Electronic Supplementary information

Elucidation of the *Streptomyces coelicolor* pathway to 4-methoxy-2,2'-bipyrrole-5-carboxaldehyde, an intermediate in prodiginine biosynthesis

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Construction of mutants

The sequences of the primers used for replacement of each gene with the *oriT-aac(3)IV* cassette in *S. coelicolor* M511 are given in the table below. The underlined sequences are complementary to universal priming sites on the *oriT-aac(3)IV* cassette.

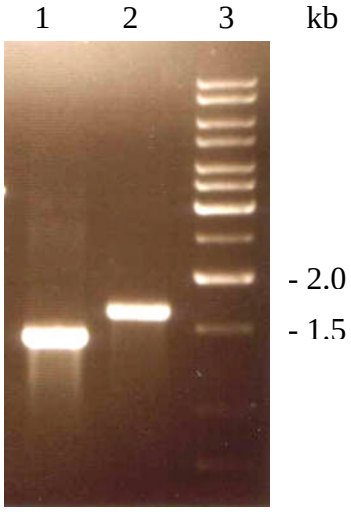
Gene	Forward Primer	Reverse Primer
redM	5'-CAACCCACGCACGTCAAGGAGAAGGGCCT GTACACCATGATTCCGGGGATCCGTCGACC-3'	5'-GTGGCTCACGTTCTTCTCCCTGCGGTTCC GGGCCATCTATGTAGGCTGGAGCTGCTTC-3'
redU	5'-CGGCCGGGACCGGTGCACGGGGCGGAACG GGGGTCGGTGATTCCGGGGATCCGTCGACC-3'	5'-CCGGAAGATCTGCAACACCCGGATGGCGG GCGCGGTTCATGTAGGCTGGAGCTGCTTC-3'
redW	5'-CACGGACGCAACGCGATGAACTTCGACTT CGACGCCGGGATTCCGGGGATCCGTCGACC-3'	5'-ATGACGTCCATCTCTCGGGTCTCCCTGAC TGTGCGTTCATGTAGGCTGGAGCTGCTTC-3'
redX^{c}	5'-ACGACCGGTCCGTTCCGCGTCCCGCGCGC GGCCGAGCCCATTCCGGGGATCCGTCGACC-3'	5'-GAGTGTGCGCAGCAGTCCGGCCGCCCCGG CCGCCGCGAATGTAGGCTGGAGCTGCTTC-3'

The sequences of the primers used to confirm that each of the genes has been replaced on the chromosome of *S. coelicolor* M511 as desired are given in the table below. The primers are designed to anneal outside the region of the chromosome that has been replaced.

Gene replacement	Forward Primer	Reverse Primer
redM::aac(3)IV	5'-AGCGGCTGTGGACCAACATC-3'	5'-CCGTGGCTCACGTTCTTCTC-3'
redU::aac(3)IV	5'-AGTTGTGGGAGGAGGGACTCAG-3'	5'-GGACCGTTTCGTGCACAACAAG-3'
redW::aac(3)IV	5'-GGCGGCATCAATTACCAC-3'	5'-CCGAGATGCTCTCGAAGTAG-3'
redX^{c}*::aac(3)IV*	5'-TGCTGATGGTCGGCCTCTAC-3'	5'-GTCCTCGGCATCATCGAAAC-3'

Agarose gel electrophoresis analyses of amplimers obtained by PCR using genomic DNA from each of the mutants as the template and the appropriate primers are shown below. The expected size of each amplimer is indicated.

redW::aac(3)IV mutant

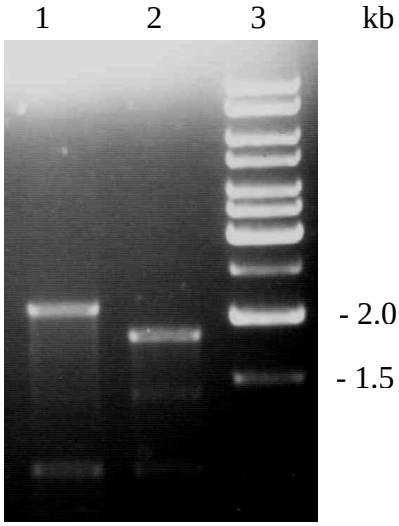


Lane 1: *S. coelicolor* M511 (expected size 1.43 kb)

Lane 2: *redW::aac(3)IV* mutant (expected size 1.65 kb)

Lane 3: size markers

redM::aac(3)IV mutant

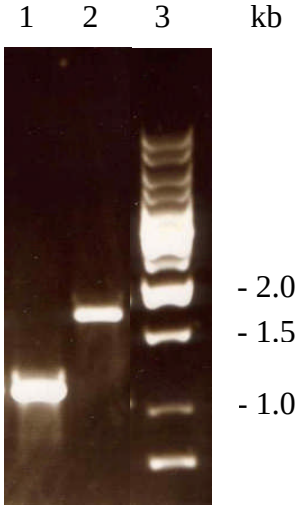


Lane 1: *S. coelicolor* M511 (expected size 2.06 kb)

Lane 2: *redM::aac(3)IV* mutant (expected size 1.80 kb)

Lane 3: size markers

*redU::aac(3)IV*mutant

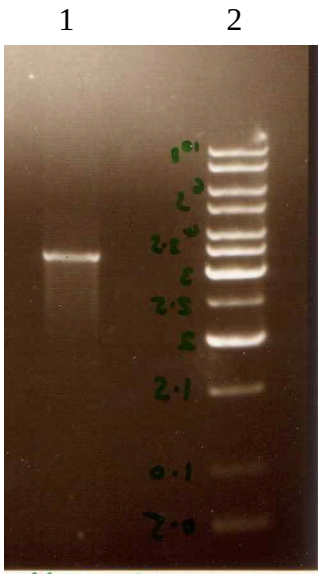


Lane 1: *S. coelicolor* M511 (expected size 1.14 kb)

Lane 2: *redU::aac(3)IV* mutant (expected size 1.66 kb)

Lane 3: size markers

*redX::aac(3)IV*mutant



Lane 1: *redX::aac(3)IV* mutant (expected size 3.38 kb)

Lane 2: size markers

Feeding of 5 and 10 to the mutants

Solid Medium:

R5 agar plates were overlaid with sterile permeable membranes (12-14000 Da molecular weight cut off, Size 20). 20 µL of a spore suspension of each mutant diluted with 200 µL sterile water was spread on a separate plate. After 3 days incubation at 30 °C, 5 x 10 µL aliquots of a solution of **5** or **10** in methanol (40-50 mg/mL) were dropped onto the plate. After a further day of incubation at 30 °C, the membrane was peeled off the plate and the mycelia were scraped off into a 15 mL tube. The mycelia were extracted with 10 mL of a 4:3:3 Diethyl ether : Acetonitrile : Methanol, (acidified with 0.15% 2M HCl) and sonicated (15 bursts of 1 second duration). The extracts were centrifuged at 4000g for 10 min and the supernatants were analysed by LC-MS/MS

Liquid Medium:

20 µL of a spore suspension of each mutant was inoculated separately into 10 mL of supplemented minimal medium. After 2 days shaking incubation at 30 °C and 180 rpm, 100 µL of a solution of **5** or **10** in methanol (5 mg/mL) was added and incubation was continued for 2-3 days. The mycelia were pelleted by centrifugation and the supernatant was decanted and discarded. The mycelial pellet was extracted as above and analysed by LC-MS/MS.

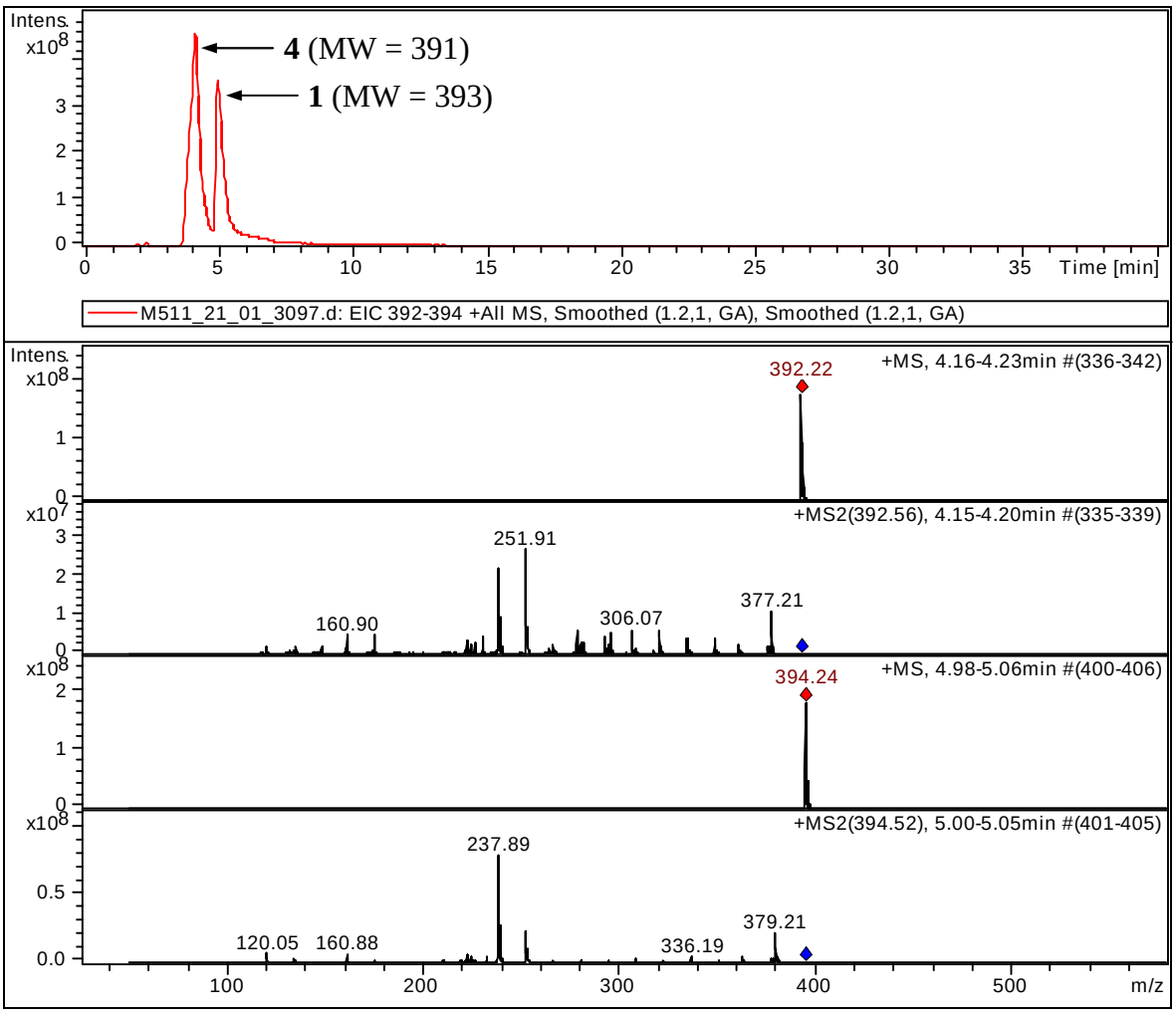
LC-MS/MS analysis of mycelial extracts of mutants with and without added 5 or 10

An Agilent 1100 instrument equipped with a binary pump and a diode array detector was used for HPLC analysis. Samples were analysed on an Agilent C8 column (150 x 4.6 mm, 5µm, column temperature 25ºC) using the gradient elution profile in the table below.

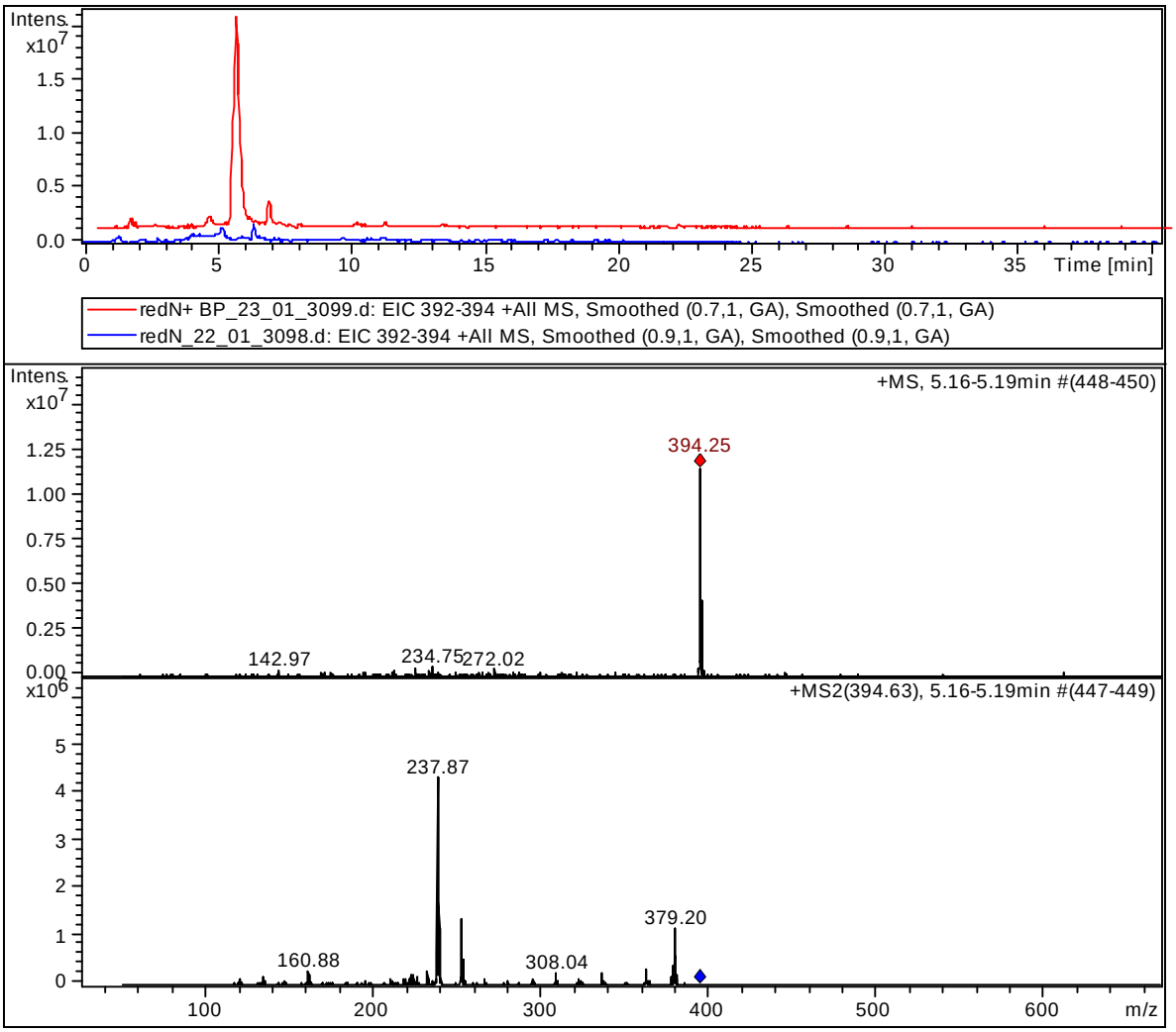
Time (min)	Water (pH3 with HCl)	Acetonitrile	Flow Rate
0	50	50	1.0
1	50	50	1.0
4	25	75	1.3
21	20	80	1.4
23	50	50	1.0

The HPLC out-flow was connected via a splitter (10% flow to MS, 90% flow to waste) to a Bruker HCT+ mass spectrometer (Bruker Daltonics, Coventry) equipped with an electrospray source operated in positive ion mode with parameters as follows: nebulizer flow 40 psi, dry gas flow 10.0 L/min, dry temperature 300ºC, capillary -4 kV, skimmer 40V, capillary exit 106 V, ion charge control target (ICC) 100,000, spectral averages 3. Auto-MS/MS was used to generate daughter ions.

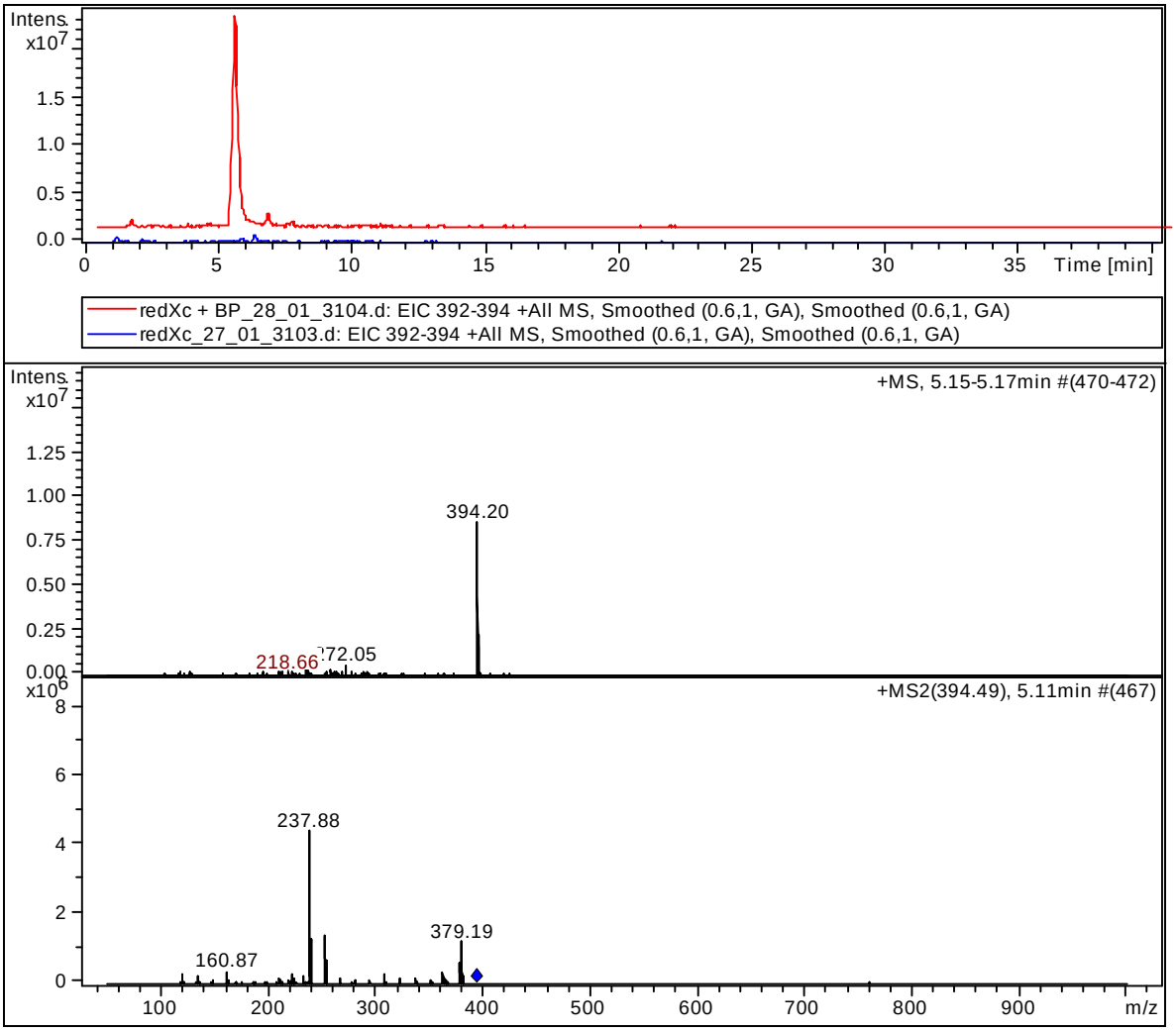
S. coelicolor M511 (wild type):



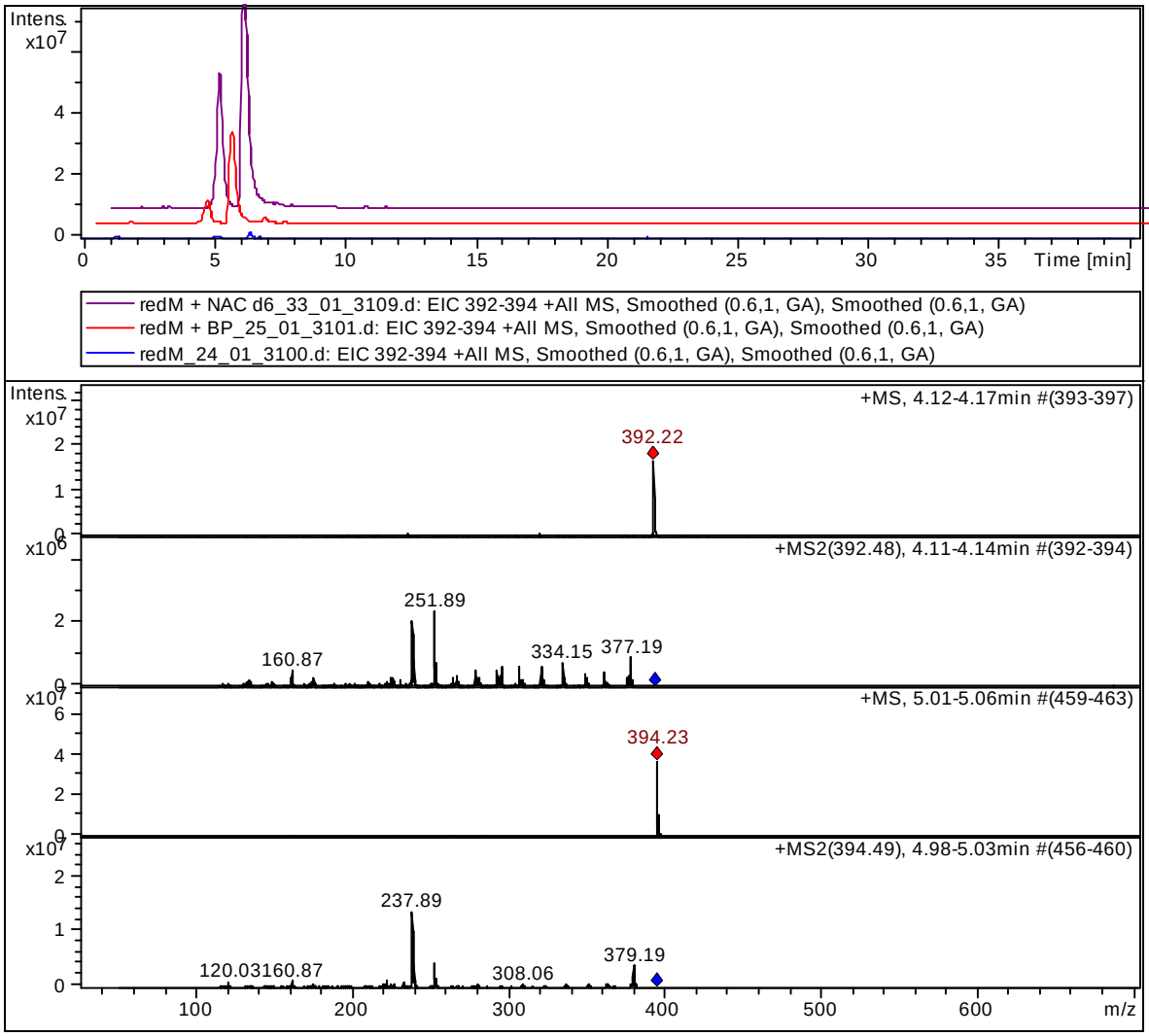
ΔredN mutant with (red) and without (blue) addition of **5**:



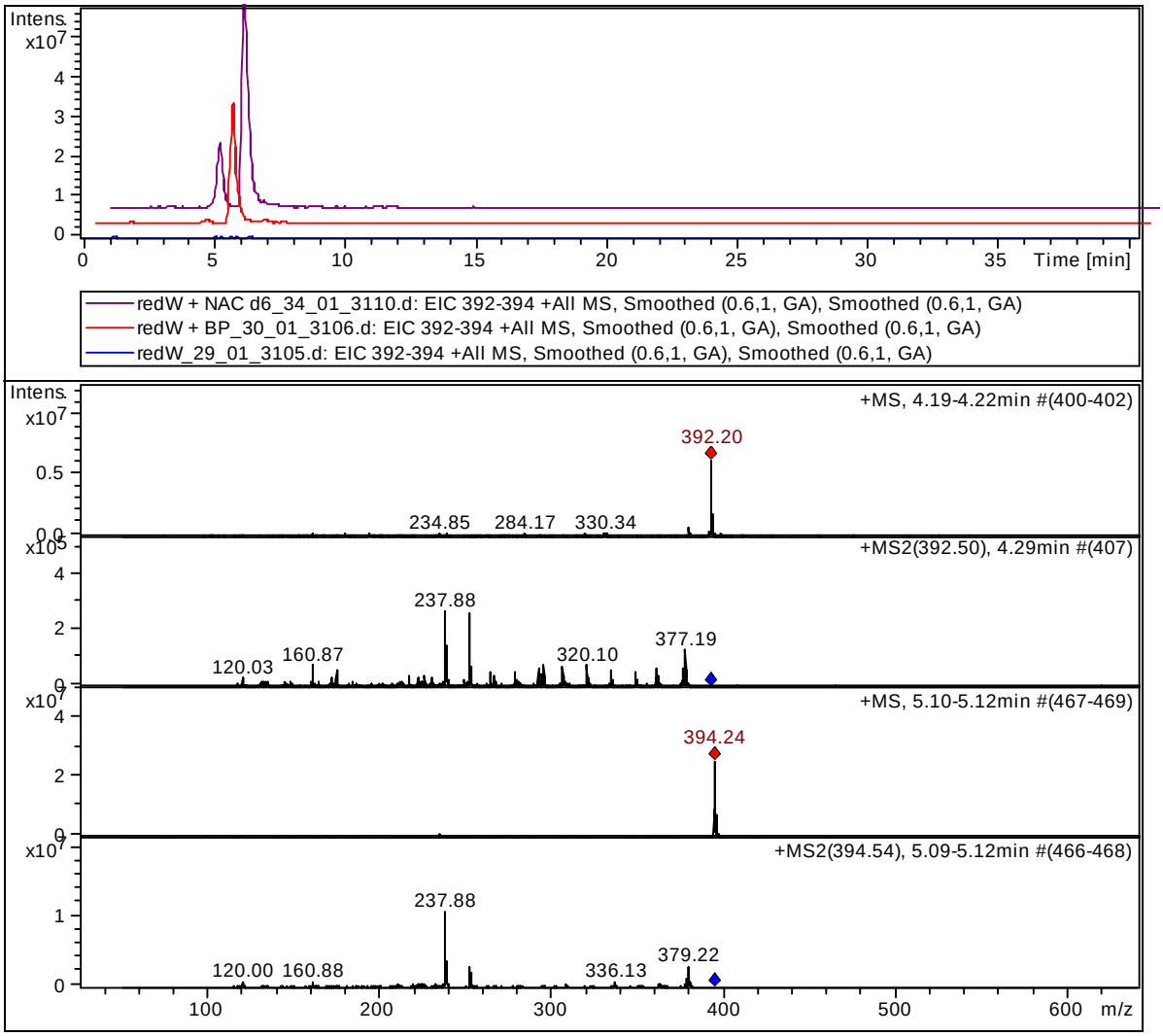
redX::aac(3)IV mutant with (red) and without (blue) addition of **5**:



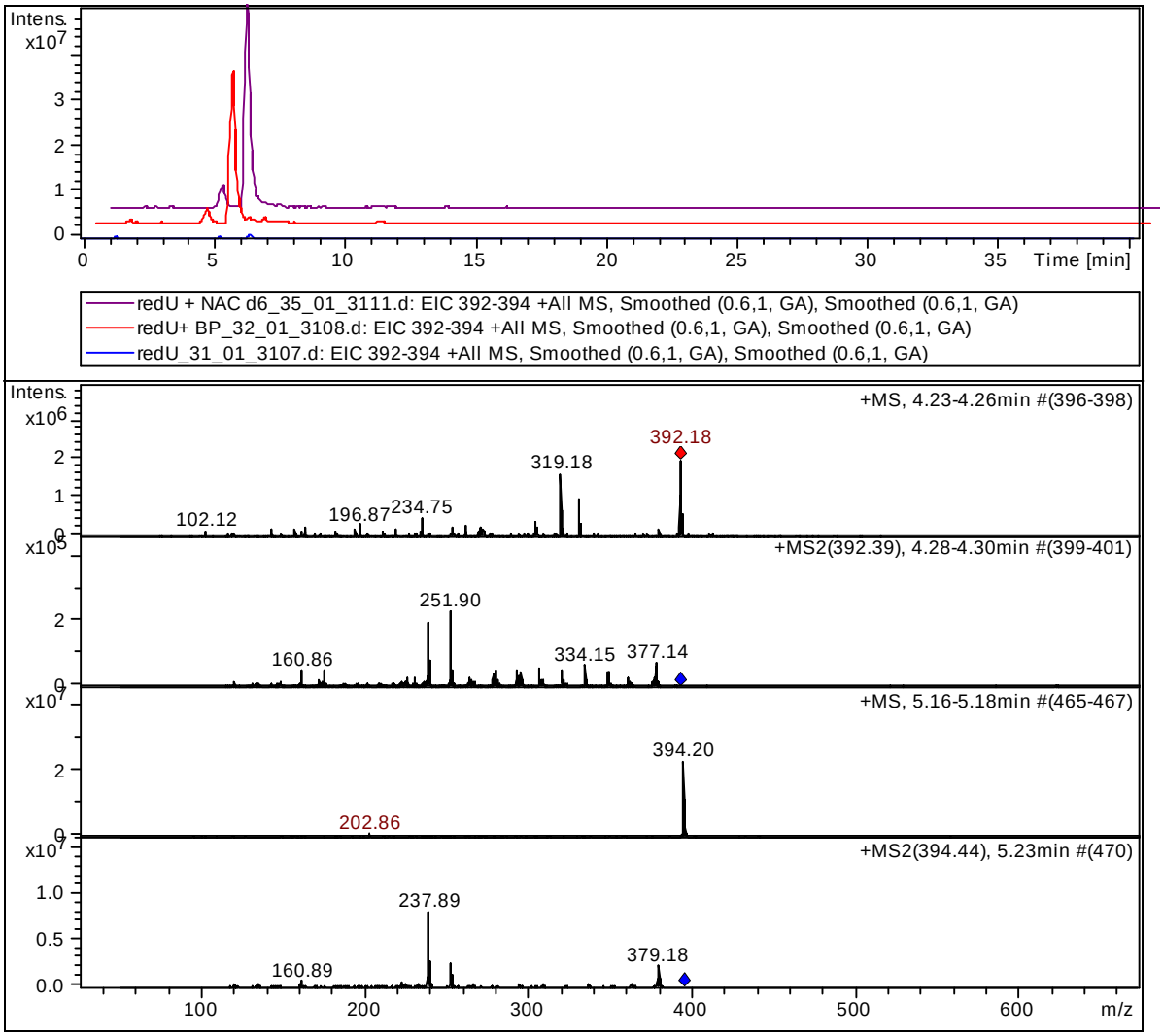
redM::aac(3)IV mutant (blue), with addition of **5** (red), or addition of **10** (purple):



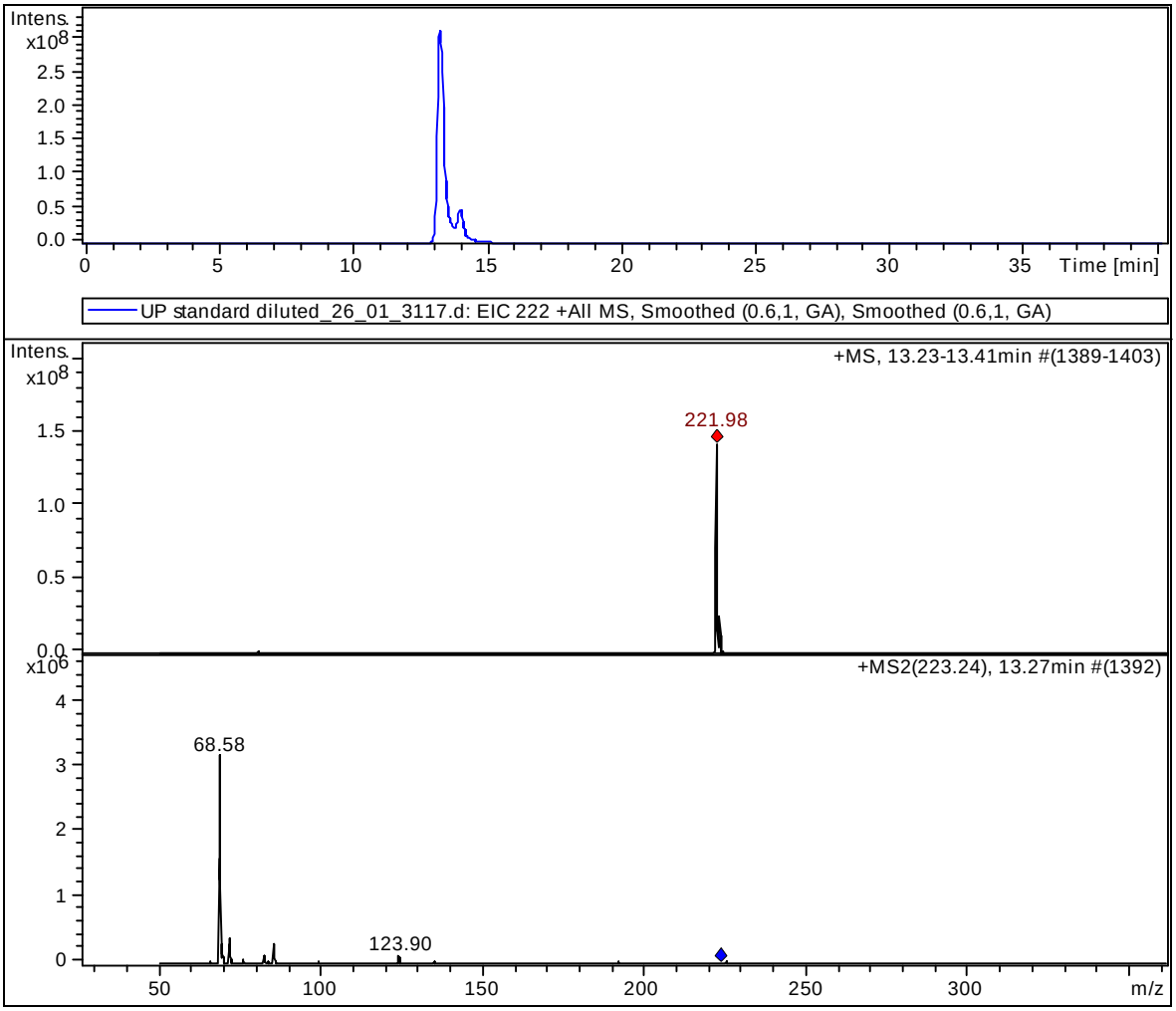
redW::aac(3)IV mutant (blue), with addition of **5** (red), or addition of **10** (purple):



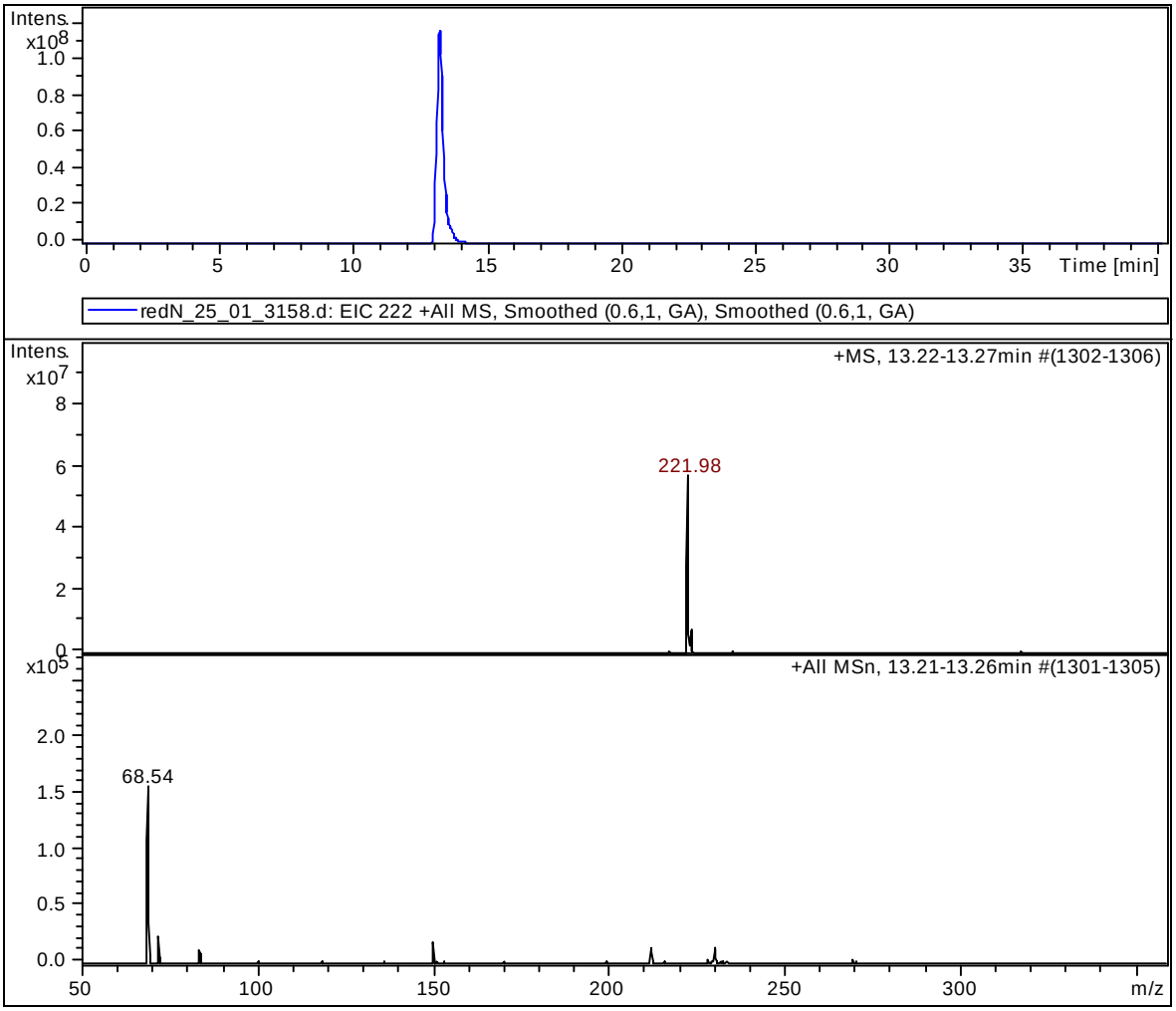
redU::aac(3)IV mutant (blue), with addition of **5** (red), or addition of **10** (purple):



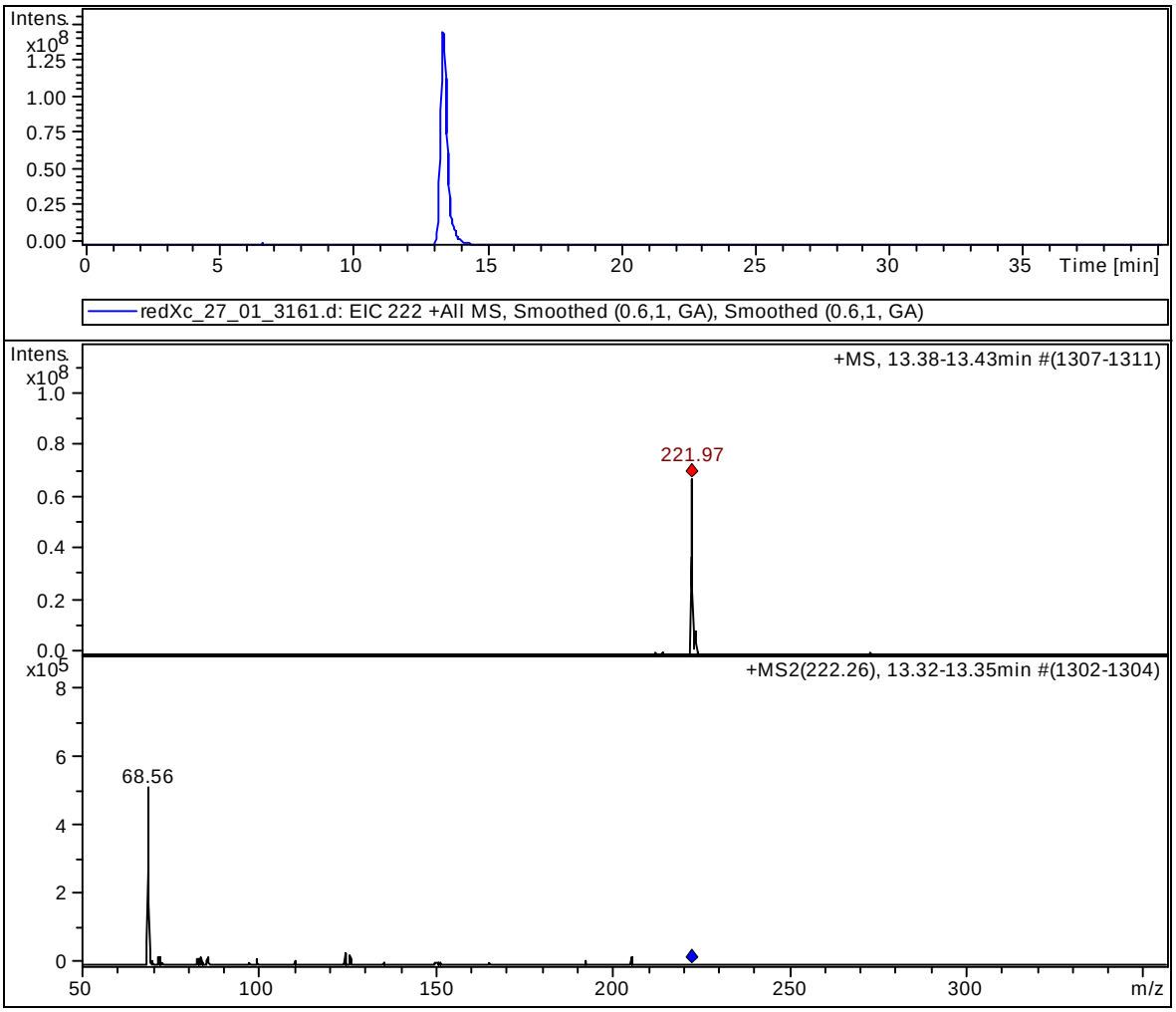
Synthetic undecylpyrrole (MW = 220):



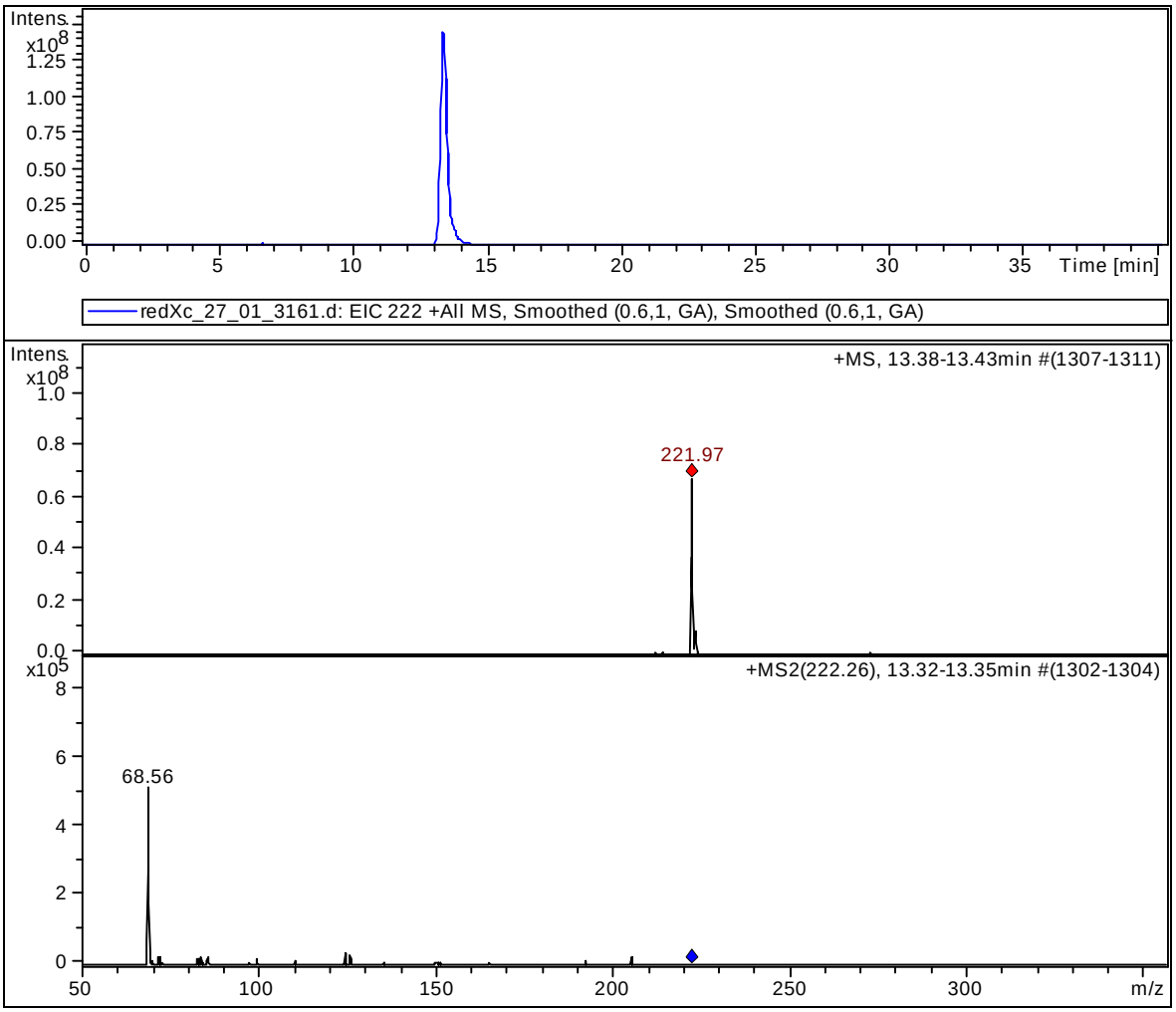
Undecylpyrrole accumulated in *ΔredN* mutant:



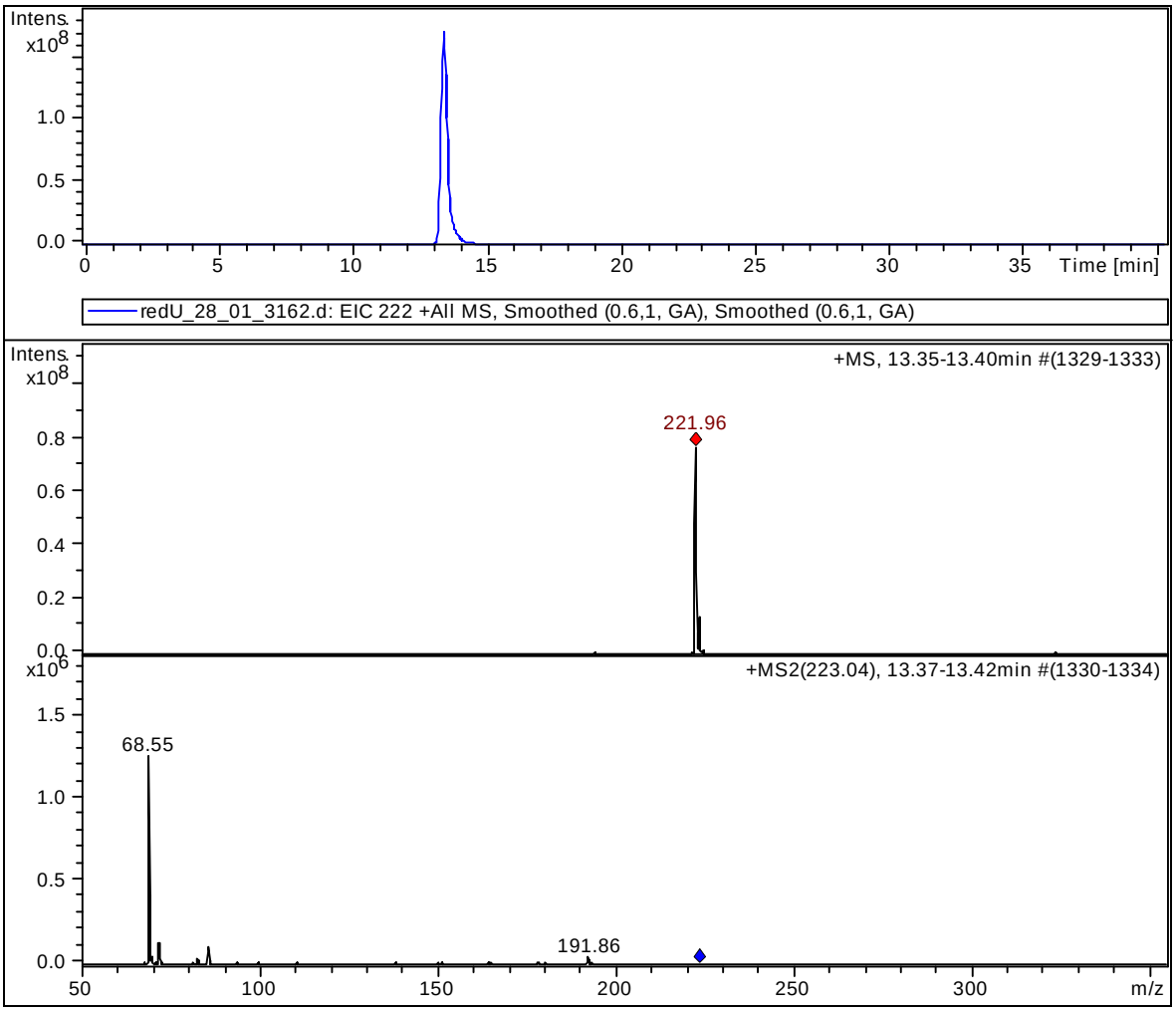
Undecylpyrrole accumulated in *redX::aac(3)IV* mutant:



Undecylpyrrole accumulated in *redM::aac(3)IV* mutant:



Undecylpyrrole accumulated in *redU::aac(3)IV* mutant:



Undecylpyrrole accumulated in *redW::aac(3)IV* mutant: