Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Supplemental Information for

Synthesis of bicyclic organo-peptide hybrids via oxime/intein-mediated macrocyclization followed by disulfide bond formation

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Supplementary Table S1: Oligonucleotide primers

Primer	Sequence
8/10mer(C@U+1)_for	5'-GCATCCCATGGCTAGTGCGGATCC-3'
8mer(C@U+2)_for	5'-GCATCCCATGGCTAGACATGCTCCGCC-3'
8mer(C@U+3)_for	5'-GCATCCCATGGCTAGACAGGATGCGCCGA-3'
8mer(C@U+4)_for	5'-CCACAGGATCCTGCGAATACGGCACC-3'
8mer(C@U+5)_for	5'-CCACAGGATCCGCCTGCTACGGCACC-3'
8mer(C@U+6)_for	5'-CCACAGGATCCGCCGAATGCGGCACC-3'
8mer(C@U+7)_for	5'-CCACAGGATCCGCCGAATACTGCACCTG-3'
10mer(C@U+2)_for	5'-GCATCCCATGGCTAGACATGCTCCAAACTG-3'
10mer(C@U+3)_for	5'-GCATCCCATGGCTAGACAGGATGCAAACTG-3'
10mer(C@U+4)_for	5'-CAACAGGATCCTGCCTGGCCGAATACGG-3'
10mer(C@U+5)_for	5'-CAACAGGATCCAAATGCGCCGAATACGG-3'
10mer(C@U+6)_for	5'-CAACAGGATCCAAACTGTGCGAATACGG-3'
10mer(C@U+7)_for	5'-CAACAGGATCCAAACTGGCCTGCTACGG-3'
10mer(C@U+8)_for	5'-CAACAGGATCCAAACTGGCCGAATGCGG-3'
10mer(C@U+9)_for	5'-CAACAGGATCCAAACTGGCCGAATACTGCACCTG-3'
T7 terminator primer	5'-GCTAGTTATTGCTCAGCGG-3'
T7_term_long	5'-GCTAGTTATTGCTCAGCGGTGGC-3'

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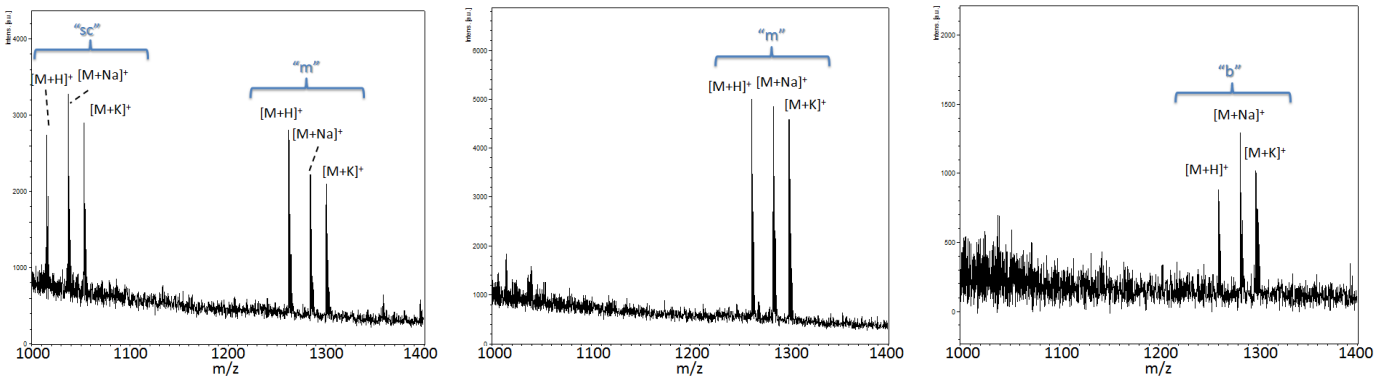
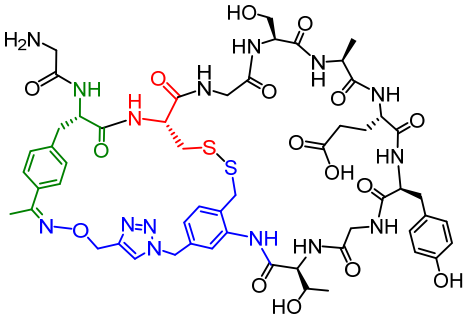
Supplementary Figure S1. MALDI-TOF MS analysis of the reactions between **1** (SP6) and the 8mer biosynthetic precursors (MG8 series, Table 1). Spectra correspond to the three hour (*left*) and 24 hour time point (*center*) after addition of **1** and one hour time point after addition of BPDS (*right*). Peaks corresponding to the proton ($[M+H]^+$), sodium ($[M+Na]^+$), and potassium ($[M+K]^+$) adducts of the MOrPH ('m'), self-cyclized thiolactone ('sc') and bicyclic product ('b') are labeled. When no self-cyclized product is formed, the 24 hr-time point spectrum is omitted as it is identical to the 3-hr spectrum. The calculated and observed *m/z* values for the proton (or sodium) adducts are provided in the table. The structure of the bicyclic product is also shown.

MG8_U+1 / SP6 (3h) **MG8_U+1 / SP6 (24h)** **MG8_U+1 / SP6 (post-oxidation)**



MG8_U+1 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1263.4	1262.8
Thiolactone ("sc")	1016.1	1015.7
Bicycle ("b")	1261.4	1260.7



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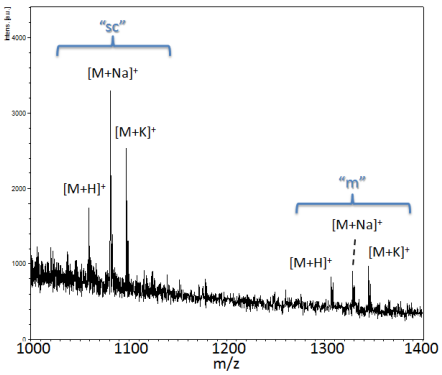
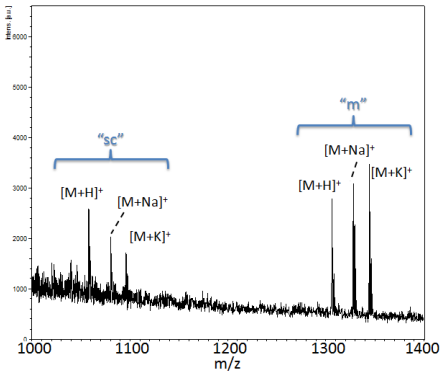
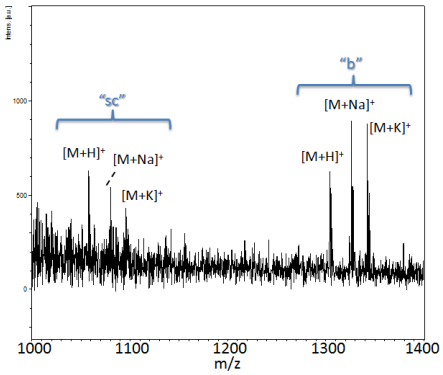
MG8_U+2 / SP6 (3h)



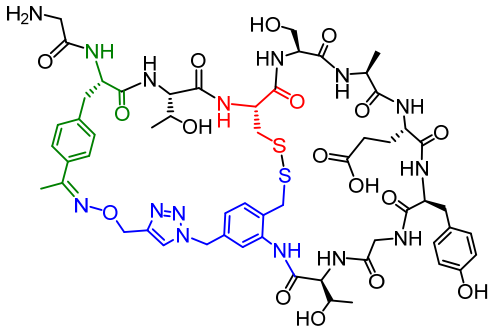
MG8_U+2 / SP6 (24h)



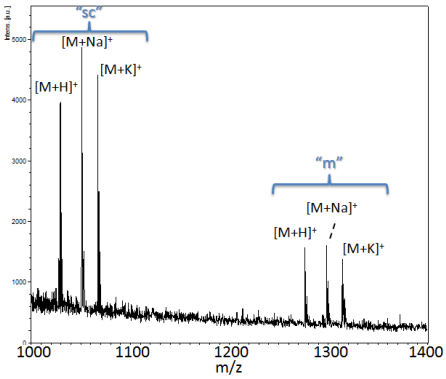
MG8_U+2 / SP6 (post-oxidation)



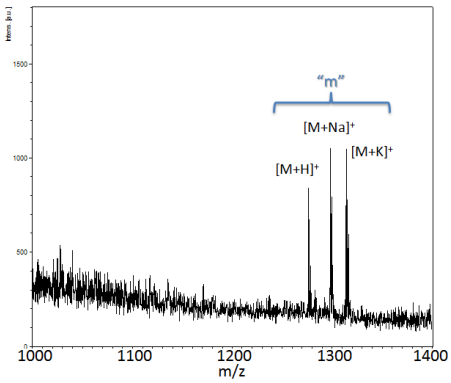
MG8_U+2 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1307.5	1306.9
Thiolactone ("sc")	1060.2	1059.8
Bicycle ("b")	1305.5	1304.9



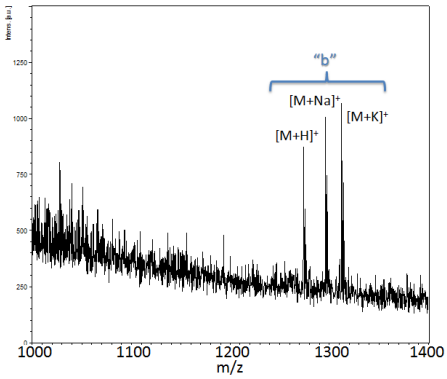
MG8_U+3 / SP6 (3h)



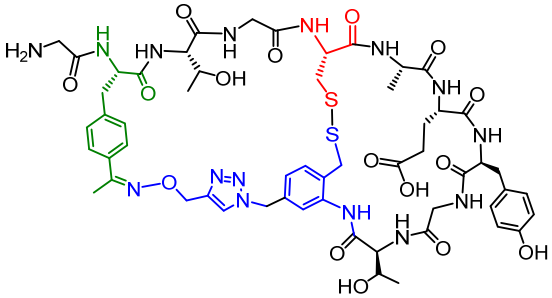
MG8_U+3 / SP6 (24h)



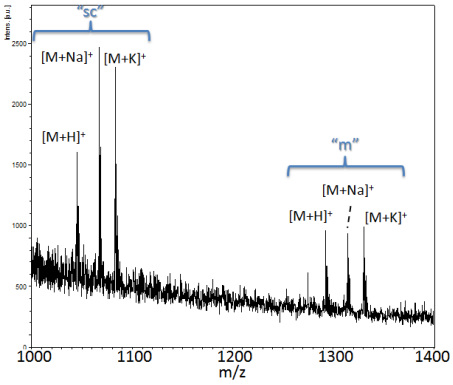
MG8_U+3 / SP6 (post-oxidation)



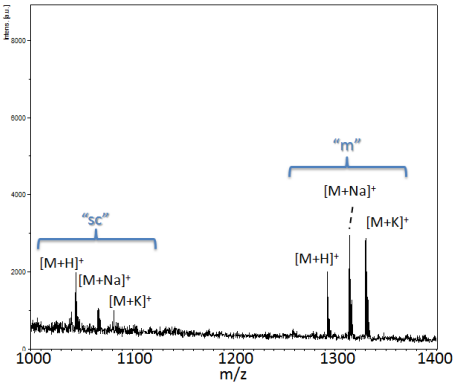
MG8_U+3 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1277.5	1276.7
Thiolactone ("sc")	1030.2	1029.6
Bicycle ("b")	1275.5	1274.7



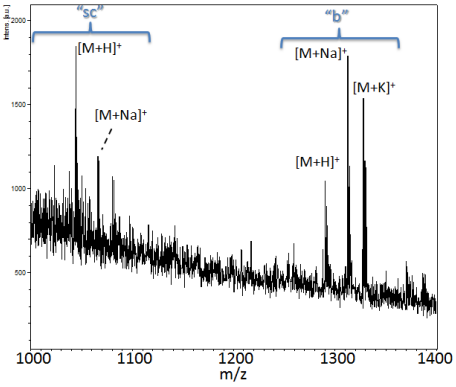
MG8_U+4 / SP6 (3h)



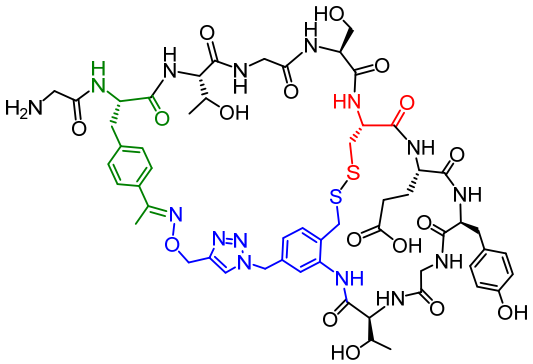
MG8_U+4 / SP6 (24h)



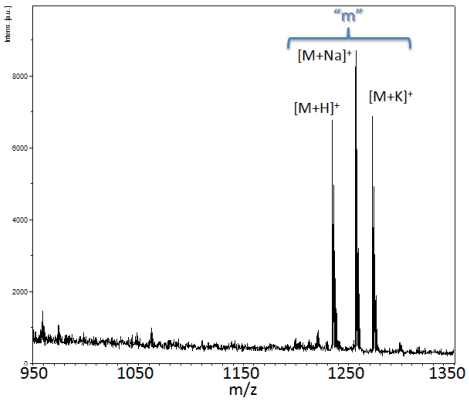
MG8_U+4 / SP6 (post-oxidation)



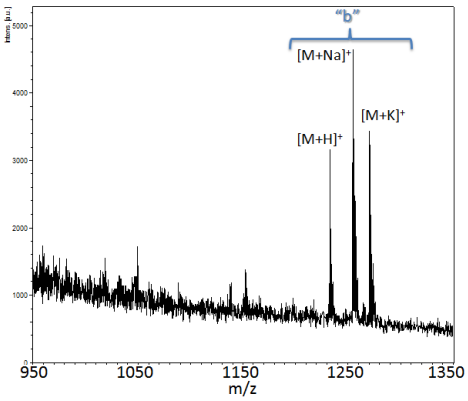
MG8_U+4 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1293.5	1292.8
Thiolactone ("sc")	1046.2	1045.8
Bicycle ("b")	1291.5	1290.8



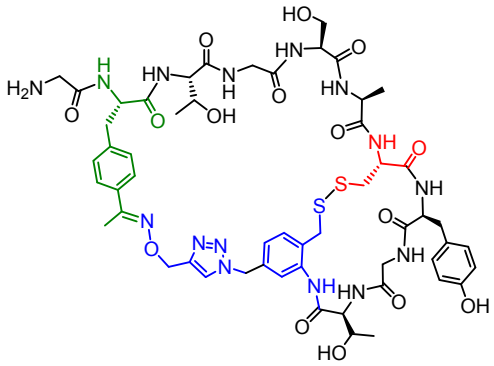
MG8_U+5 / SP6 (3h)



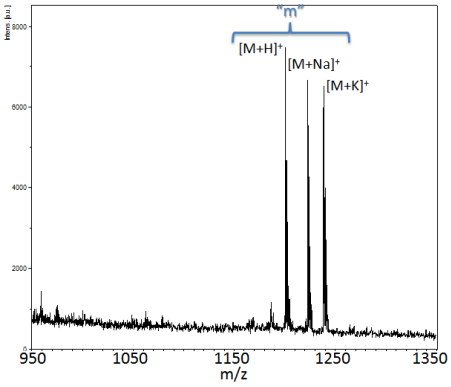
MG8_U+5 / SP6 (post-oxidation)



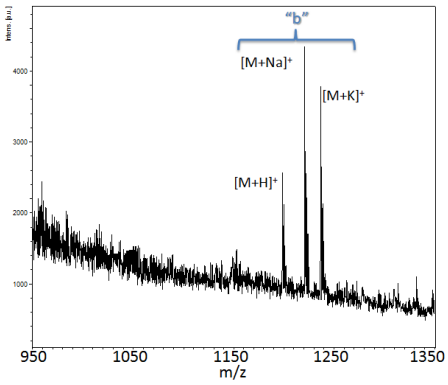
MG8_U+5 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1235.4	1234.7
Thiolactone ("sc")	988.1	not obs.
Bicycle ("b")	1233.4	1232.6



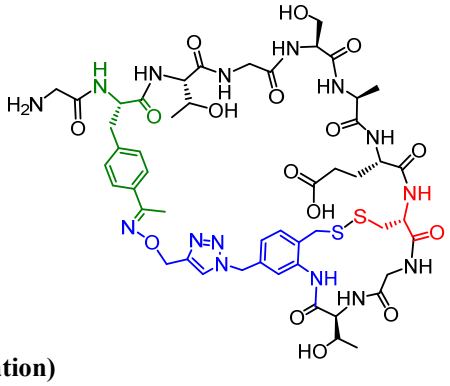
MG8_U+6 / SP6 (3h)



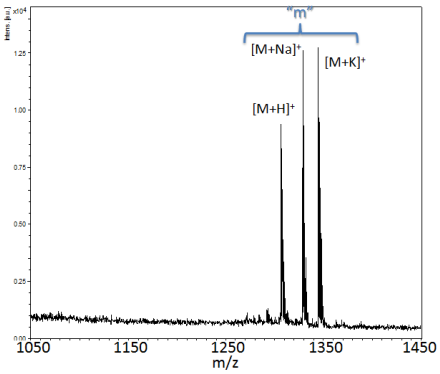
MG8_U+6 / SP6 (post-oxidation)



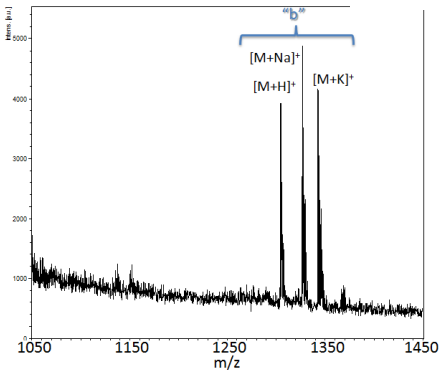
MG8_U+6 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1201.4	1200.7
Thiolactone ("sc")	954.1	not obs.
Bicycle ("b")	1199.4	1198.7



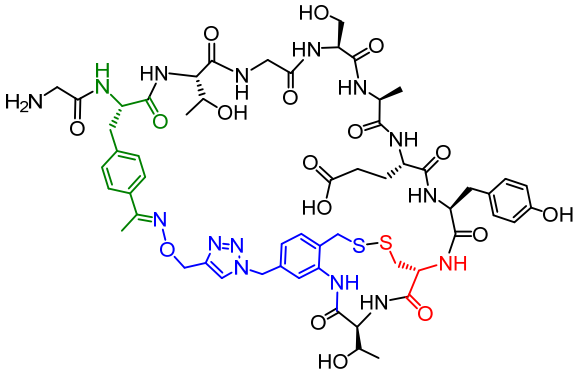
MG8_U+7 / SP6 (3h)



MG8_U+7 / SP6 (post-oxidation)



MG8_U+7 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1307.5	1306.8
Thiolactone ("sc")	1060.2	not obs.
Bicycle ("b")	1305.5	1304.8



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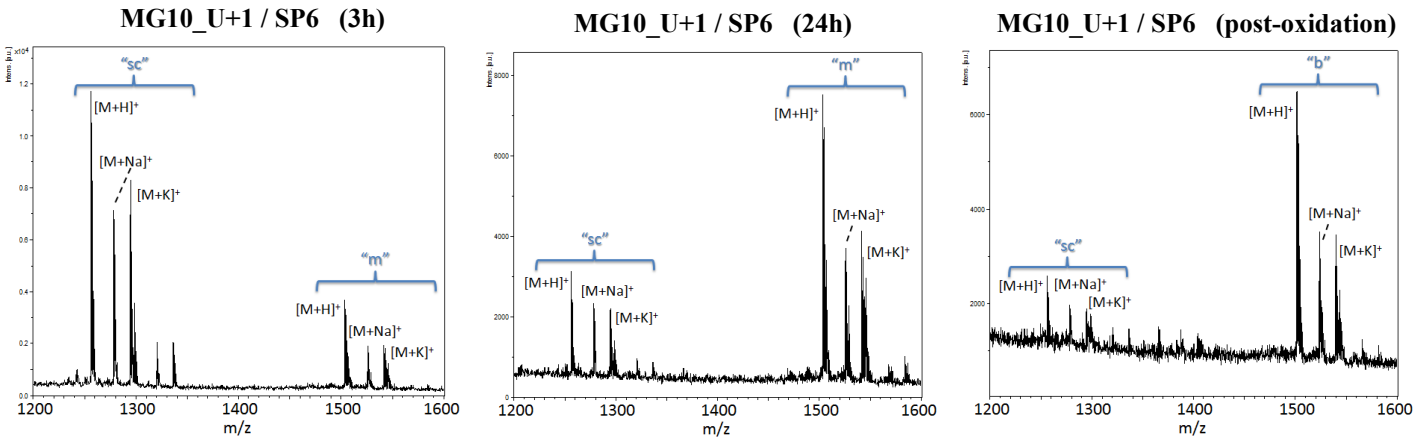
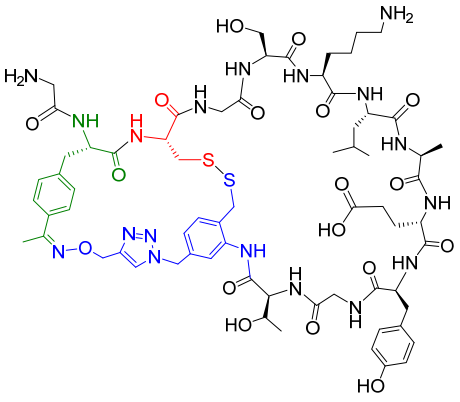
Supplementary Figure S2. MALDI-TOF MS analysis of the reactions between **1** (SP6) and the 10mer biosynthetic precursors (MG10 series, Table 1). Spectra correspond to the three hour (*left*) and 24 hour time point (*center*) after addition of **1** and one hour time point after addition of BPDS (*right*). Peaks corresponding to the proton ($[M+H]^+$), sodium ($[M+Na]^+$), and potassium ($[M+K]^+$) adducts of the MOrPH ('m'), self-cyclized thiolactone ('sc') and bicyclic product ('b') are labeled. When no self-cyclized product is formed, the 24 hr-time point spectrum is omitted as it is identical to the 3-hr spectrum. The calculated and observed *m/z* values for the proton (or sodium) adducts are provided in the table. The structure of the bicyclic product is also shown.



MG10_U+1 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1504.6	1504.1
Thiolactone ("sc")	1257.3	1256.9
Bicycle ("b")	1502.6	1502.1



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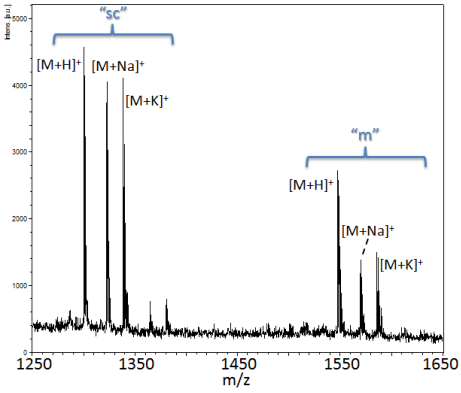
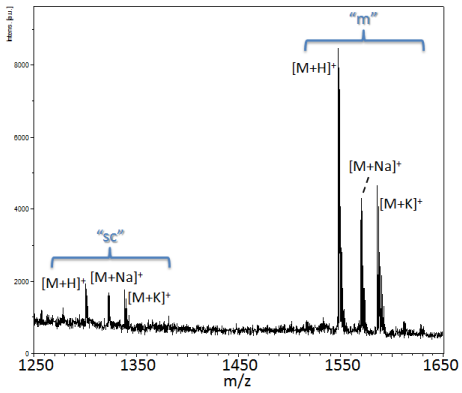
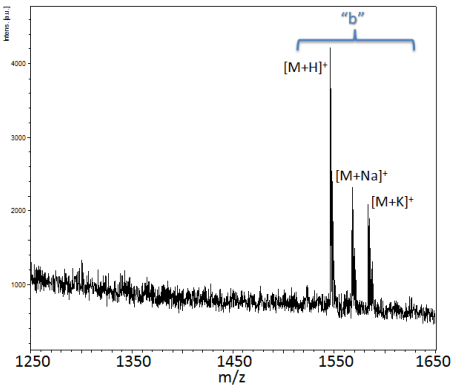
MG10_U+2 / SP6 (3h)



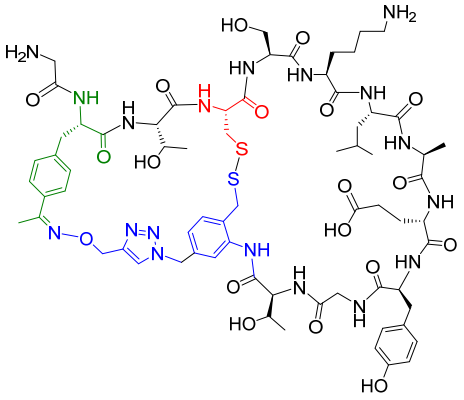
MG10_U+2 / SP6 (24h)



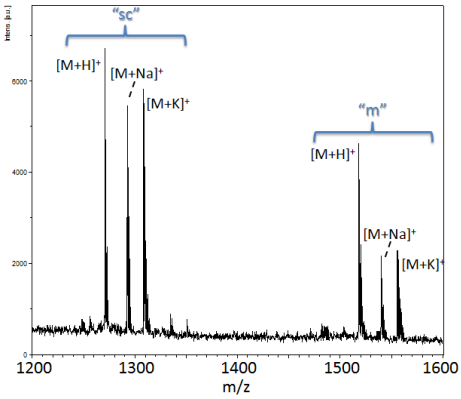
MG10_U+2 / SP6 (post-oxidation)



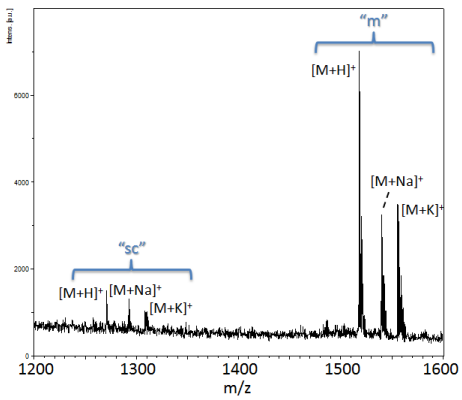
MG10_U+2 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1504.6	1503.9
Thiolactone ("sc")	1257.3	1256.8
Bicycle ("b")	1502.6	1501.9



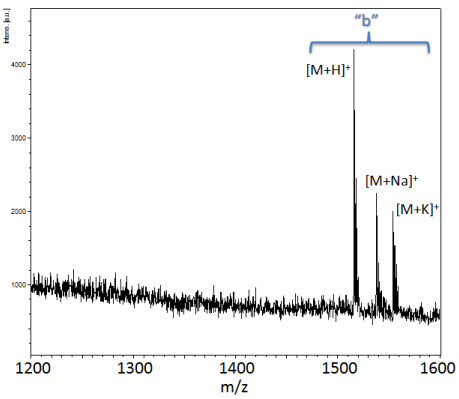
MG10_U+3 / SP6 (3h)



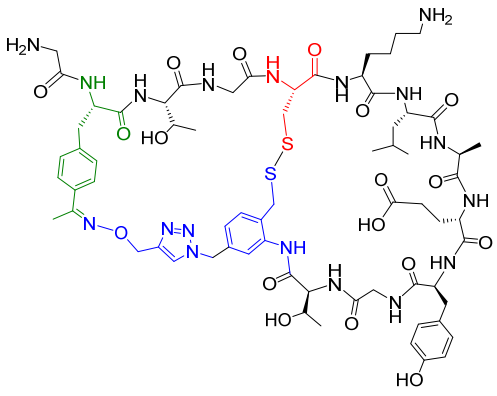
MG10_U+3 / SP6 (24h)



MG10_U+3 / SP6 (post-oxidation)



MG10_U+3 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1518.7	1517.9
Thiolactone ("sc")	1271.4	1270.8
Bicycle ("b")	1516.7	1515.9



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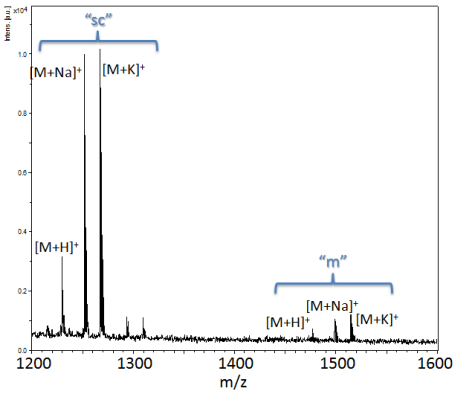
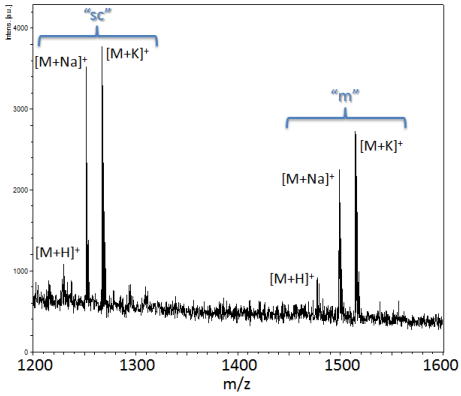
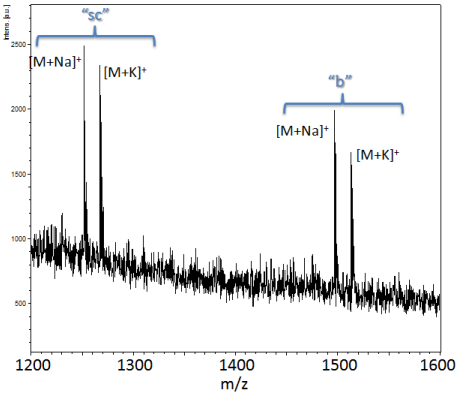
MG10_U+4 / SP6 (3h)



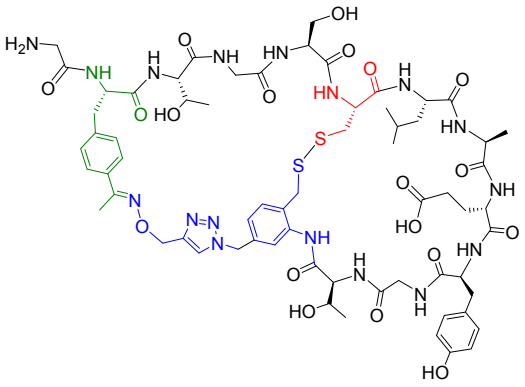
MG10_U+4 / SP6 (24h)



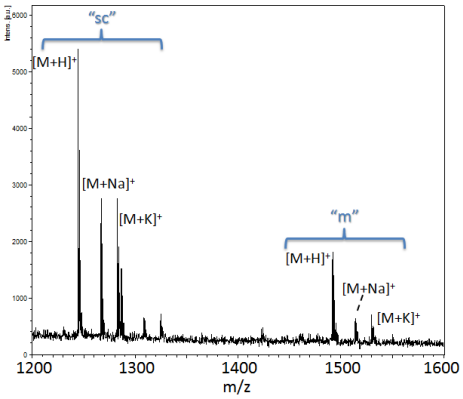
MG10_U+4 / SP6 (post-oxidation)



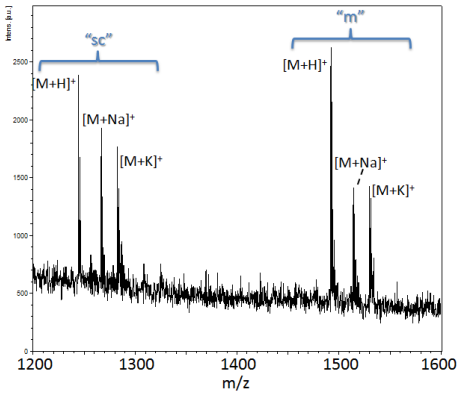
MG10_U+4 and SP6	Calc $[M+Na]^+$	Obs $[M+Na]^+$
MOrPH ("m")	1499.7	1498.9
Thiolactone ("sc")	1252.4	1251.8
Bicycle ("b")	1497.7	1496.9



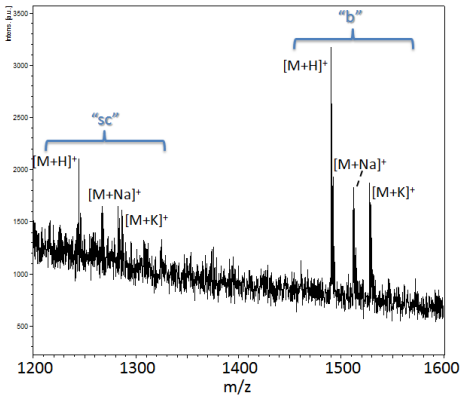
MG10_U+5 / SP6 (3h)



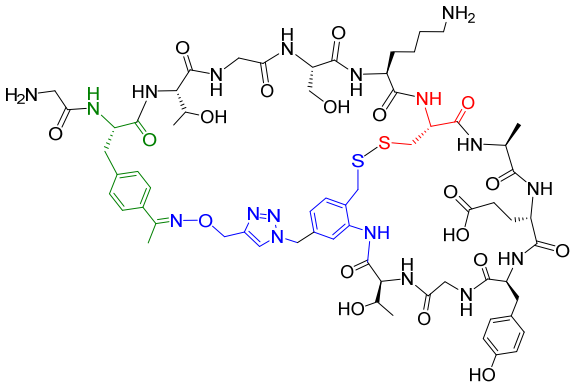
MG10_U+5 / SP6 (24h)



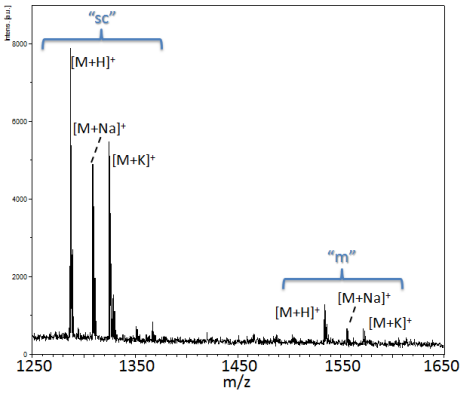
MG10_U+5 / SP6 (post-oxidation)



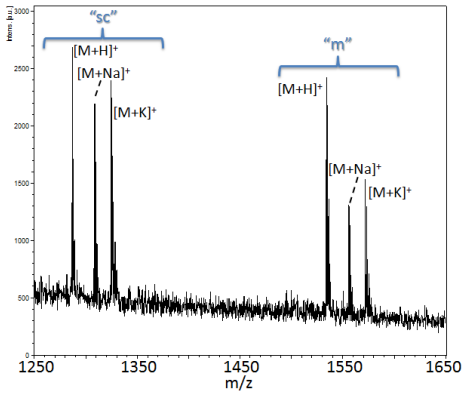
MG10_U+5 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1492.7	1492.0
Thiolactone ("sc")	1245.4	1244.8
Bicycle ("b")	1490.7	1489.9



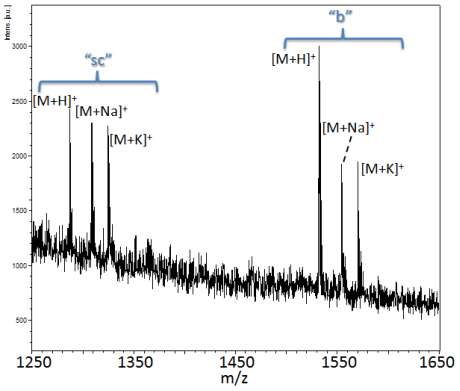
MG10_U+6 / SP6 (3h)



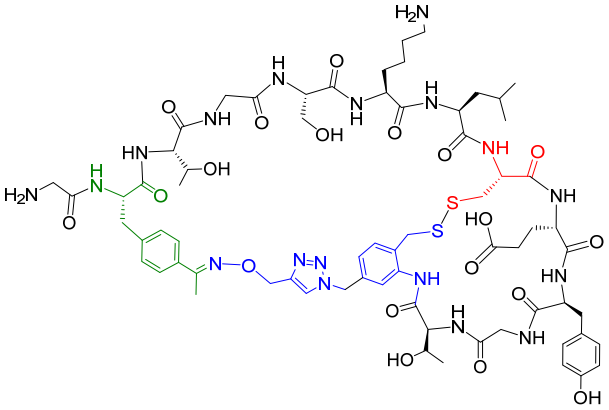
MG10_U+6 / SP6 (24h)



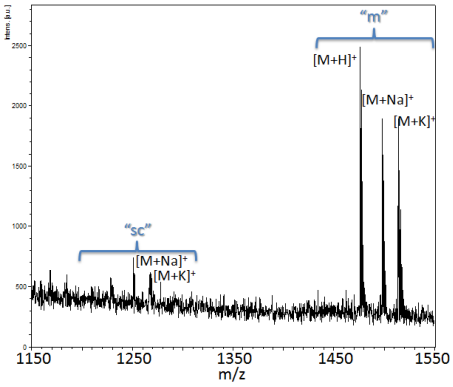
MG10_U+6 / SP6 (post-oxidation)



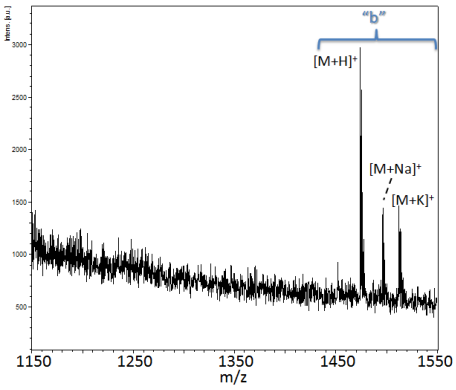
MG10_U+6 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1534.8	1534.1
Thiolactone ("sc")	1287.5	1286.9
Bicycle ("b")	1532.8	1532.1



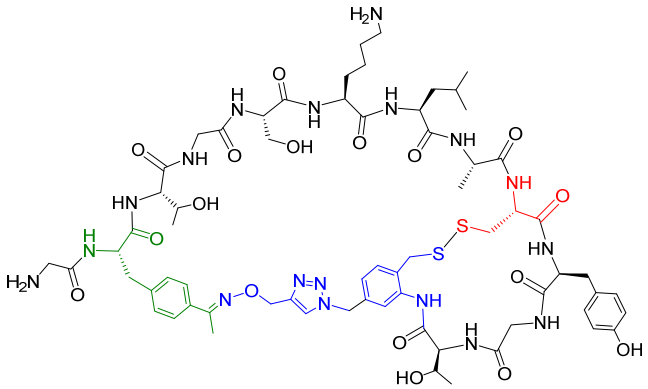
MG10_U+7 / SP6 (3h)



MG10_U+7 / SP6 (post-oxidation)



MG10_U+7 and SP6	Calc $[M+Na]^+$	Obs $[M+Na]^+$
MOrPH ("m")	1498.8	1498.1
Thiolactone ("sc")	1251.5	1250.9
Bicycle ("b")	1496.8	1496.1



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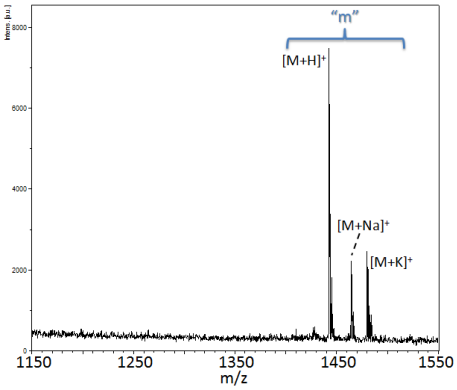
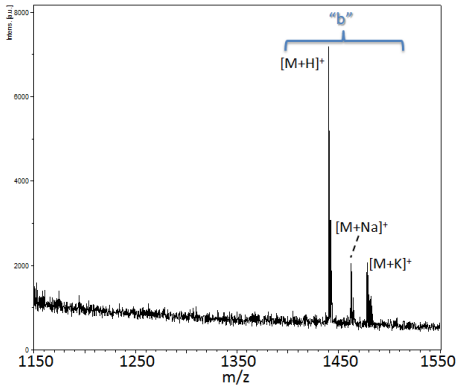
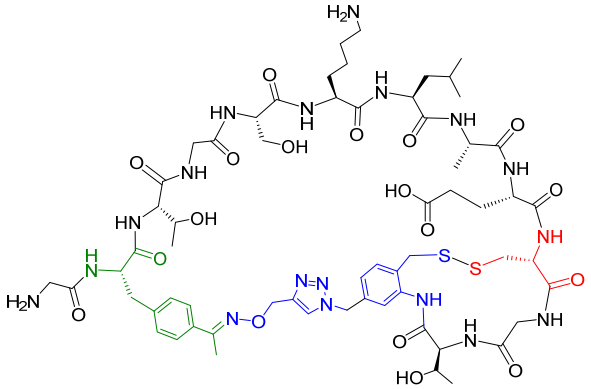
MG10_U+8 / SP6 (3h)



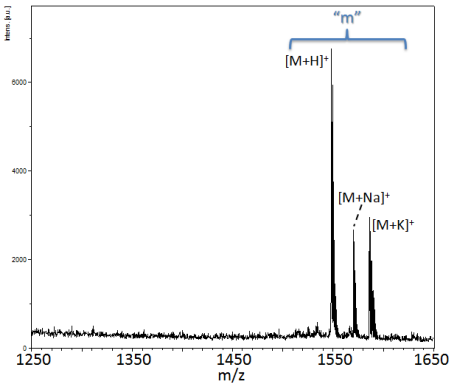
MG10_U+8 / SP6 (post-oxidation)



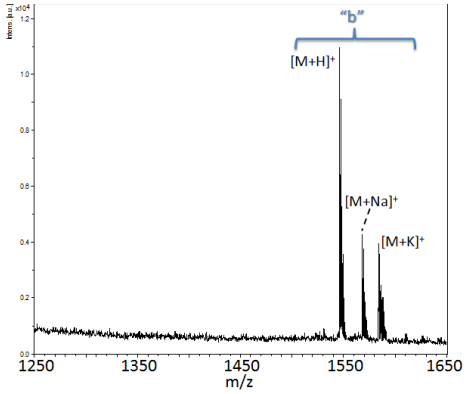
MG10_U+8 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1442.7	1442.1
Thiolactone ("sc")	1195.4	n/o
Bicycle ("b")	1440.7	1440.1



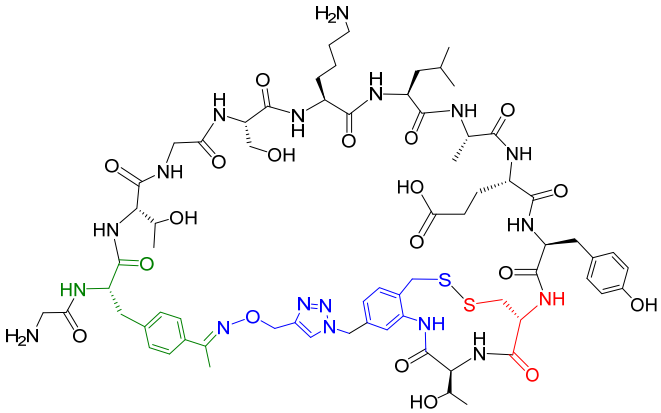
MG10_U+9 / SP6 (3h)



MG10_U+9 / SP6 (post-oxidation)

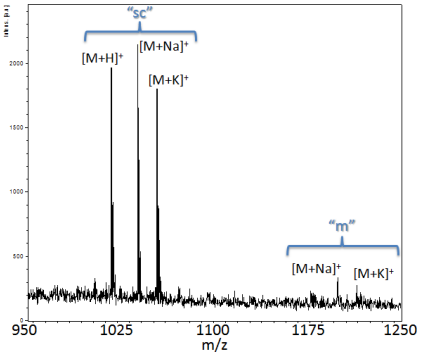


MG10_U+9 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1548.8	1548.2
Thiolactone ("sc")	1301.5	n/o
Bicycle ("b")	1546.8	1546.2

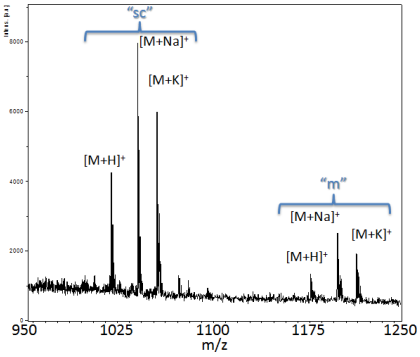


Supplementary Figure S3. MALDI-TOF MS analysis of the reactions between **2** (SP4) and the 8mer biosynthetic precursors (MG8 series, Table 1). Spectra correspond to the three hour (*left*) and 24 hour time point (*center*) after addition of **1** and one hour time point after addition of BPDS (*right*). Peaks corresponding to the proton ($[M+H]^+$), sodium ($[M+Na]^+$), and potassium ($[M+K]^+$) adducts of the MOrPH ('m'), self-cyclized thiolactone ('sc') and bicyclic product ('b') are labeled. When no self-cyclized product is formed, the 24 hr-time point spectrum is omitted as it is identical to the 3-hr spectrum. The calculated and observed *m/z* values for the proton (or sodium) adducts are provided in the table. The structure of the bicyclic product is also shown.

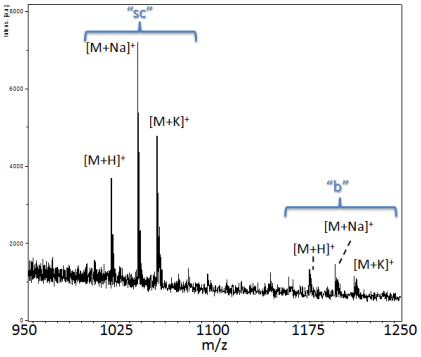
MG8_U+1 / SP4 (3h)



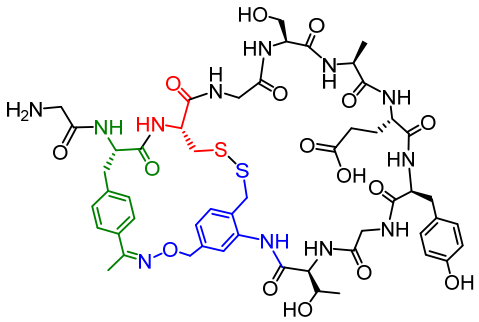
MG8_U+1 / SP4 (24h)



MG8_U+1 / SP4 (post-oxidation)



MG8_U+1 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1182.4	1181.6
Thiolactone ("sc")	1016.1	1015.6
Bicycle ("b")	1180.4	1179.8



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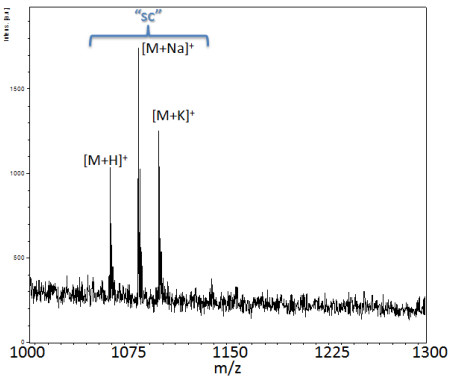
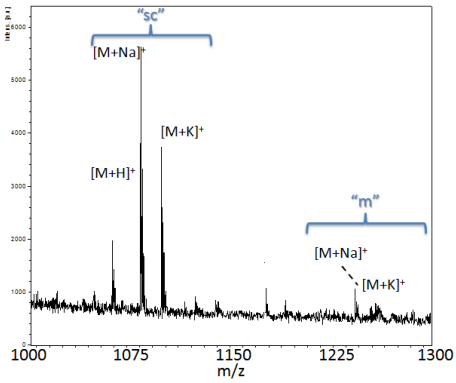
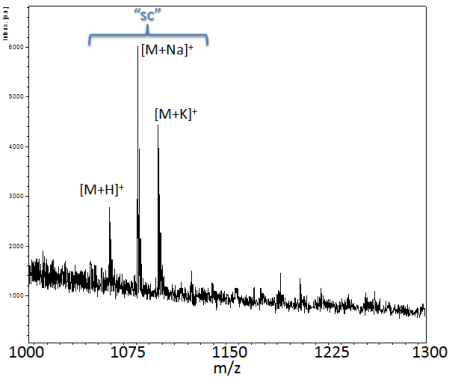
MG8_U+2 / SP4 (3h)



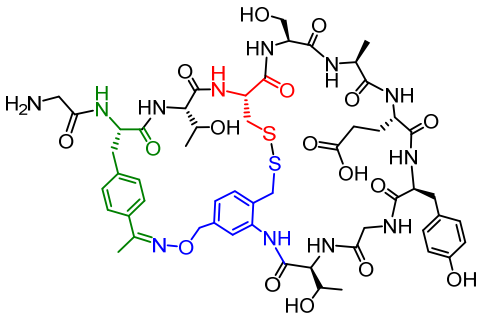
MG8_U+2 / SP4 (3h)



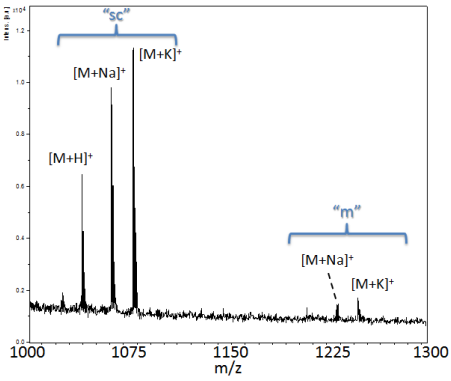
MG8_U+2 / SP4 (post-oxidation)



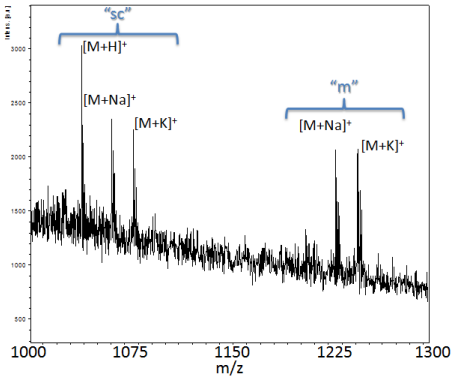
MG8_U+2 and SP4	Calc $[M+Na]^+$	Obs $[M+Na]^+$
MOrPH ("m")	1248.5	1247.6
Thiolactone ("sc")	1082.2	1081.5
Bicycle ("b")	1246.5	n/o



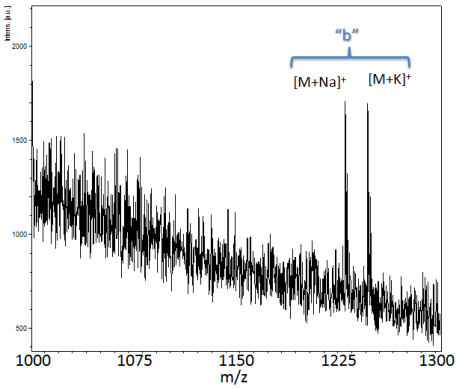
MG8_U+3 / SP4 (3h)



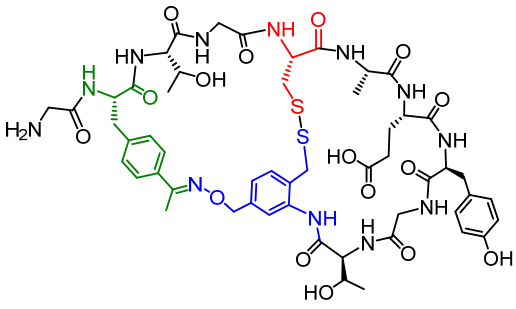
MG8_U+3 / SP4 (24h)



MG8_U+3 / SP4 (post-oxidation)



MG8_U+3 and SP4	Calc $[M+Na]^+$	Obs $[M+Na]^+$
MOrPH ("m")	1218.5	1217.8
Thiolactone ("sc")	1032.2	1051.7
Bicycle ("b")	1216.5	1215.8



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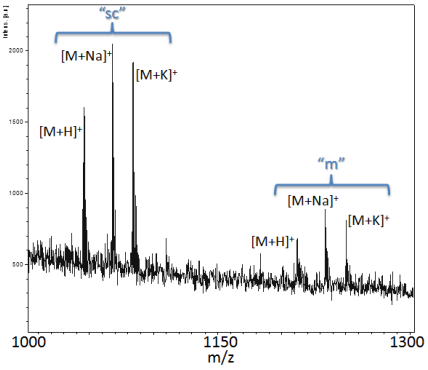
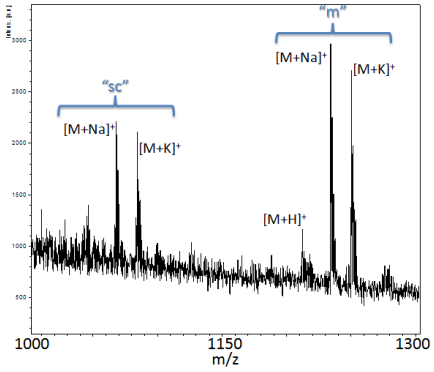
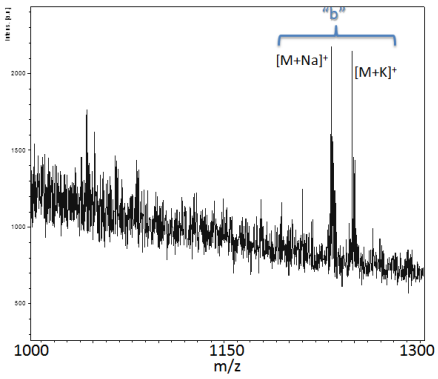
MG8_U+4 / SP4 (3h)



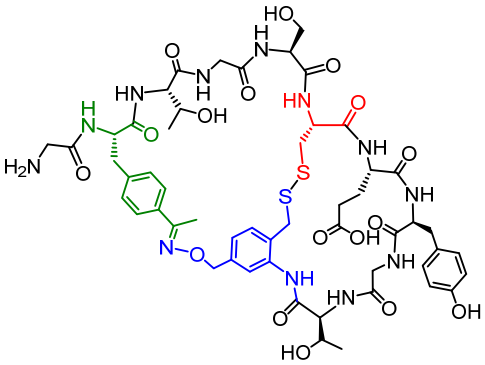
MG8_U+4 / SP4 (24h)



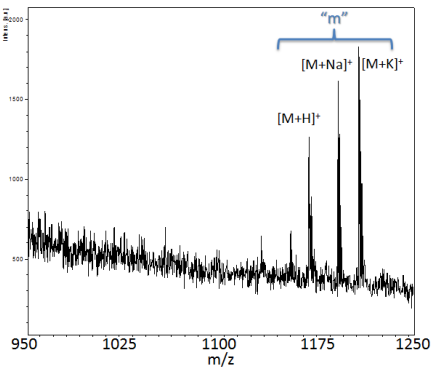
MG8_U+4 / SP4 (post-oxidation)



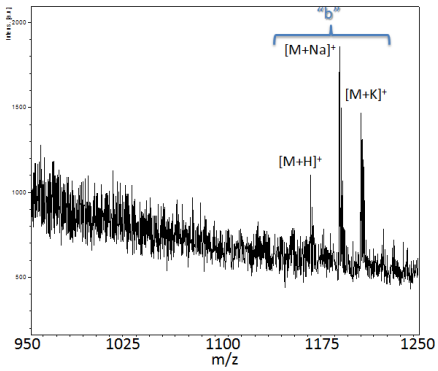
MG8_U+4 and SP4	Calc $[M+Na]^+$	Obs $[M+Na]^+$
MOrPH ("m")	1234.5	1234.3
Thiolactone ("sc")	1068.2	1068.1
Bicycle ("b")	1232.5	1232.2



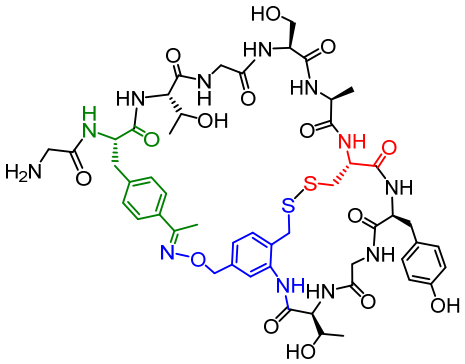
MG8_U+5 / SP4 (3h)



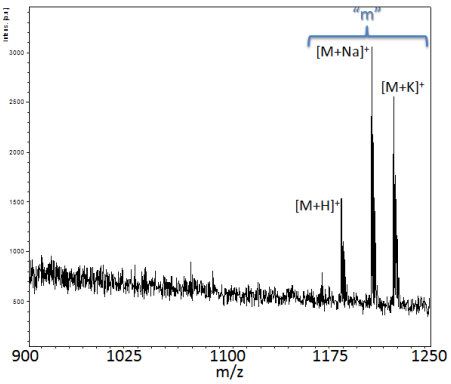
MG8_U+5 / SP4 (post-oxidation)



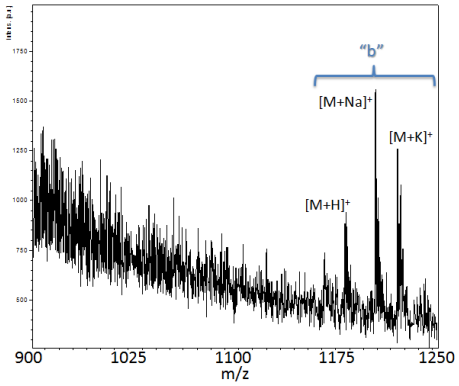
MG8_U+5 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH "m"	1154.4	1154.4
Thiolactone "sc"	988.1	n/o
Bicyclic MOrPH "b"	1152.4	1152.5



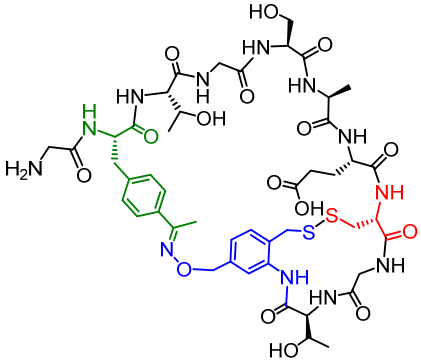
MG8_U+6 / SP4 (3h)



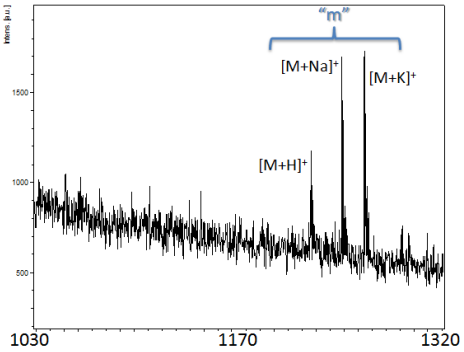
MG8_U+6 / SP4 (post-oxidation)



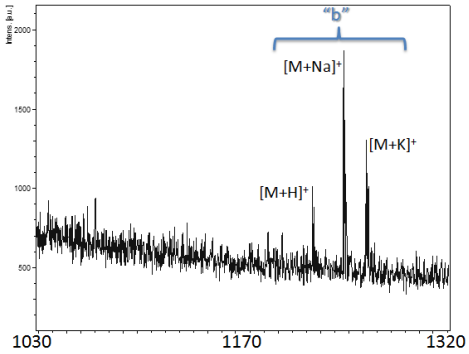
MG8_U+6 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1120.4	1120.4
Thiolactone ("sc")	954.1	n/o
Bicycle ("b")	1118.4	1118.2



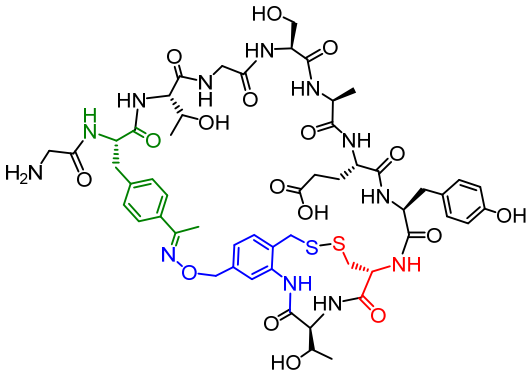
MG8_U+7 / SP4 (3h)



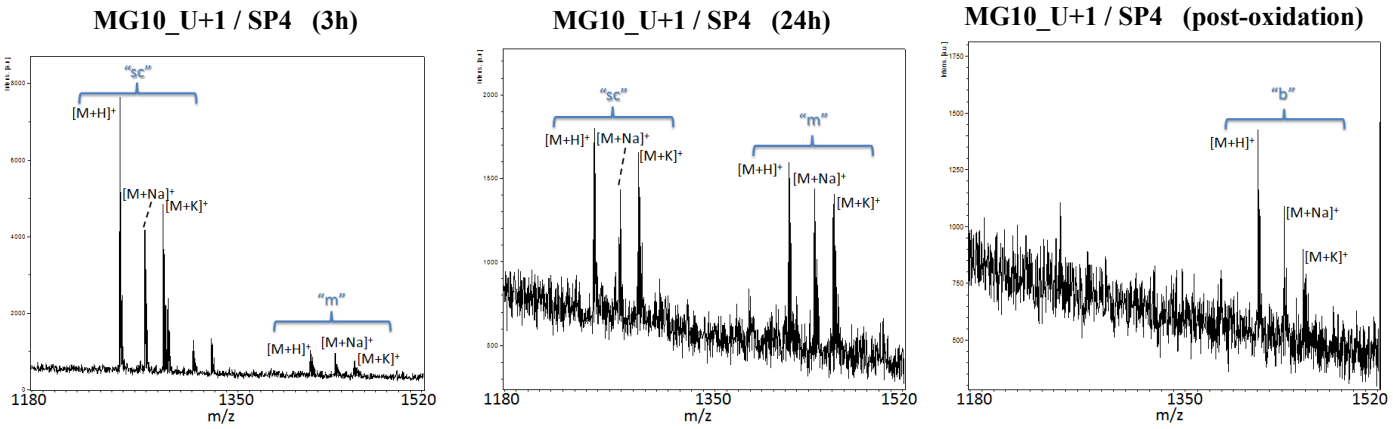
MG8_U+7 / SP4 (post-oxidation)



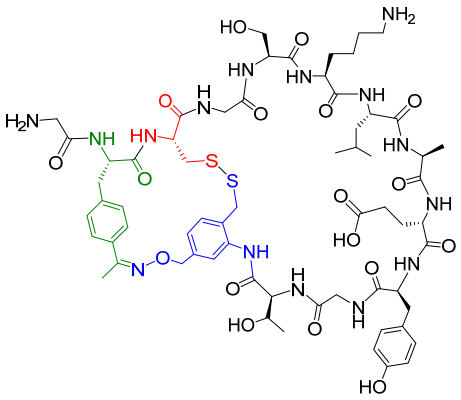
MG8_U+7 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1226.5	1226.0
Thiolactone ("sc")	1060.2	n/o
Bicycle ("b")	1224.5	1224.1



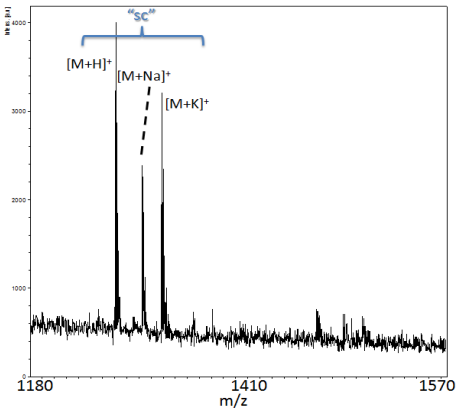
Supplementary Figure S4. MALDI-TOF MS analysis of the reactions between **2** (SP4) and the 10mer biosynthetic precursors (MG10 series, Table 1). Spectra correspond to the three hour (*left*) and 24 hour time point (*center*) after addition of **1** and one hour time point after addition of BPDS (*right*). Peaks corresponding to the proton ($[M+H]^+$), sodium ($[M+Na]^+$), and potassium ($[M+K]^+$) adducts of the MOrPH ('m'), self-cyclized thiolactone ('sc') and bicyclic product ('b') are labeled. When no self-cyclized product is formed, the 24 hr-time point spectrum is omitted as it is identical to the 3-hr spectrum. The calculated and observed *m/z* values for the proton (or sodium) adducts are provided in the table. The structure of the bicyclic product is also shown.



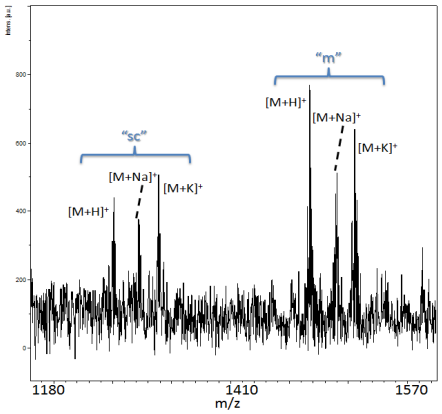
MG10_U+1 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1423.6	1422.9
Thiolactone ("sc")	1257.3	1256.8
Bicycle ("b")	1421.6	1420.9



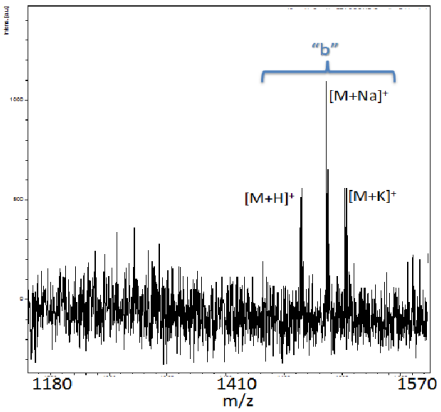
MG10_U+2 / SP4 (3h)



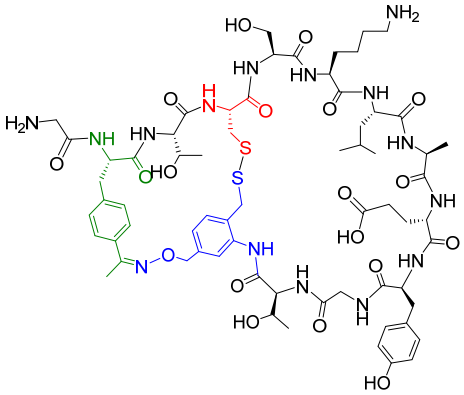
MG10_U+2 / SP4 (24h)



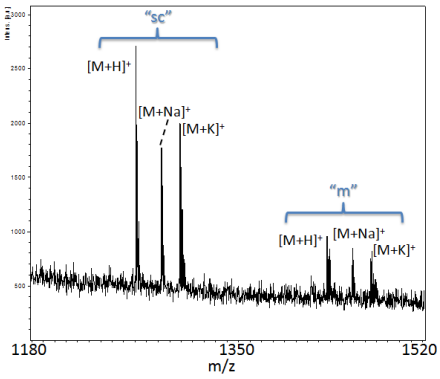
MG10_U+2 / SP4 (post-oxidation)



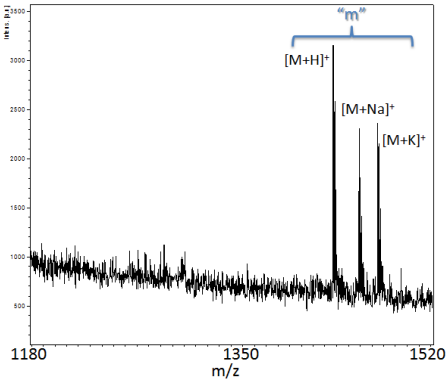
MG10_U+2 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1466.7	1467.1
Thiolactone ("sc")	1300.4	1300.9
Bicycle ("b")	1464.7	1465.0



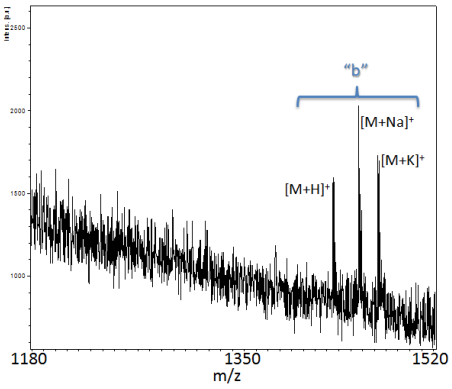
MG10_U+3 / SP4 (3h)



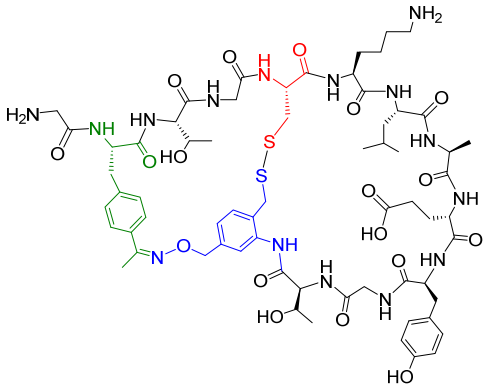
MG10_U+3 / SP4 (24h)



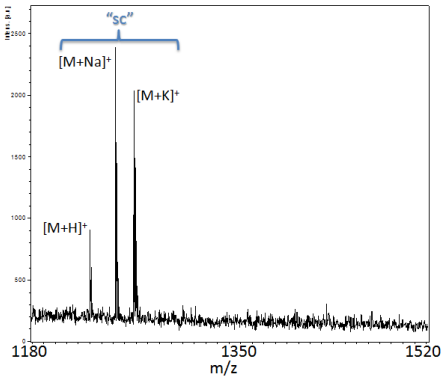
MG10_U+3 / SP4 (post-oxidation)



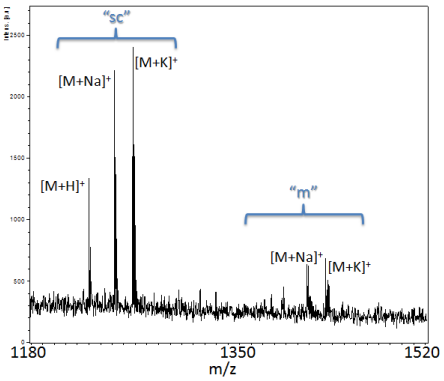
MG10_U+3 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1437.7	1437.1
Thiolactone ("sc")	1271.4	1271.0
Bicycle ("b")	1435.7	1435.0



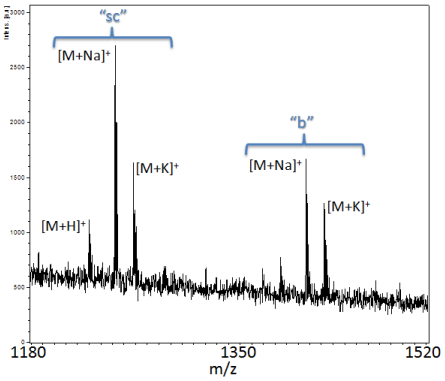
MG10_U+4 / SP4 (3h)



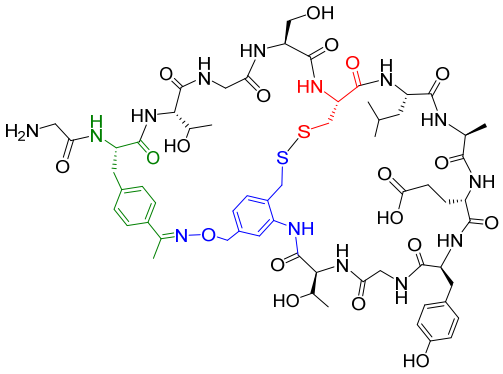
MG10_U+4 / SP4 (24h)



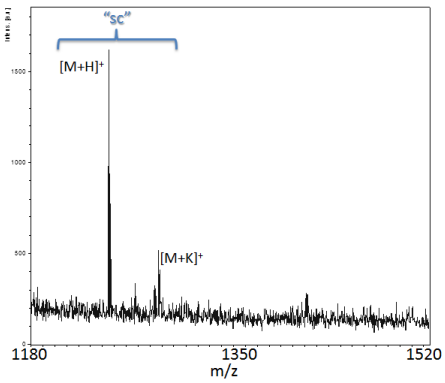
MG10_U+4 / SP4 (post-oxidation)



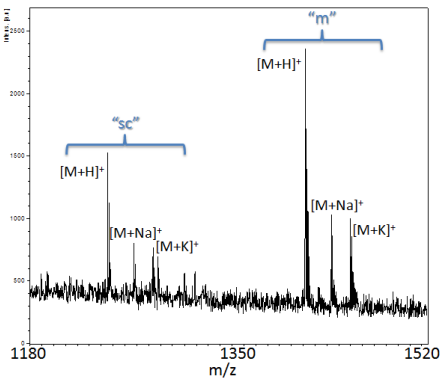
MG10_U+4 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1396.7	1396.7
Thiolactone ("sc")	1230.4	1230.6
Bicycle ("b")	1394.7	1394.9



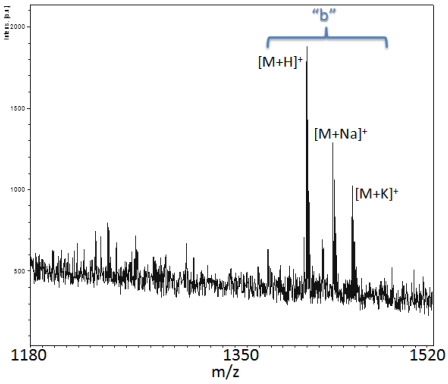
MG10_U+5 / SP4 (3h)



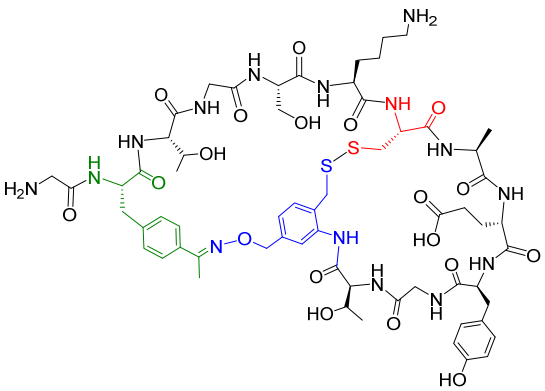
MG10_U+5 / SP4 (24h)



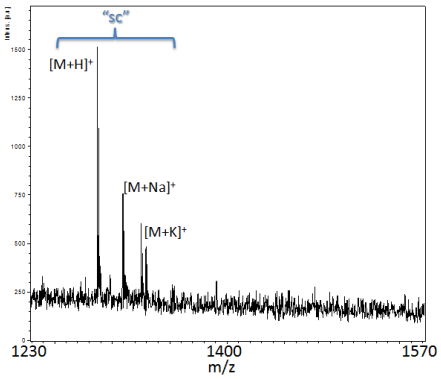
MG10_U+5 / SP4 (post-oxidation)



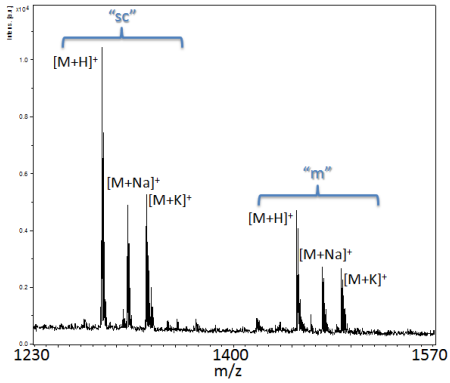
MG10_U+5 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1410.7	1411.4
Thiolactone ("sc")	1244.4	1245.2
Bicycle ("b")	1408.7	1409.3



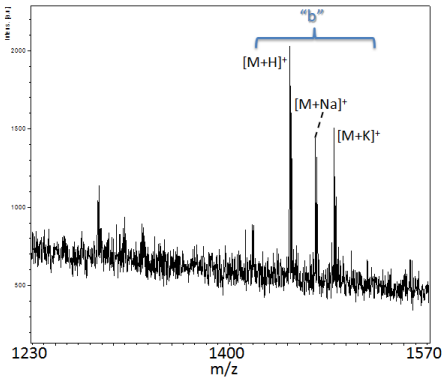
MG10_U+6 / SP4 (3h)



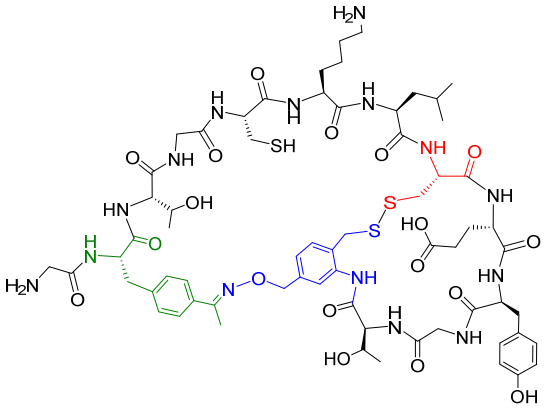
MG10_U+6 / SP4 (24h)



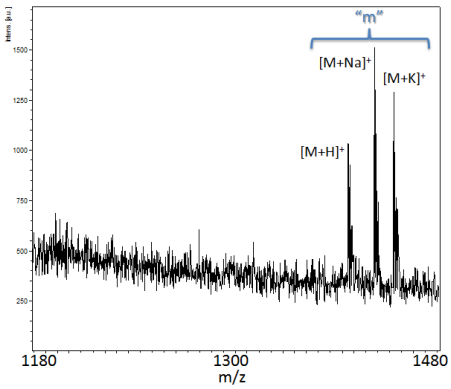
MG10_U+6 / SP4 (post-oxidation)



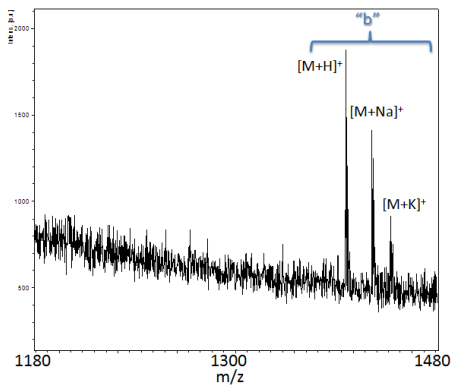
MG10_U+6 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1453.8	1453.9
Thiolactone ("sc")	1287.5	1287.7
Bicycle ("b")	1451.8	1451.9



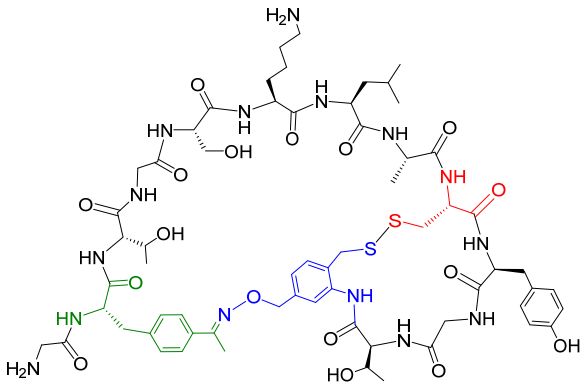
MG10_U+7 / SP4 (3h)



MG10_U+7 / SP4 (post-oxidation)



MG10_U+7 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1395.8	1395.9
Thiolactone ("sc")	1229.5	1287.7
Bicycle ("b")	1393.8	1393.9



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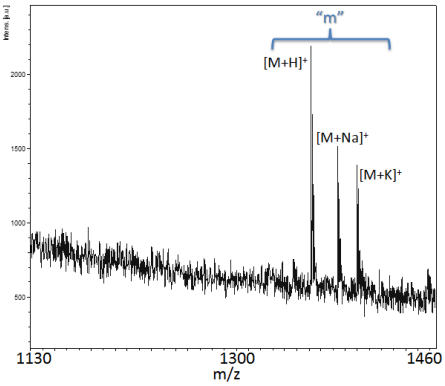
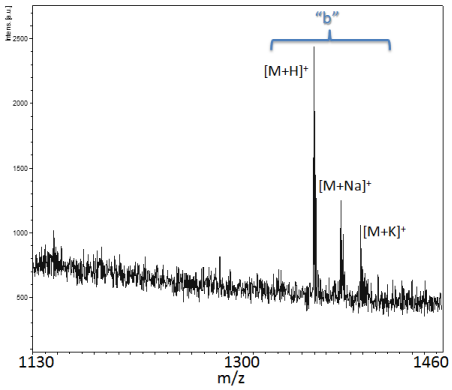
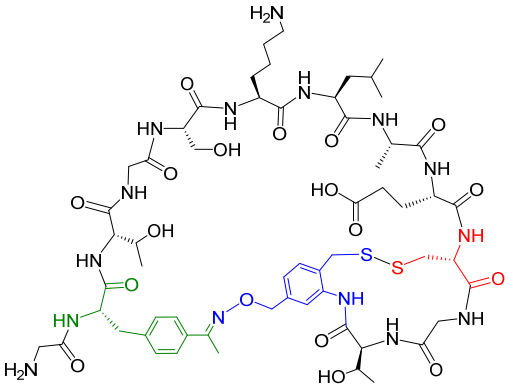
MG10_U+8 / SP4 (3h)



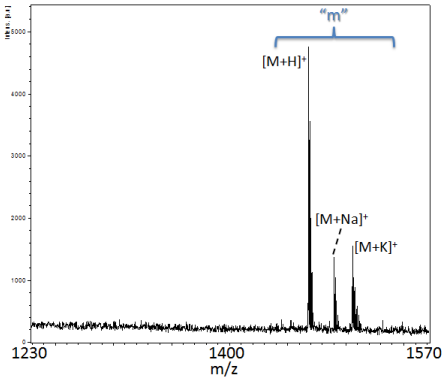
MG10_U+8 / SP4 (post-oxidation)



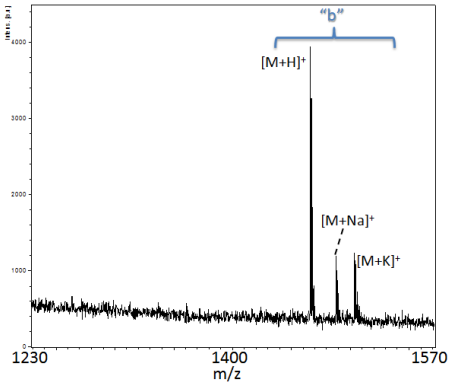
MG10_U+8 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1361.6	1361.9
Thiolactone ("sc")	1195.3	n/o
Bicycle ("b")	1359.6	1359.8



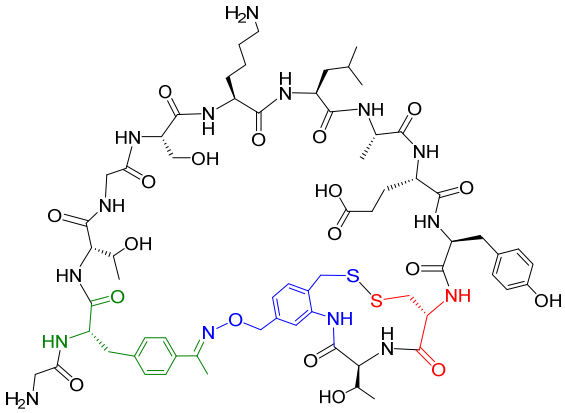
MG10_U+9 / SP4 (3h)



MG10_U+9 / SP4 (post-oxidation)



MG10_U+9 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH ("m")	1467.8	1467.6
Thiolactone ("sc")	1301.5	n/o
Bicycle ("b")	1465.8	1465.5



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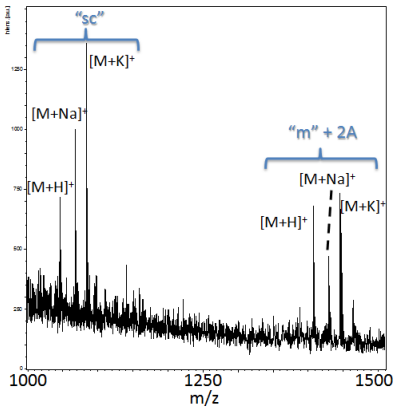
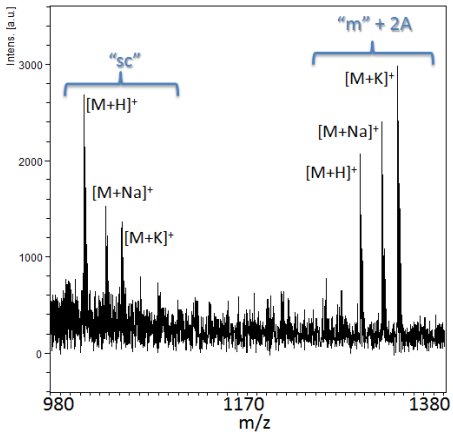
Supplementary Figure S5. Iodoacetamide thiol alkylation experiments. MALDI-TOF spectrum of post-cyclization products (6 hours) from the reaction between (A) MG8_U+4 + **1** (SP6), and (B) MG8_U+1 + **2** (SP4), after incubation with iodoacetamide (20 mM, 1 hour). Calculated and observed masses for self-cyclized and di-alkylated MOrPH products are provided.

A **MG8_U+4 / SP6 + iodoacetamide**



MG8_U+4 and SP6	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH + 2A ("m" + 2 acetamide)	1407.5	1407.5
Thiolactone ("sc")	1046.2	1046.1

B



MG8_U+1 and SP4	Calc $[M+H]^+$	Obs $[M+H]^+$
MOrPH + 2A ("m" + 2 acetamide)	1296.4	1295.8
Thiolactone ("sc")	1016.1	1015.5