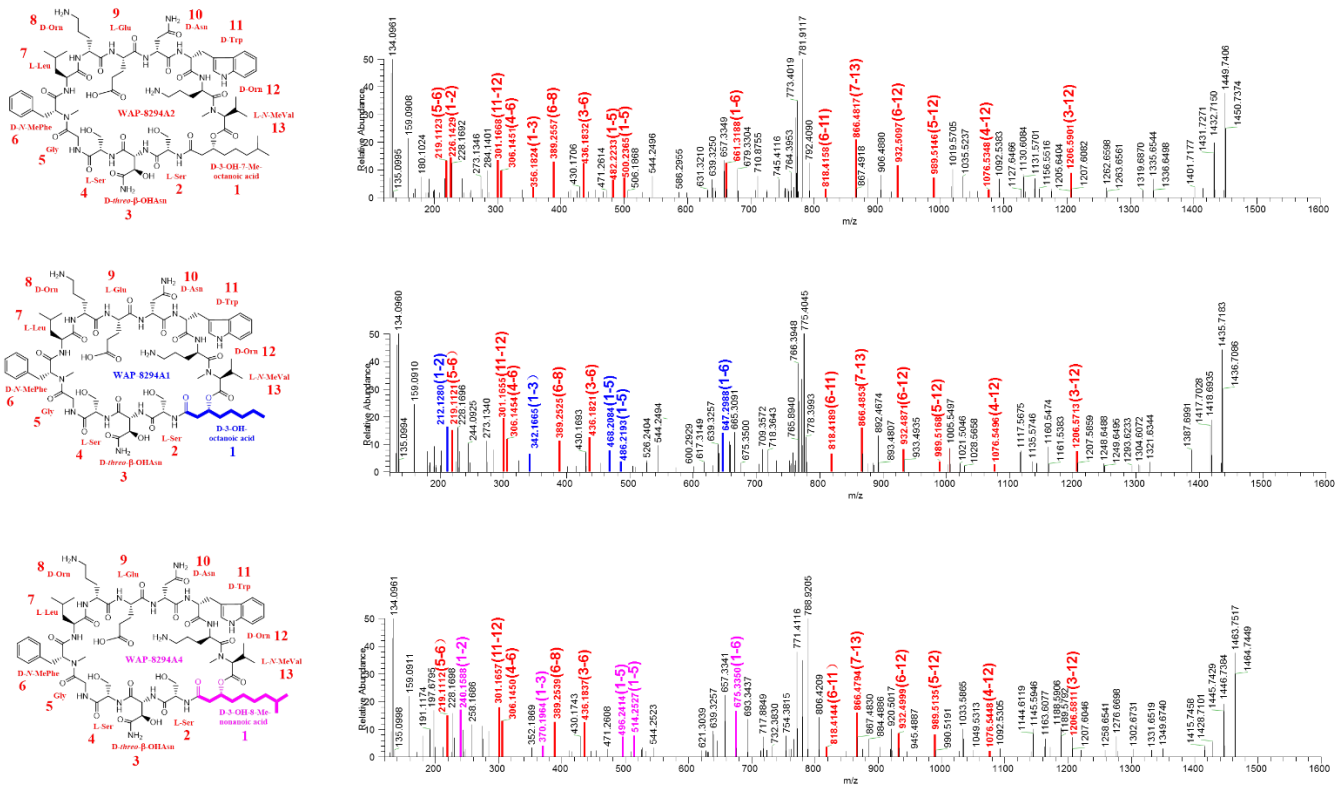
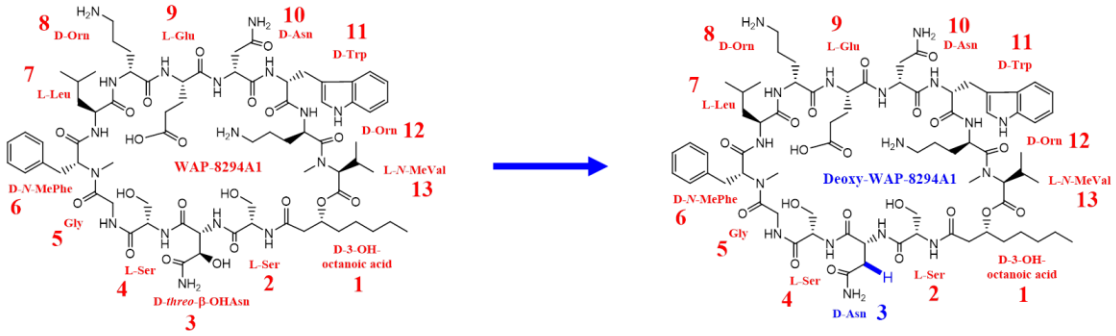
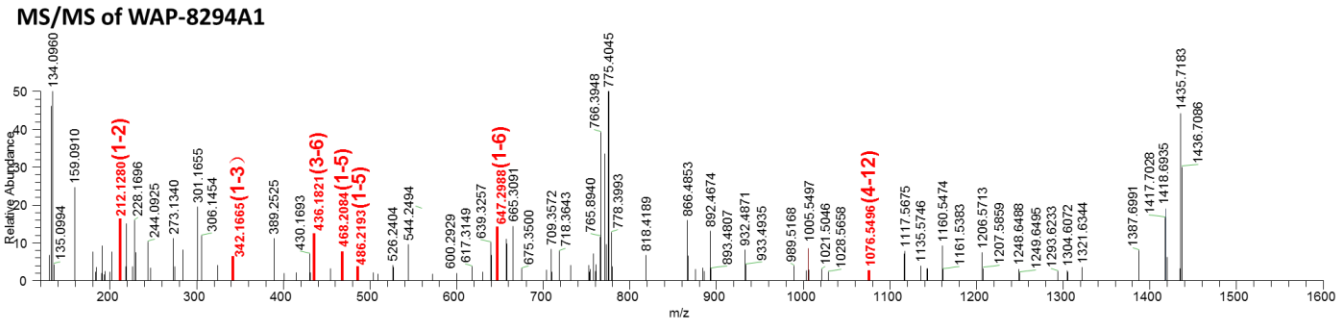
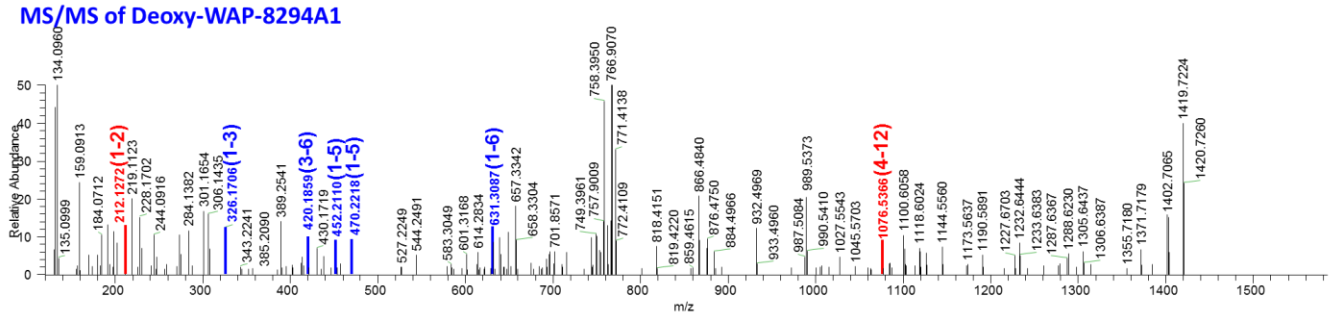



**Fig. S3** High resolution MS/MS fragments of WAP-8294A1, A2, and A4 (from top to bottom). The numbers inside the parentheses indicate the position of the building blocks, the 12 amino acid residues and D-3-OH-fatty acid moiety.

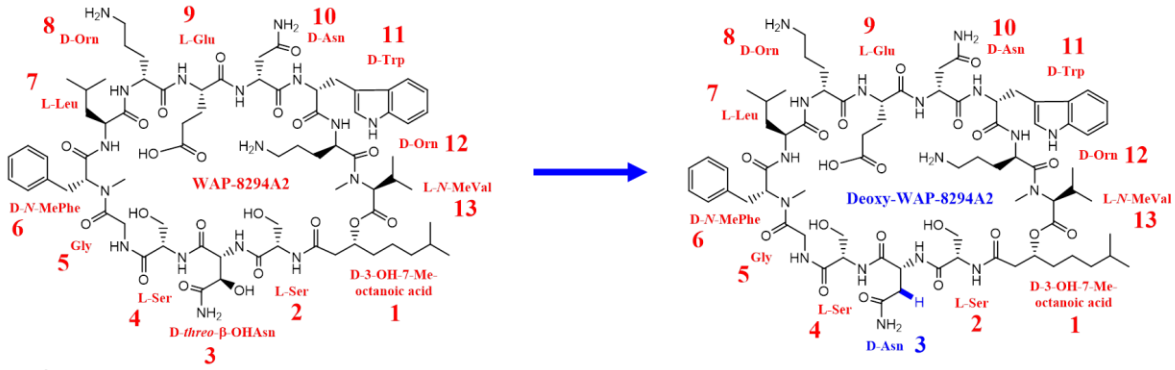
(a)


WAP-8294A1
Deoxy-WAP-8294A1
D-Orn
L-Glu
D-Asn
D-Trp
L-Leu
D-N-MePhe
Gly
L-Ser
D-threo-β-OHAsn
D-3-OH-octanoic acid
L-N-MeVal

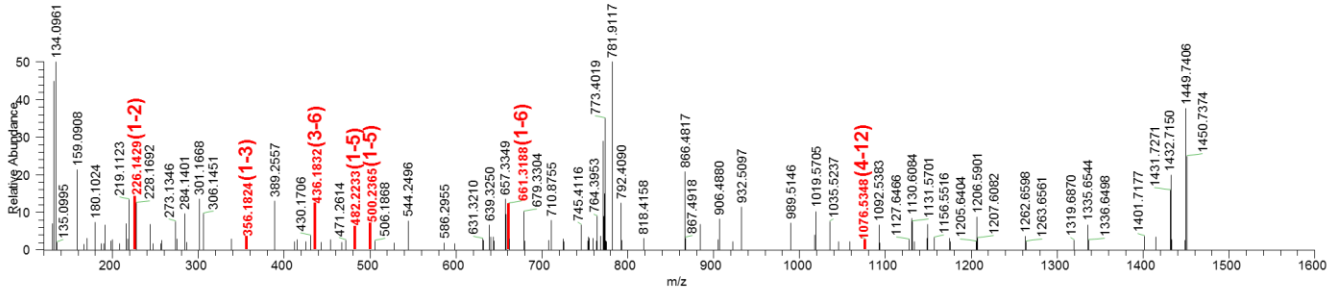

MS/MS of WAP-8294A1
Relative Abundance
m/z


MS/MS of Deoxy-WAP-8294A1
Relative Abundance
m/z

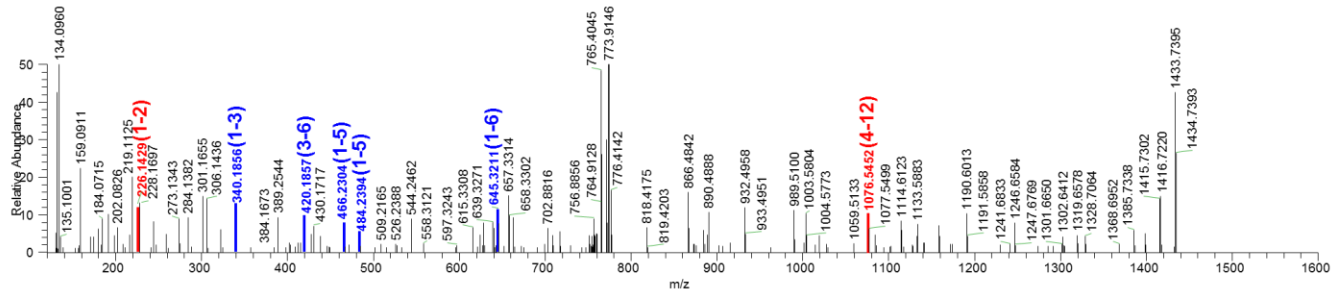
(b)




**MS/MS of WAP-8294A2**




**MS/MS of Deoxy-WAP-8294A2**



(c)

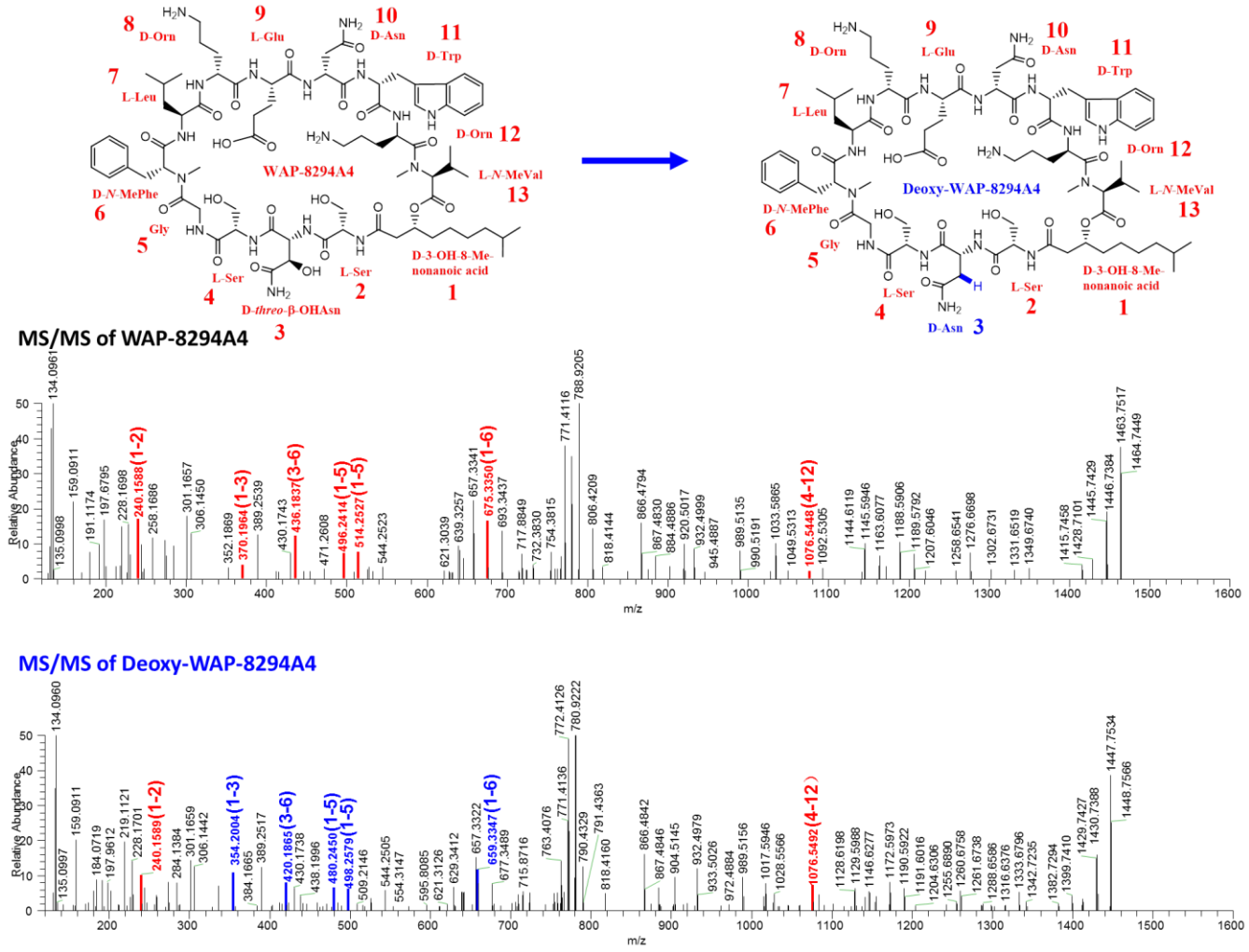



**Fig. S4** Comparison of the high resolution MS/MS fragment patterns of the deoxy analogs with the parent WAP-8294A1 (a), A2 (b), and A4 (c). The numbers inside the parentheses indicate the position of the building blocks, the 12 amino acid residues and D-3-OH-fatty acid moiety. The data confirmed that the deoxy position is at D-*threo*-β-OH-Asn, rather than at D-3-OH-fatty acid moiety.