

**Omission of Column Washing Operations in the Solid Phase Synthesis of
Oligonucleotides**

Supporting Information

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Experimental

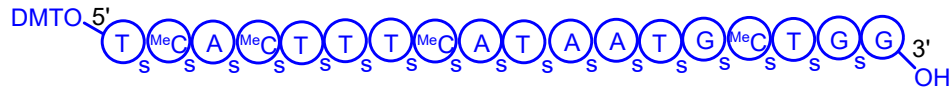
Oligonucleotide Synthesis. Syntheses of ASOs were performed using an AKTA oligopilot plus100 solid-phase synthesizer using Nittophase HL Unylinker (Kinovate) as the solid support on a Fineline 35 oligonucleotide column at ambient temperature (~ 23 °C). The syntheses were conducted at 2.2 mmol scales based on the loading density of the DMT group. The three-reaction synthesis cycle process consists of detritylation, coupling, and sulfurization/oxidation. The detritylation step was carried out using a 10% dichloroacetic acid in toluene as the reagent with a typical reaction time (3.85 min for the 1st cycle, 2.75 min for cycles 2-16 and 3.00 min for the 16-last cycles). The coupling step was performed by simultaneous delivery of the same volume of corresponding phosphoramidite (1.7 eq, 0.2 M in acetonitrile) and activator solution (8.5 eq, 1.0 M 4,5-dicyanoimidazole in acetonitrile containing 0.1 M N-methylimidazole) and then circulating the mixed solutions in the synthesis column. The total reaction time for a coupling step was 4.95 min. The sulfurization step was carried out by delivery of xanthane hydride (6 equiv, 0.2 M in pyridine). For Protocol F, a 3 min sulfurization reagent circulation was performed subsequentially using a 0.2 M xanthane hydride in pyridine solution. The oxidation step in the synthesis of 20mer ASO II was performed by delivery of oxidation reagent (3.0 eq 0.05 M iodine in pyridine/water=90:10 (v:v)). After the synthetic sequence was completed, the phosphorus protecting group was removed by circulating a mixture of triethylamine and acetonitrile (1:1, (v/v)). Cleavage and deprotection of the ASOs were completed in concentrated ammonium hydroxide at 55 °C for 11h.

UHPLC MS analysis. S UHPLC MS analysis. Sample preparation: A crude DMT-on sample (76 µL, ~30 mg/mL in ~15% aqueous NH₄OH) was evaporated under vacuum to dryness.

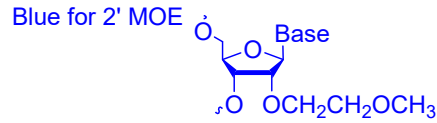
The residue was then dissolved in 0.1% triethylamine in water solution in a 25-mL volumetric flask. The sample solution was injected on an ion-pairing reversed phase UHPLC column (Waters Acquity BEH C18, 1.7 μm , 130 \AA , 2.1 x 100 mm) and the signal of the separated species was detected by both UV at 260 nm and MS on an Agilent Infinity II coupled with a 6100 series single quadrupole MS. The composition of mobile phase A was 10% ACN, 5 mM TBuAA, 1 μM EDTA and that of mobile phase B was 80% ACN, 5 mM TBuAA, 1 μM EDTA. Separation of the oligonucleotide sample by IPRP UHPLC was performed with a linear gradient from 45% B to 80% B in 14.67 min at a flow rate of 0.25 mL/min. Mass spectra were acquired for the -4 charge state of the species. The UHPLC chromatograms and MS spectra of the product generated under different wash conditions were overlaid (normalized to the main product peak(s)) to show and quantitate the levels of the impurities.

This section provides the RP-UPLC-UV-MS chromatograms and spectra of the DMT-on ASO I and ASO II products obtained from the syntheses with Reduced Wash Protocols A through J. Zoomed-in chromatograms and spectra are provided to show details of the failure sequences (the small peaks eluting before the main peaks) and the critical impurities. By comparing with the references of standard, the DMT-on product yield was obtained by the RP-UPLC-UV-MS analysis and reported.

Sequence of ASO I:

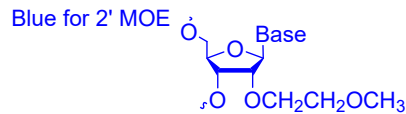
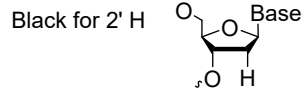


MeC = 5-Me Cytosine
s for PS linkage



ASO I: A 18-mer 2' methoxyethoxy (MOE) ribose phosphorothioate oligonucleotide.

Sequence of ASO II:



MeC = 5-Me Cytosine
s for PS linkage and o for PO linkage

ASO II: A 20-mer 2' methoxyethoxy (MOE) and deoxy mixed ribose phosphorothioate oligonucleotide.

DMT-on crude ASO I synthesized by Protocol A (Batch 1)

Figure S1. RP-UPLC UV chromatograms of DMT-on crude ASO I synthesized by Synthesis 1

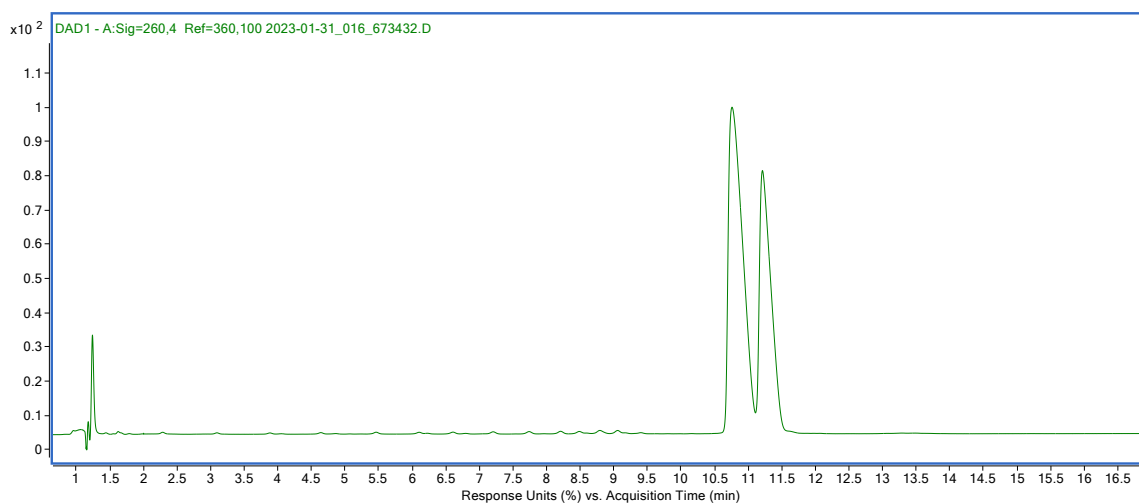


Figure S2. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I synthesized by Batch 1

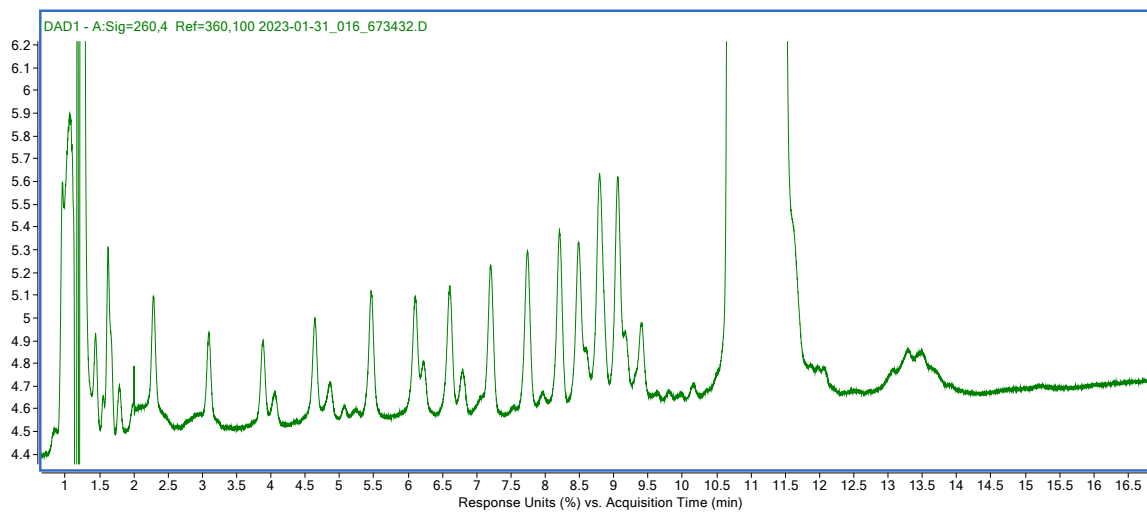


Figure S3. MS spectra of crude of DMT-on crude ASO I synthesized by Batch 1

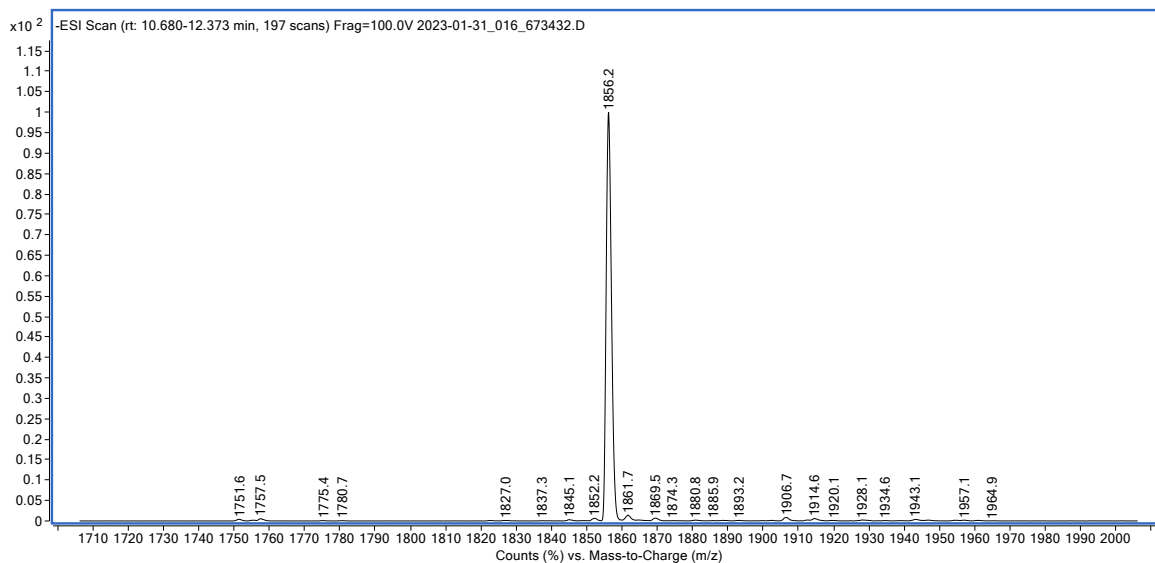
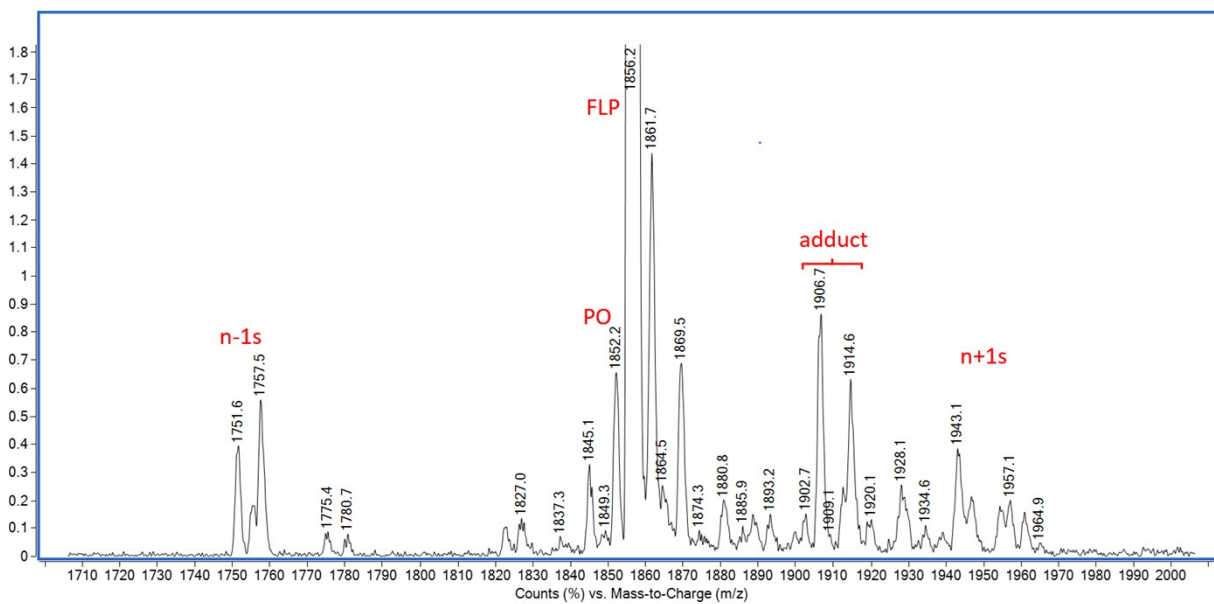


Figure S4. Zoomed in MS spectra of DMT-on crude ASO I synthesized by Batch 1



DMT-on crude ASO I synthesized by Protocol B (Batch 2)

Figure S5. RP-UPLC UV chromatograms of DMT-on crude ASO I synthesized by Batch 2

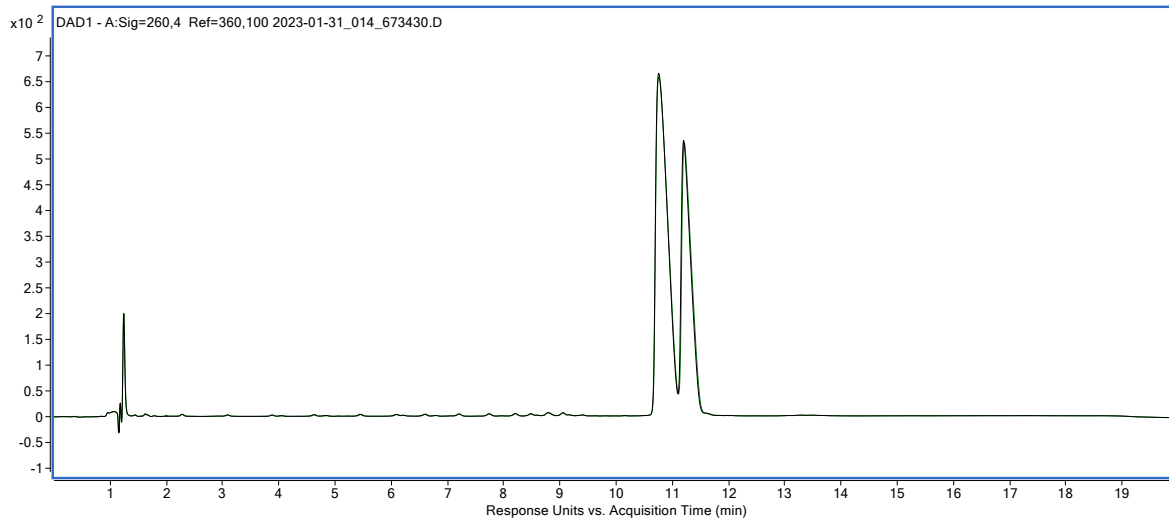


Figure S6. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I synthesized by Batch 2

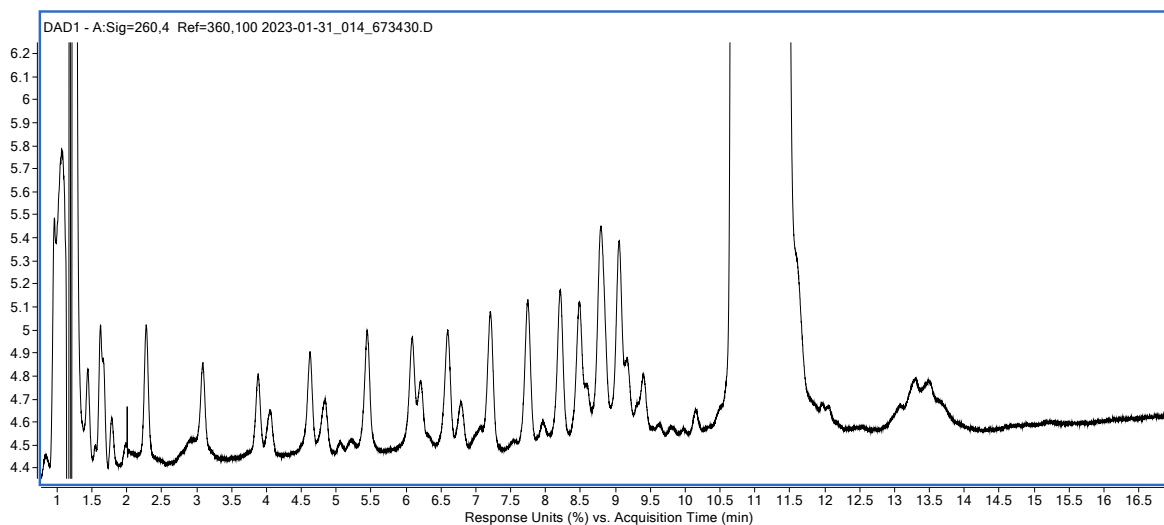


Figure S7. MS spectra of DMT-on crude ASO I synthesized by Batch 2

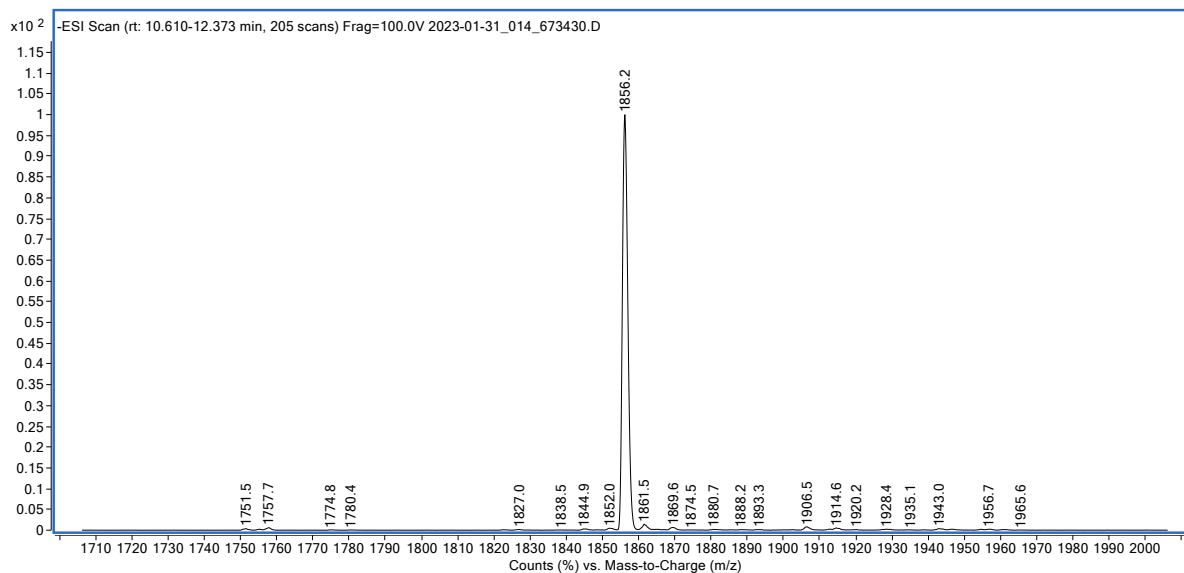
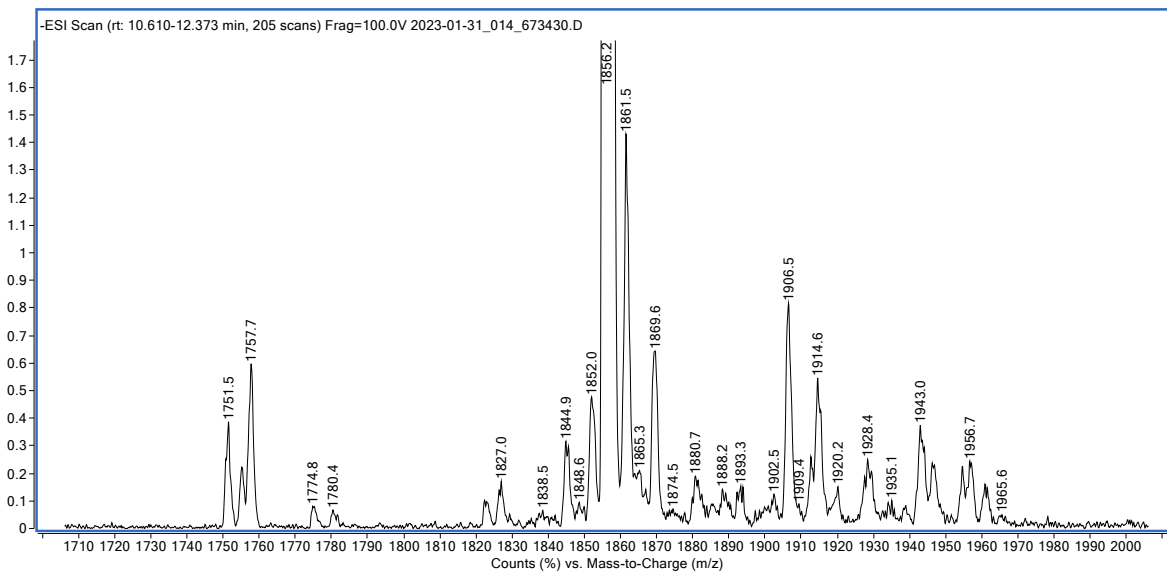


Figure S8. Zoomed in MS spectra of DMT-on crude ASO I synthesized by Batch 2



DMT-on crude ASO I synthesized by Protocol C (Batch 3)

Figure S9. RP-UPLC UV chromatograms of DMT-on crude ASO I synthesized by Batch 3

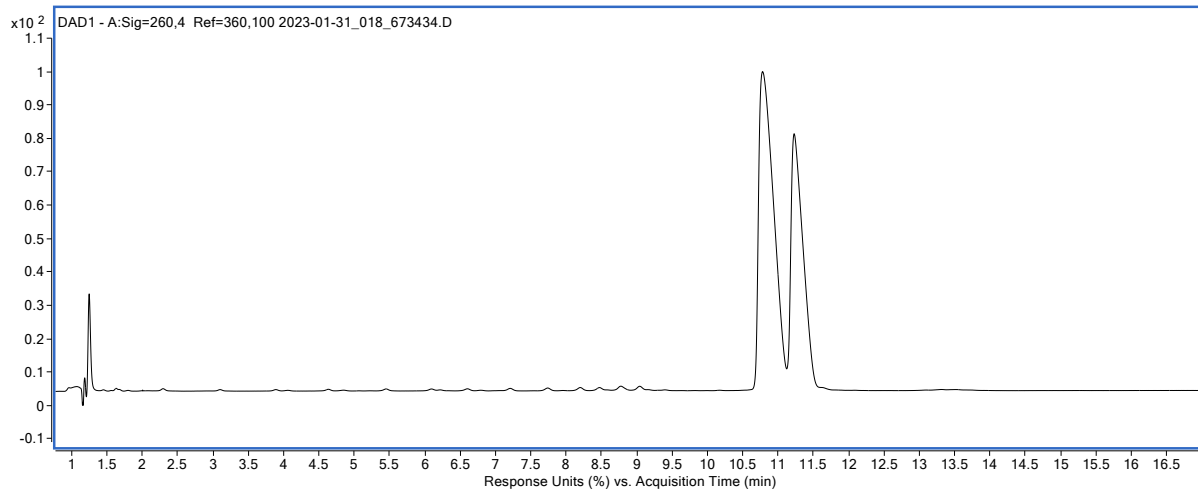


Figure S10. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I synthesized by Batch 3

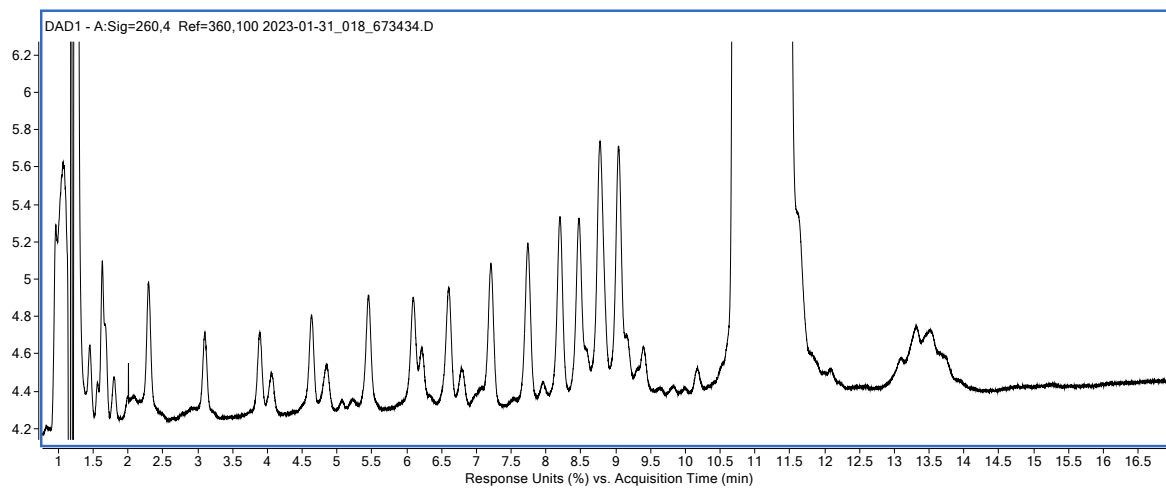


Figure S11. MS spectra of DMT-on crude ASO I synthesized by Batch 3

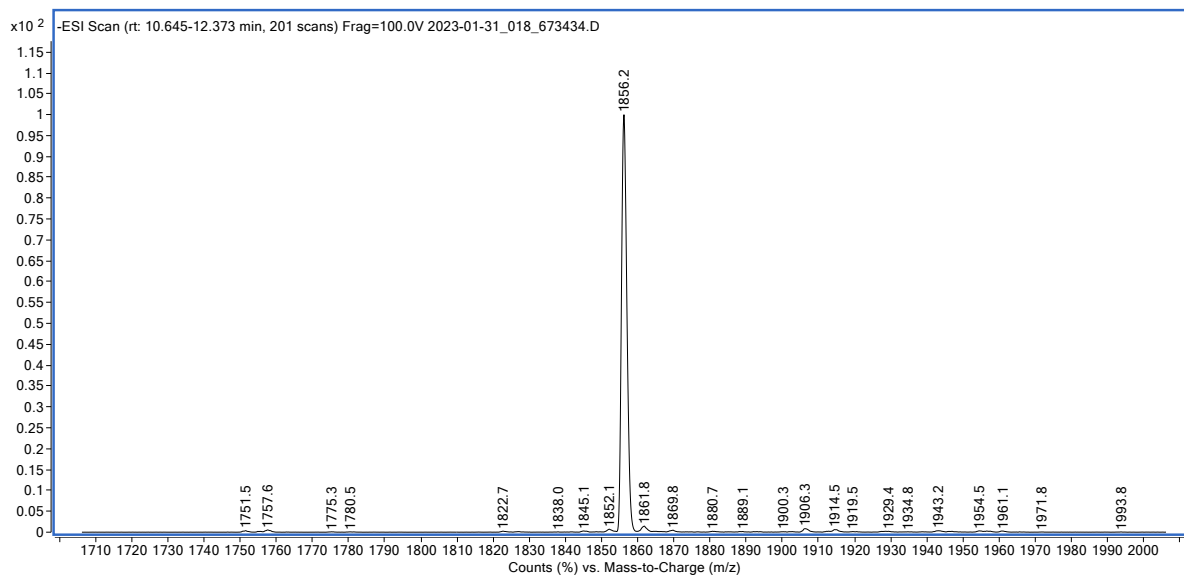
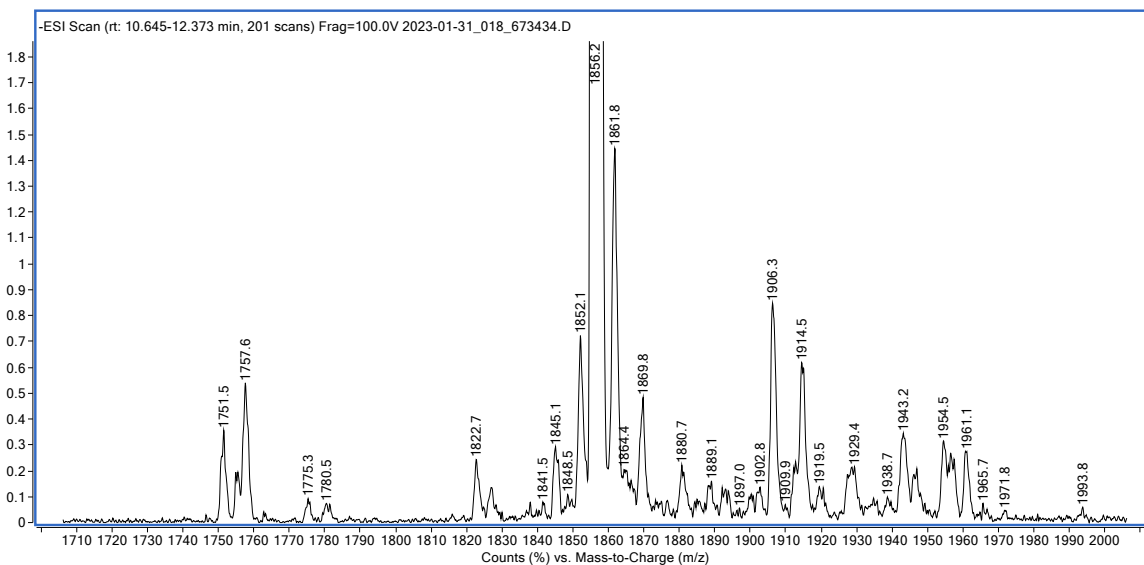


Figure S12. Zoomed in MS spectra of DMT-on crude ASO I synthesized by Batch 3



DMT-on crude ASO I synthesized by Protocol D (Batch 4)

Figure S13. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 4

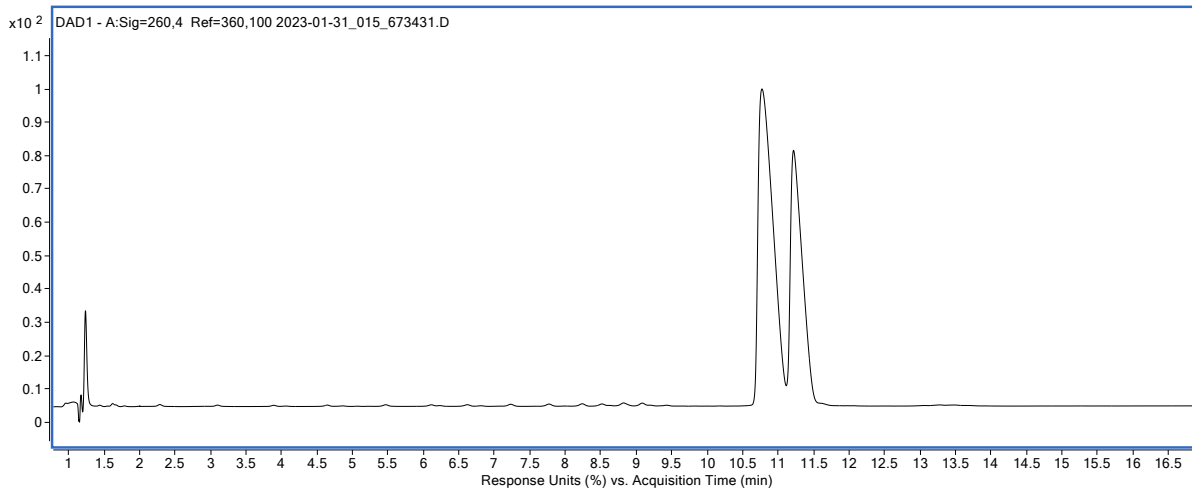


Figure S14. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 4

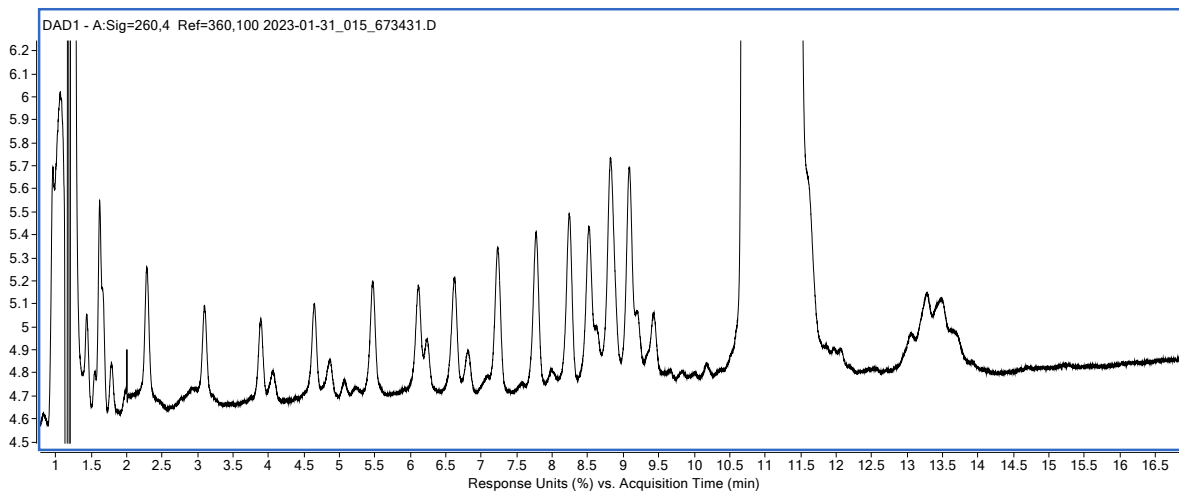


Figure S15. MS spectra of DMT-on crude ASO I from Batch 4

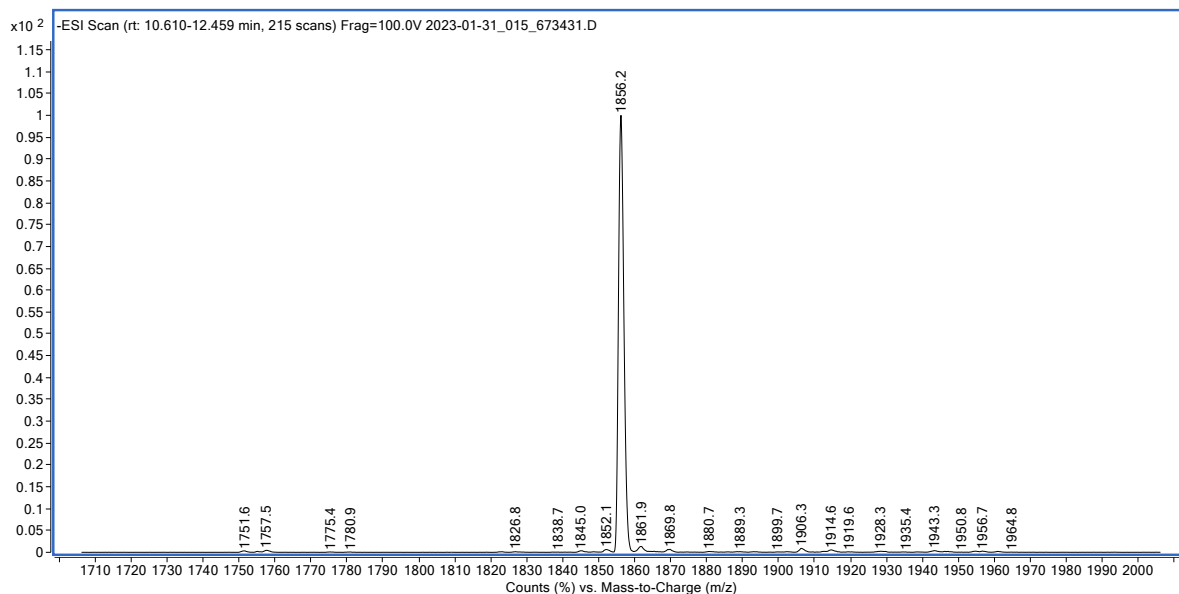
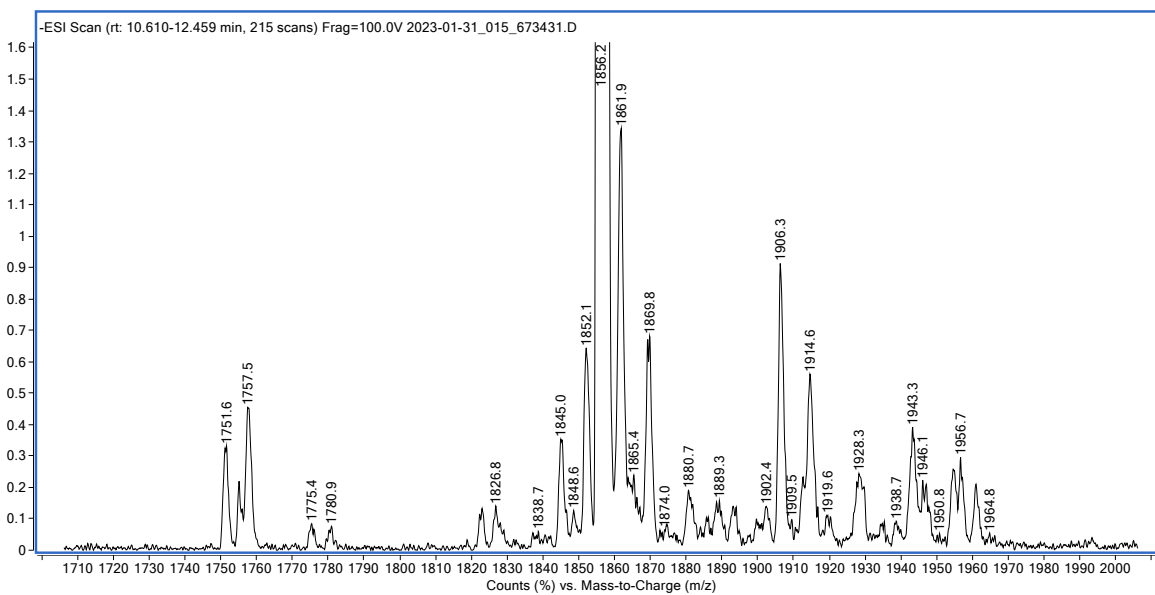


Figure S16. Zoomed in MS spectra of DMT-on crude ASO I from Batch 4



DMT-on crude ASO I synthesized by Protocol E (Batch 5 and 6)

Figure S17. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 5

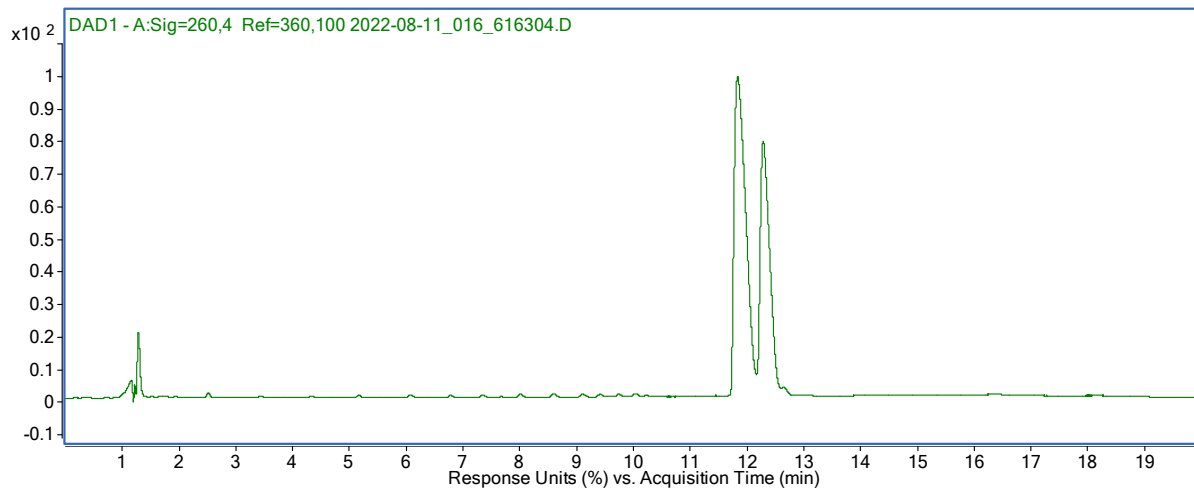


Figure S18. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 5

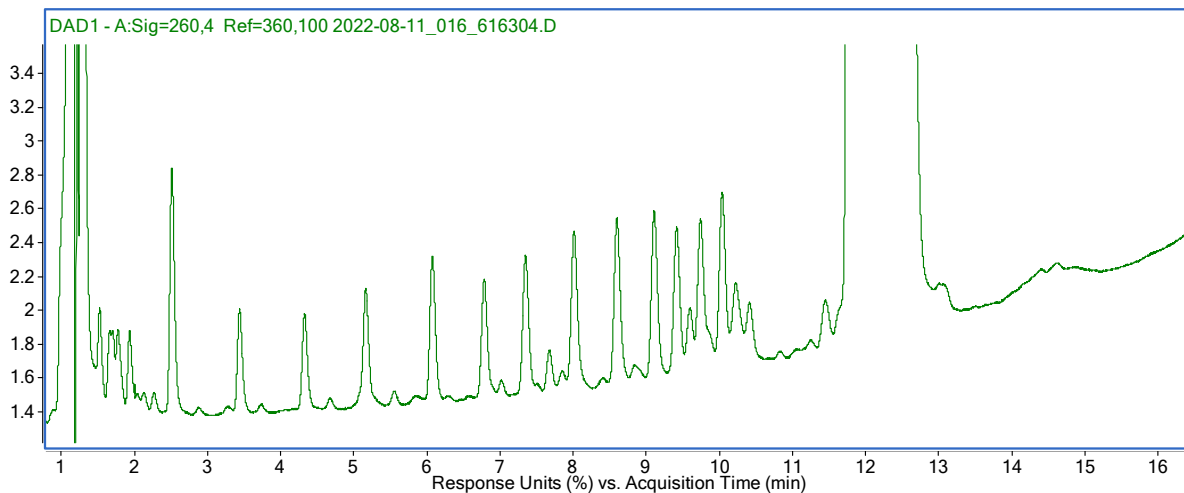


Figure S19. MS spectra of DMT-on crude ASO I from Batch 5

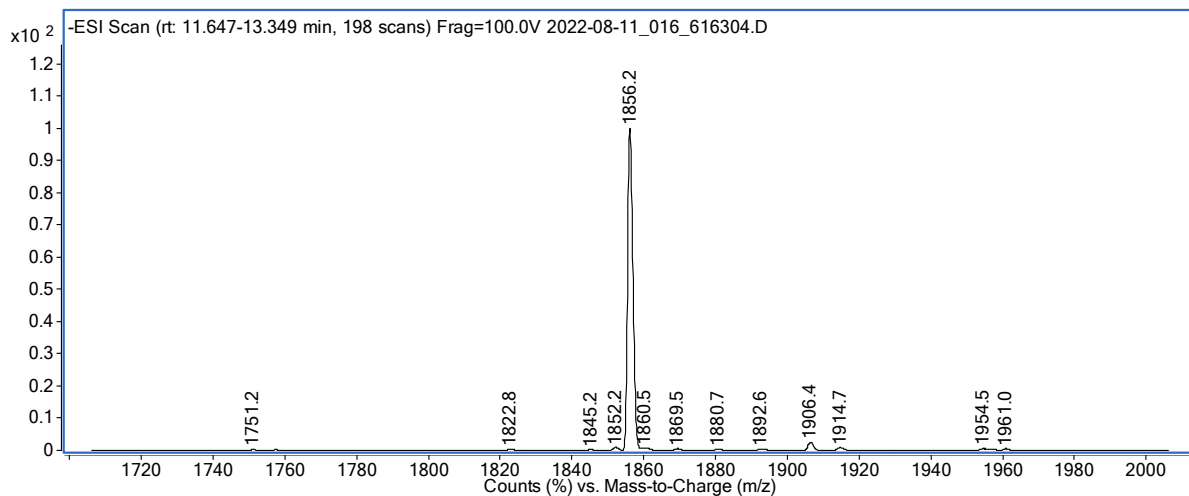


Figure S20. Zoomed in MS spectra of DMT-on crude ASO I from Batch 5

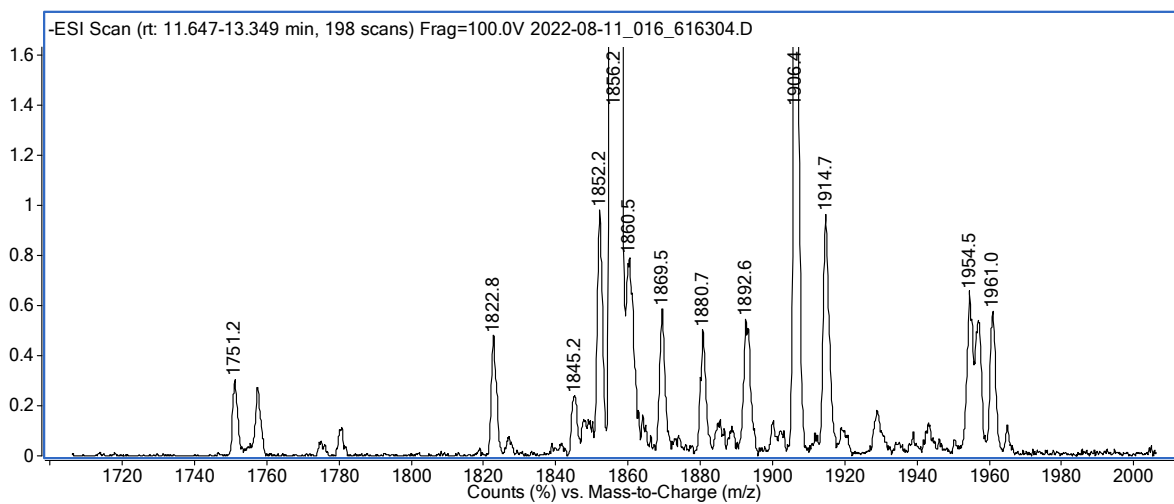


Figure S21. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 6

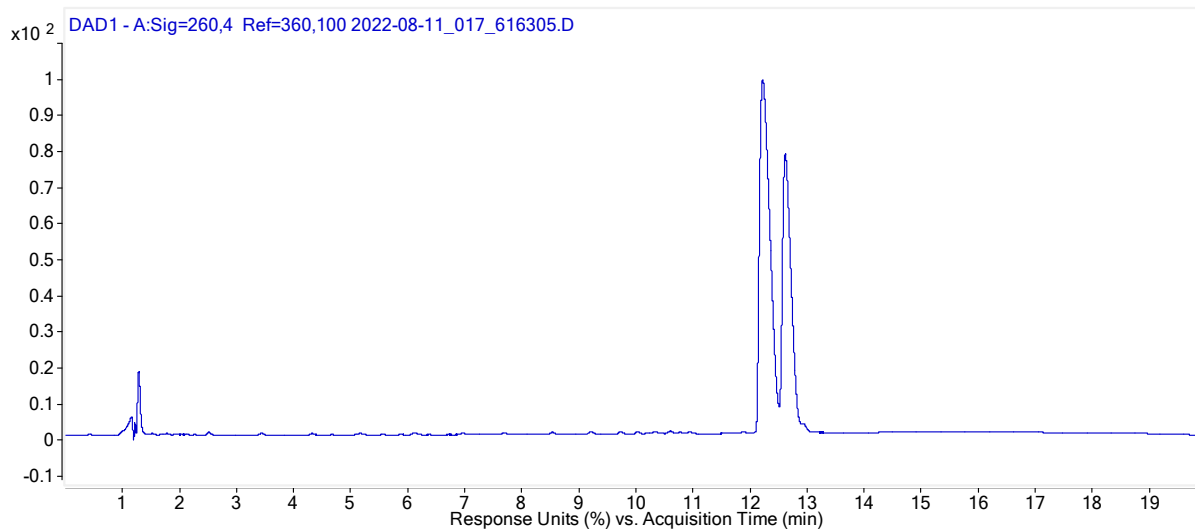


Figure S22. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 6

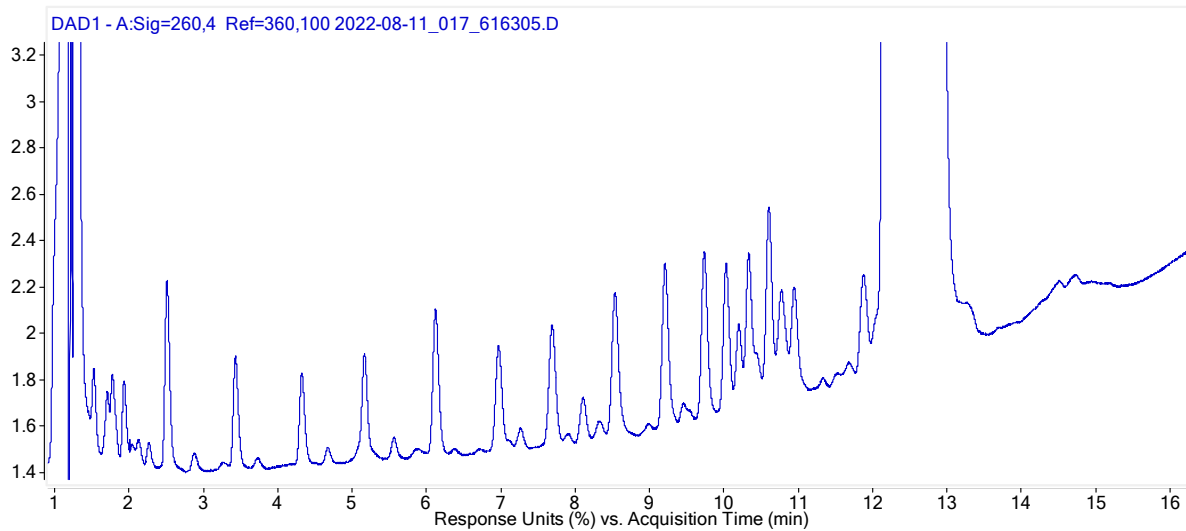


Figure S23. MS spectra of DMT-on crude ASO I from Batch 6

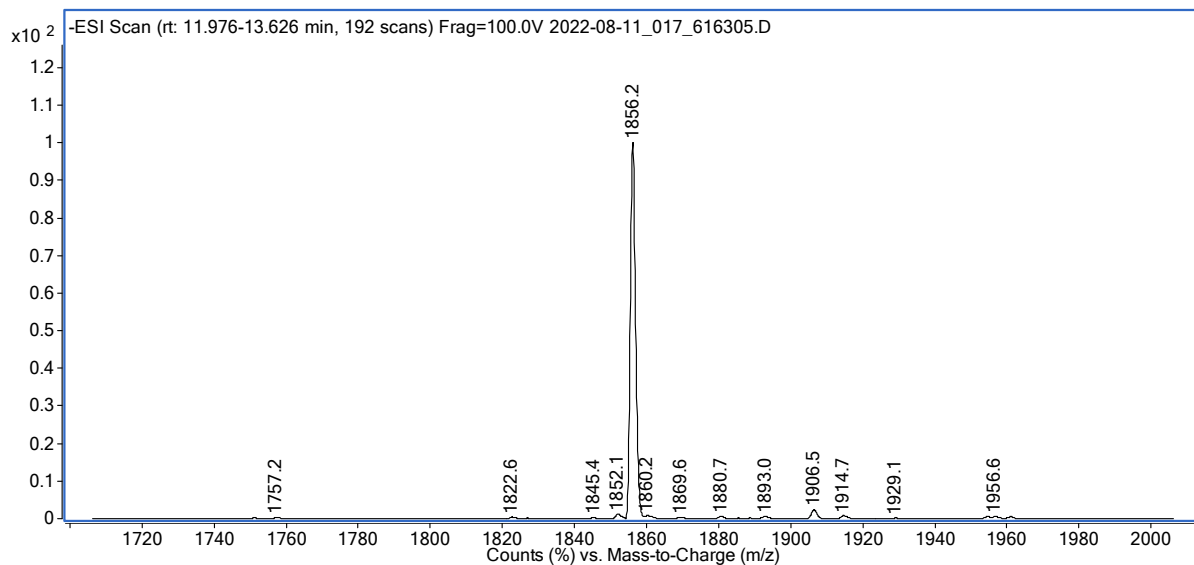


Figure S24. Zoomed in MS spectra of DMT-on crude ASO I from Batch 6

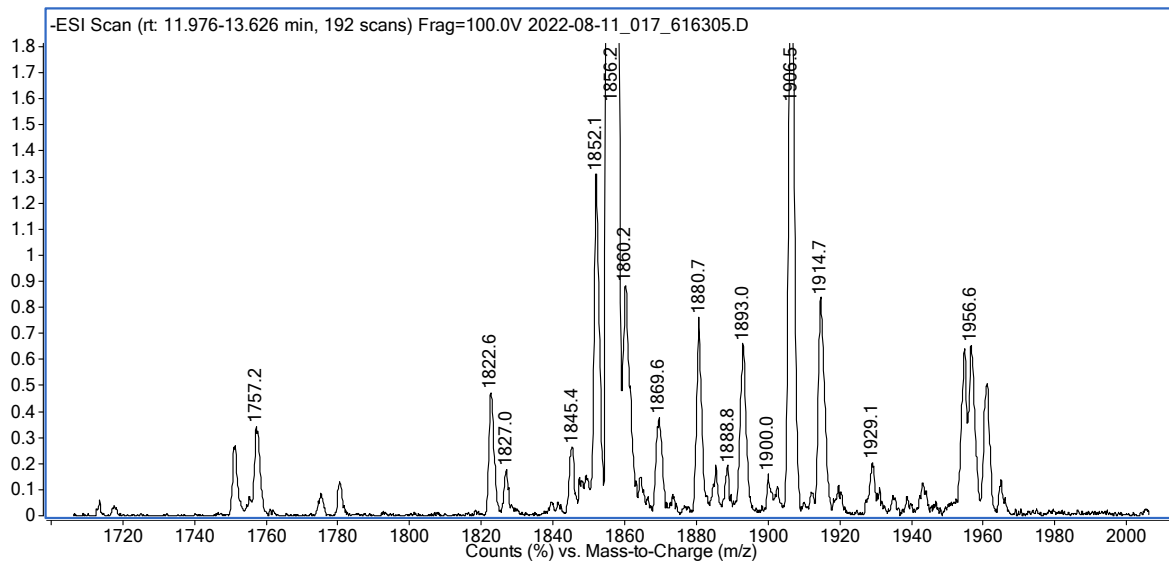


Figure S25. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 7

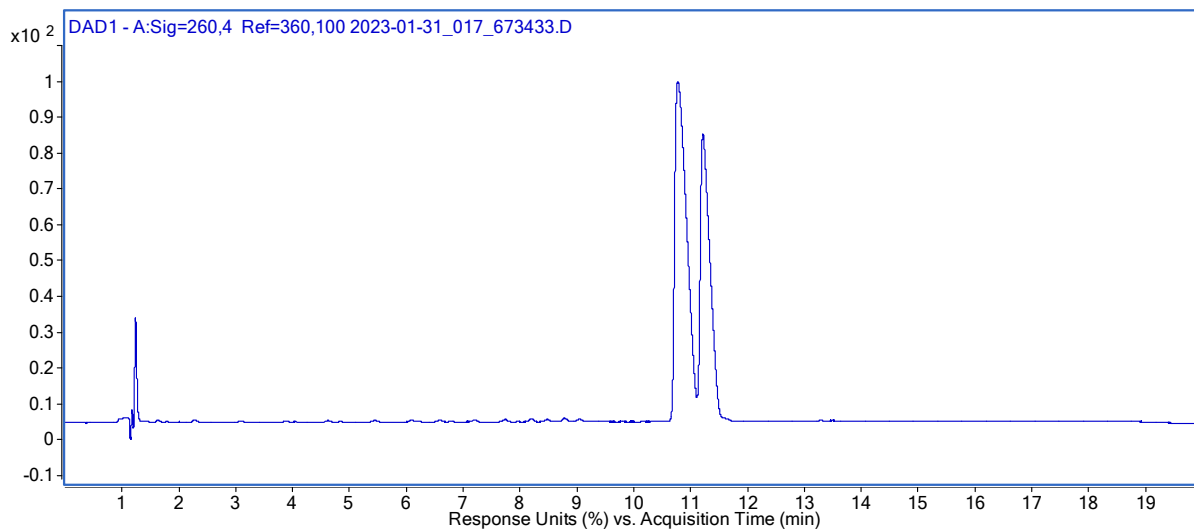


Figure S26. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 7

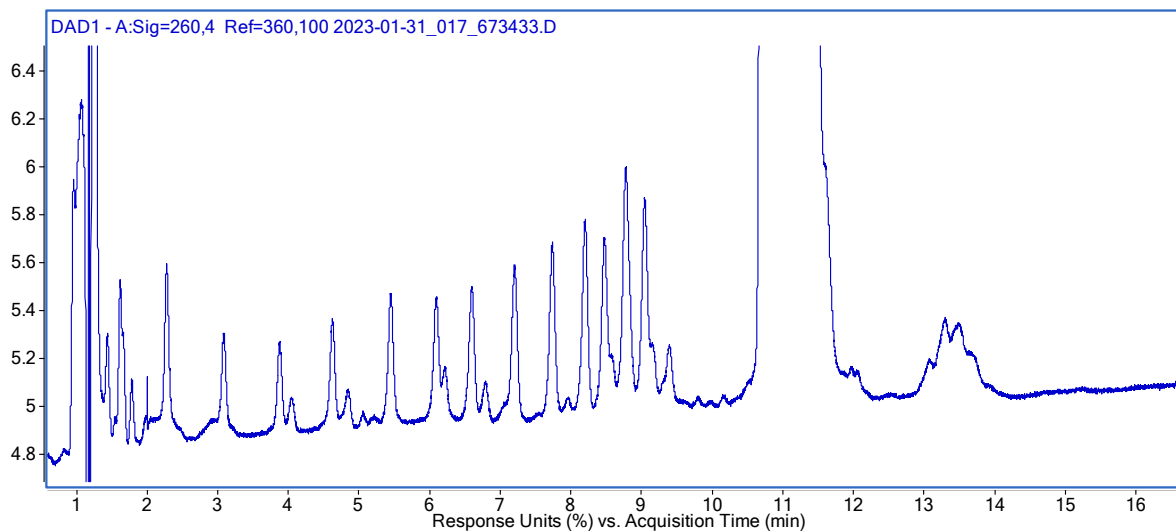


Figure S27. MS spectra of DMT-on crude ASO I from Batch 7

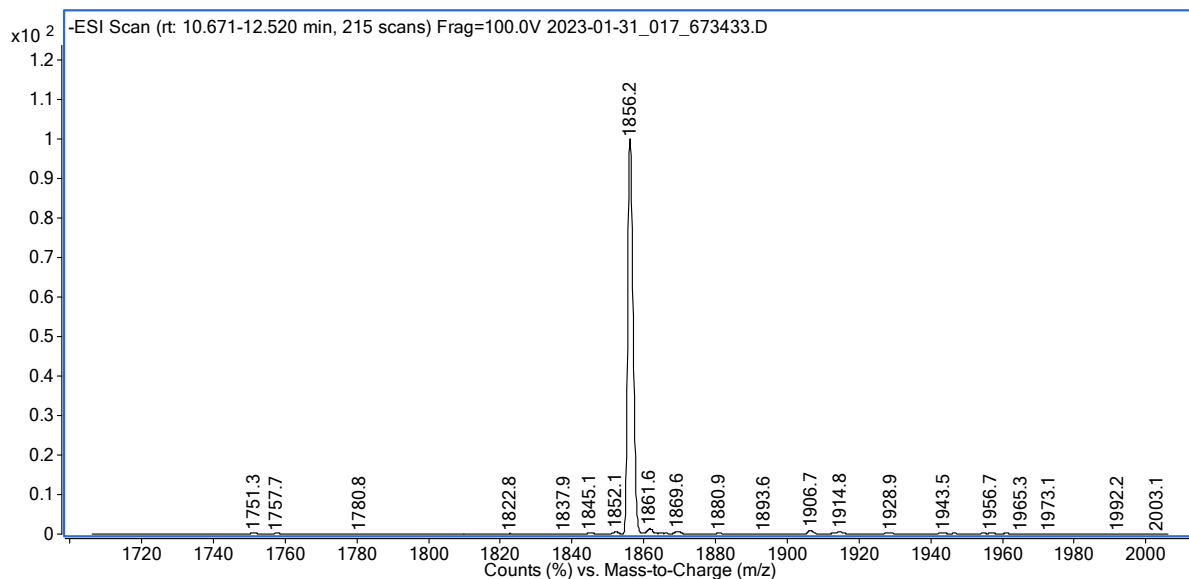


Figure S28. Zoomed in MS spectra of DMT-on crude ASO I from Batch 7

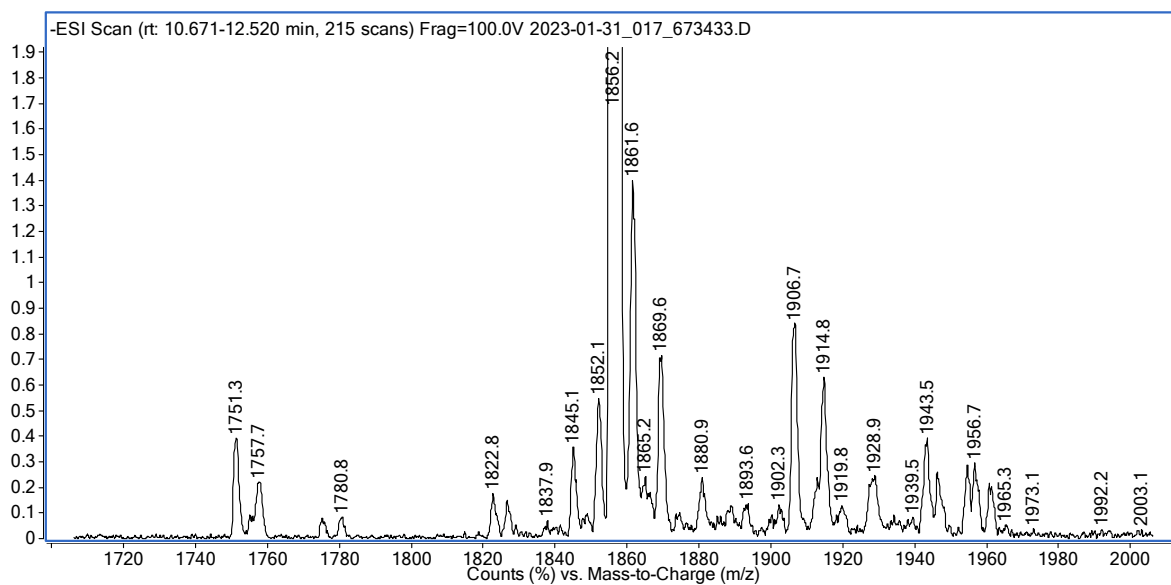


Figure S29. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 8

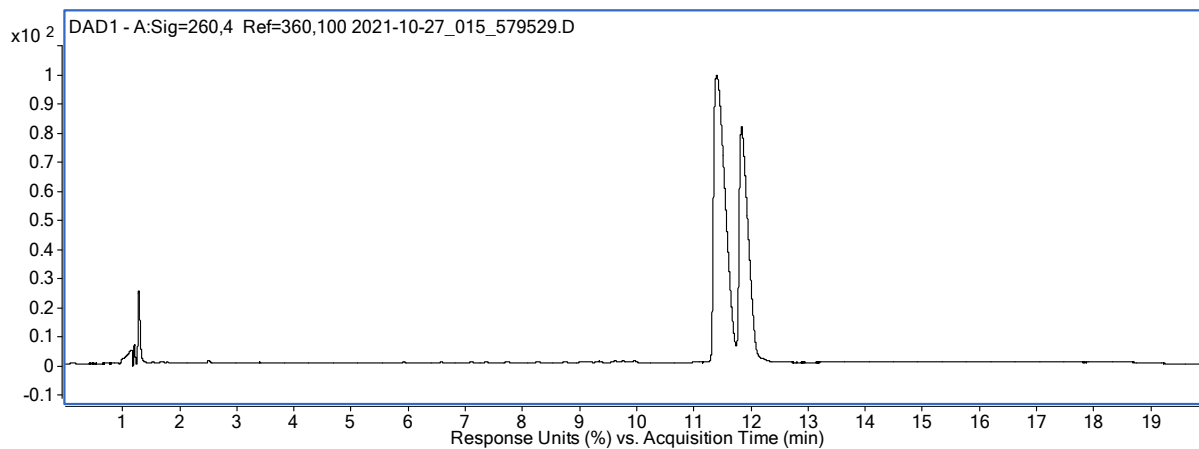


Figure S30. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 8

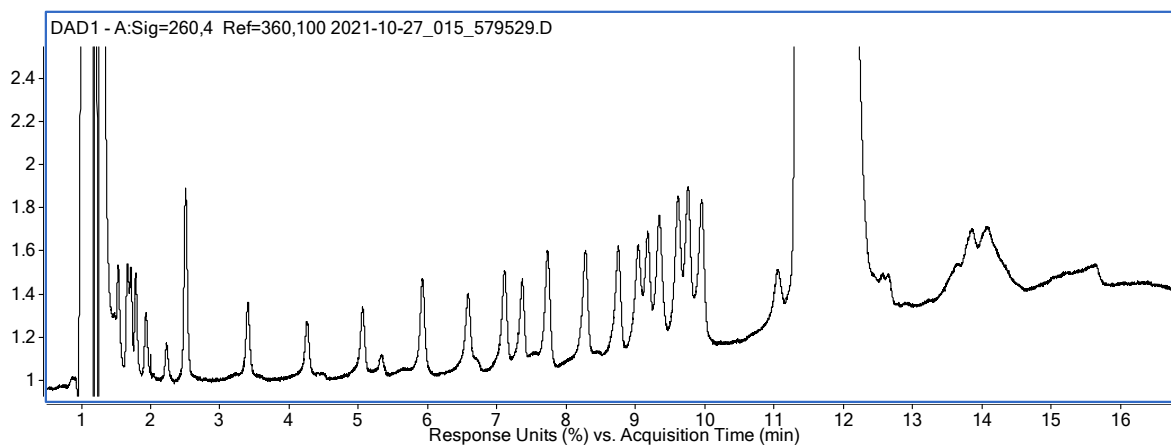


Figure S31. MS spectra of DMT-on crude ASO I from Batch 8

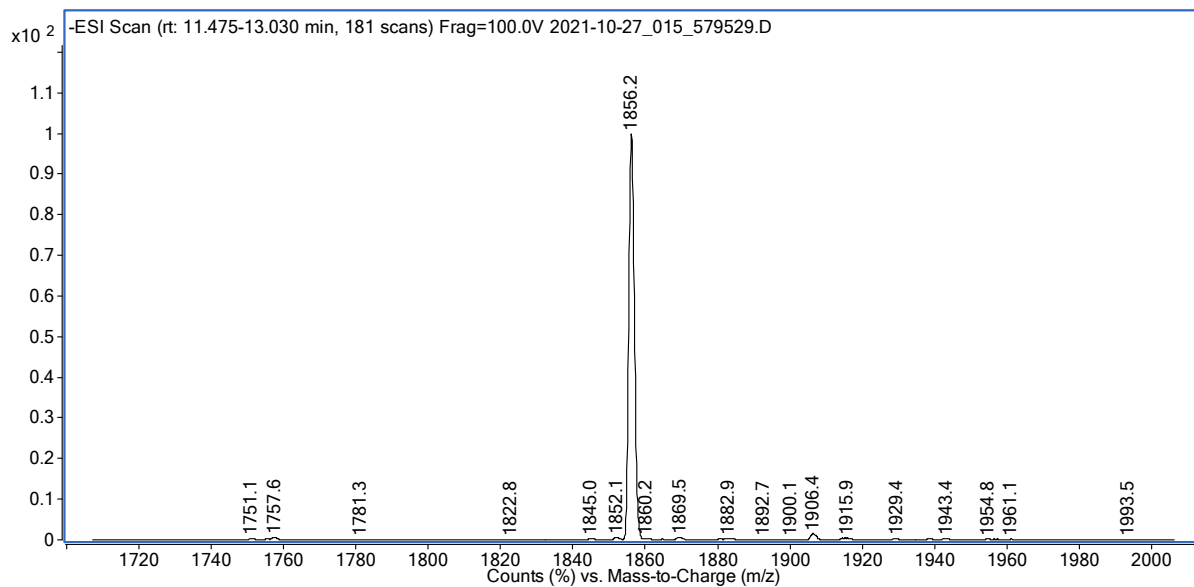


Figure S32. Zoomed in MS spectra of DMT-on crude ASO I from Batch 8

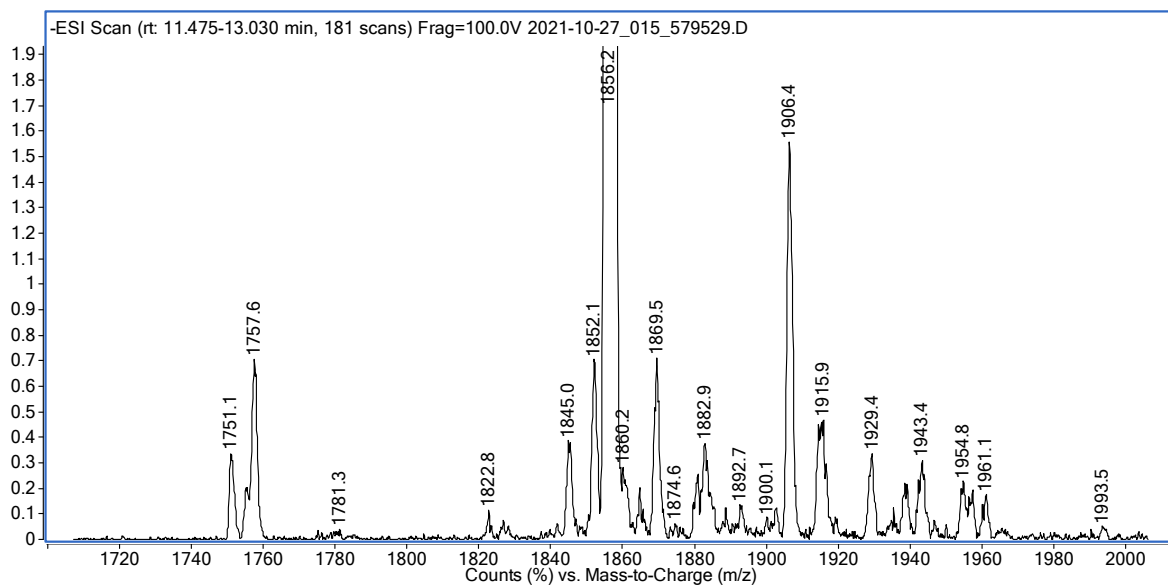


Figure S33. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 9

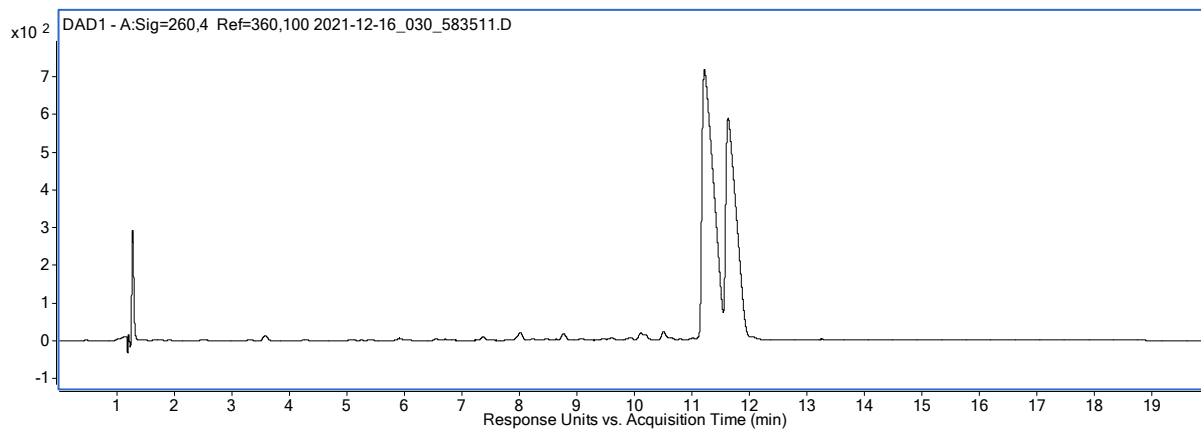


Figure S34. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 9

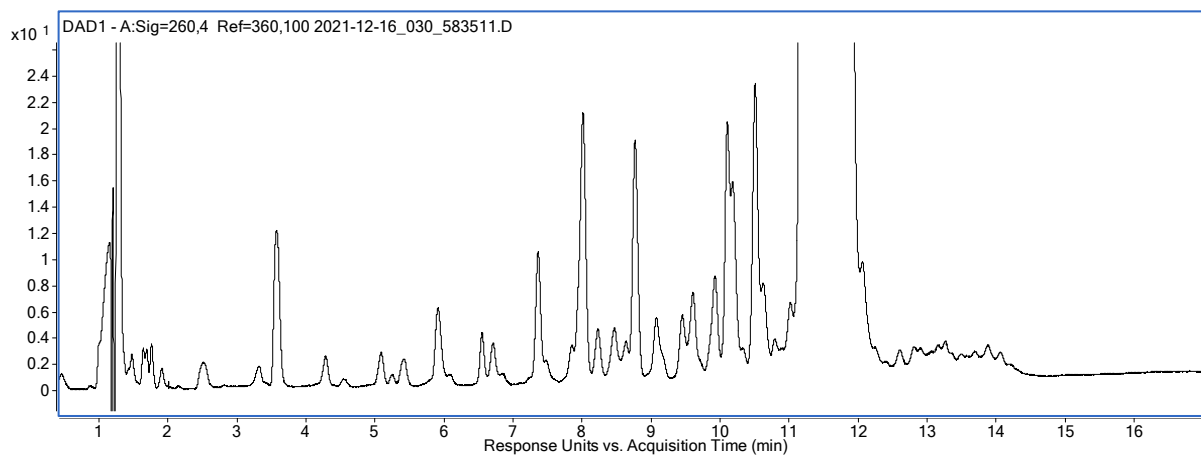


Figure S35. MS spectra of DMT-on crude ASO I from Batch 9

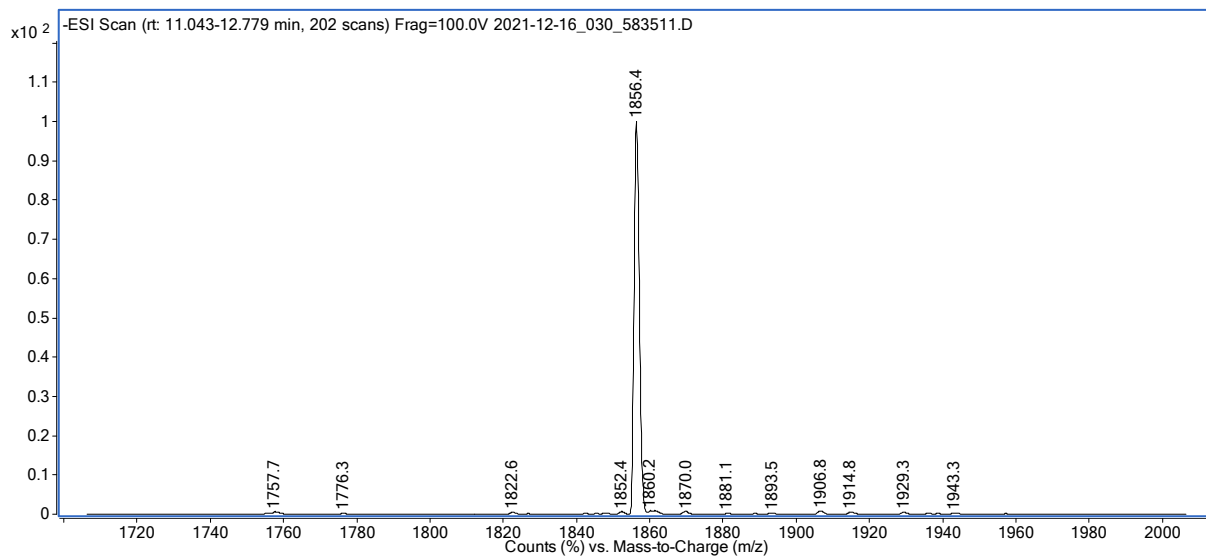


Figure S36. Zoomed in MS spectra of DMT-on crude ASO I from Batch 9

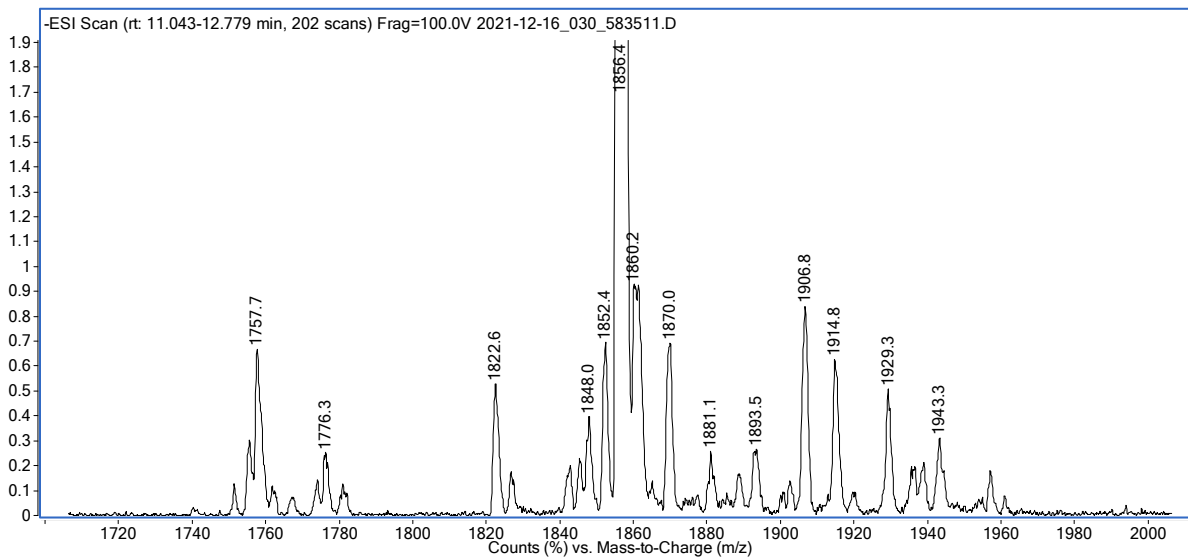


Figure S37. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 10

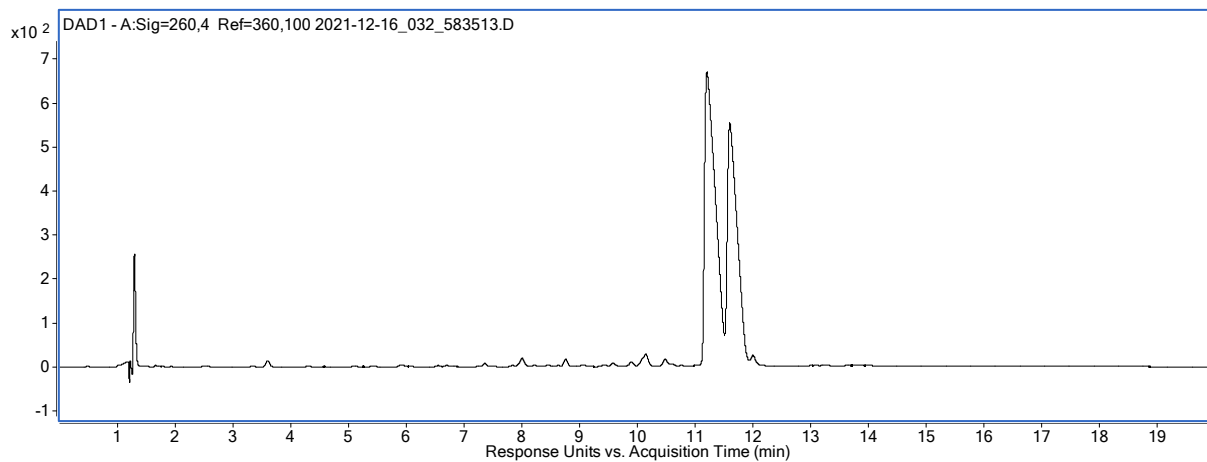


Figure S38. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 10

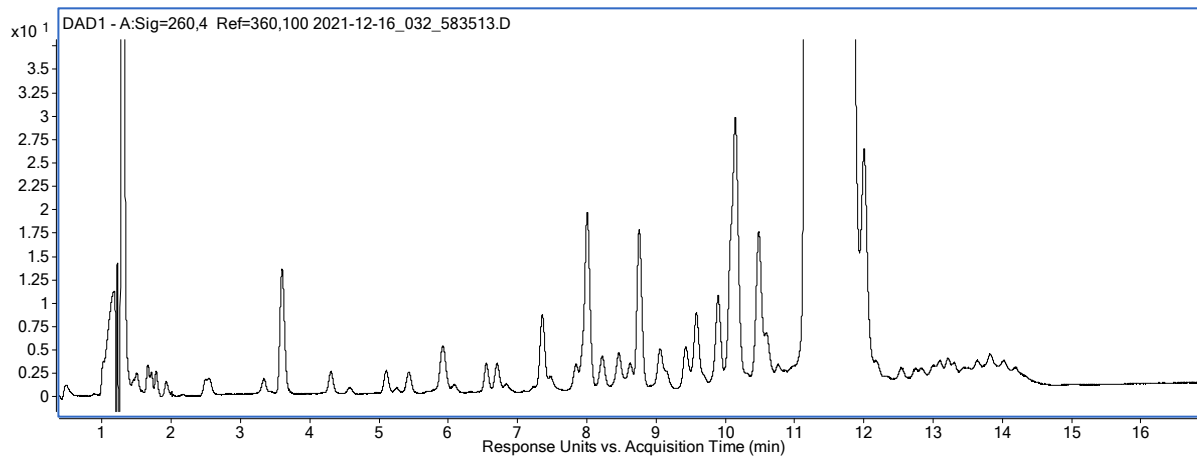


Figure S39. MS spectra of DMT-on crude ASO I from Batch 10

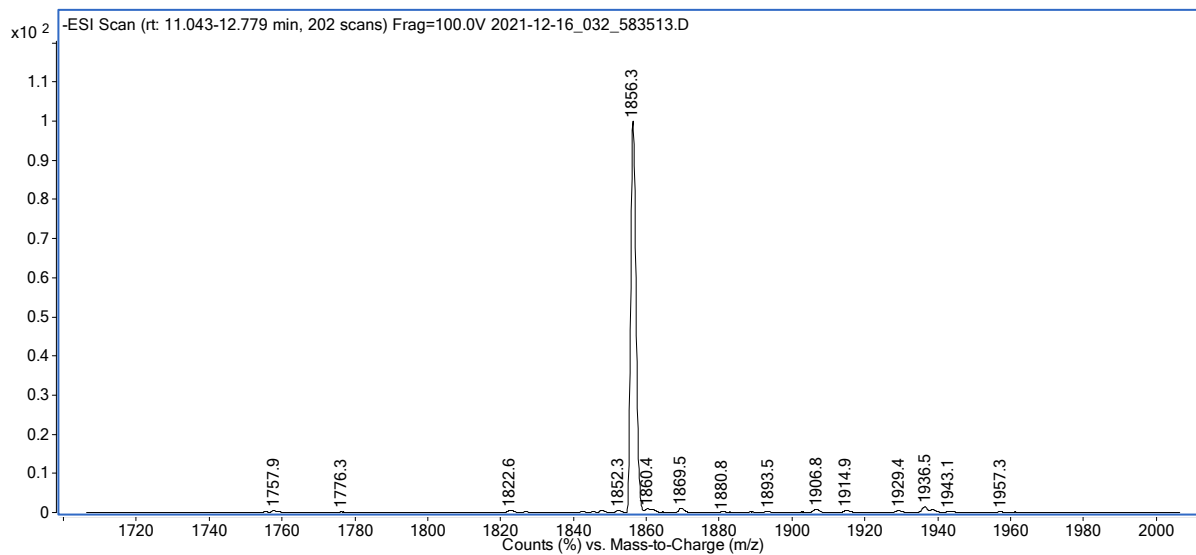


Figure S40. Zoomed in MS spectra of DMT-on crude ASO I from Batch 10

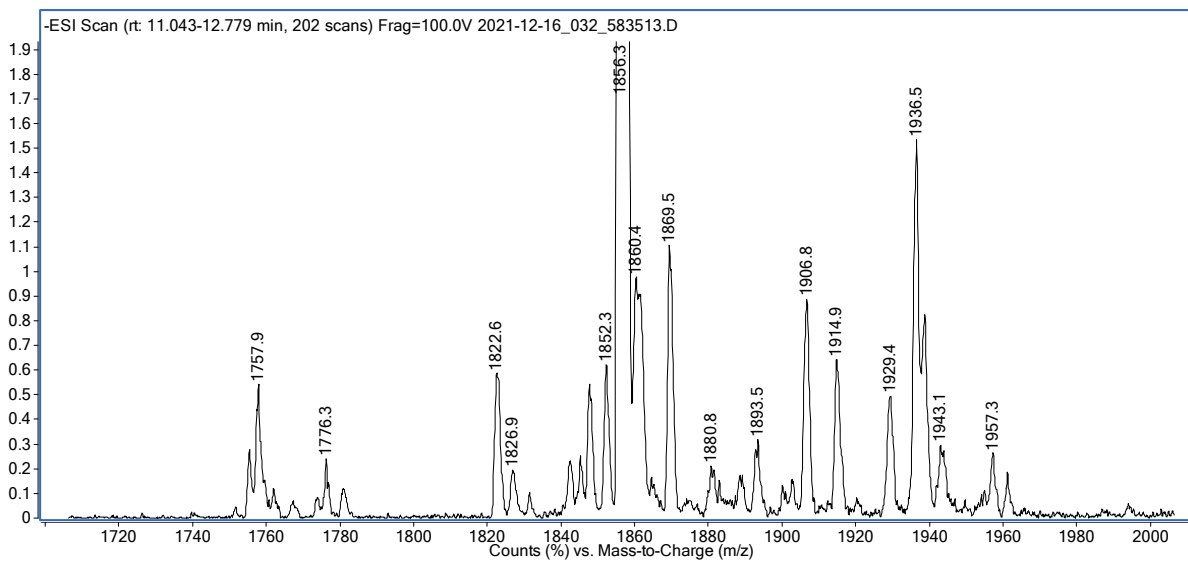


Figure S41. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 11

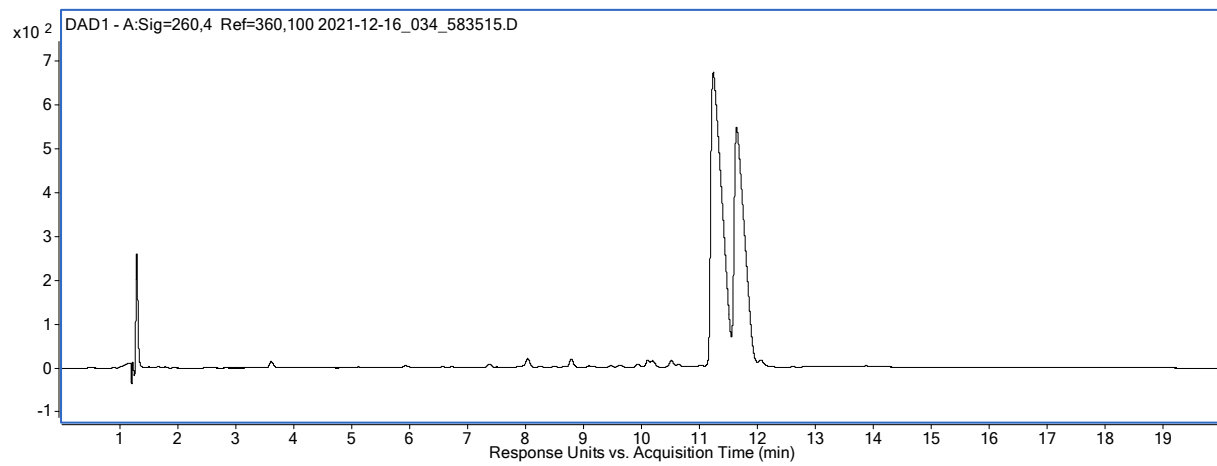


Figure S42. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 11

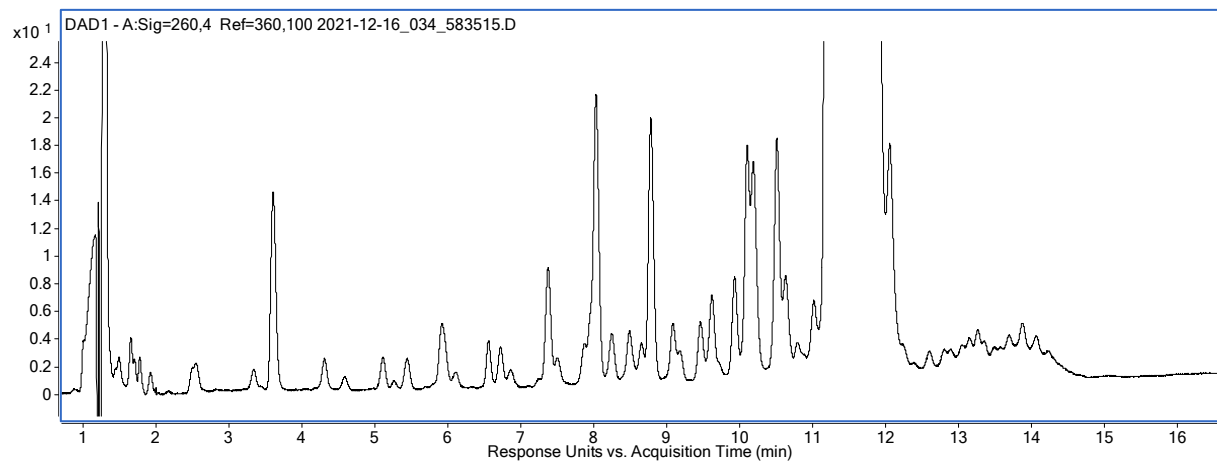


Figure S43. MS spectra of DMT-on crude ASO I from Batch 11

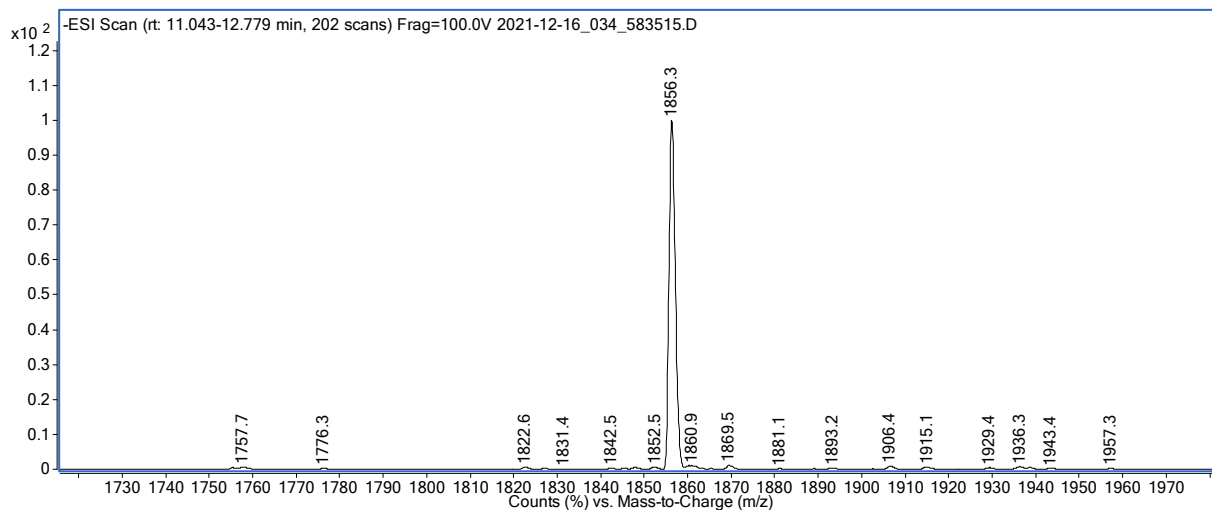


Figure S44. Zoomed in MS spectra of DMT-on crude ASO I from Batch 11

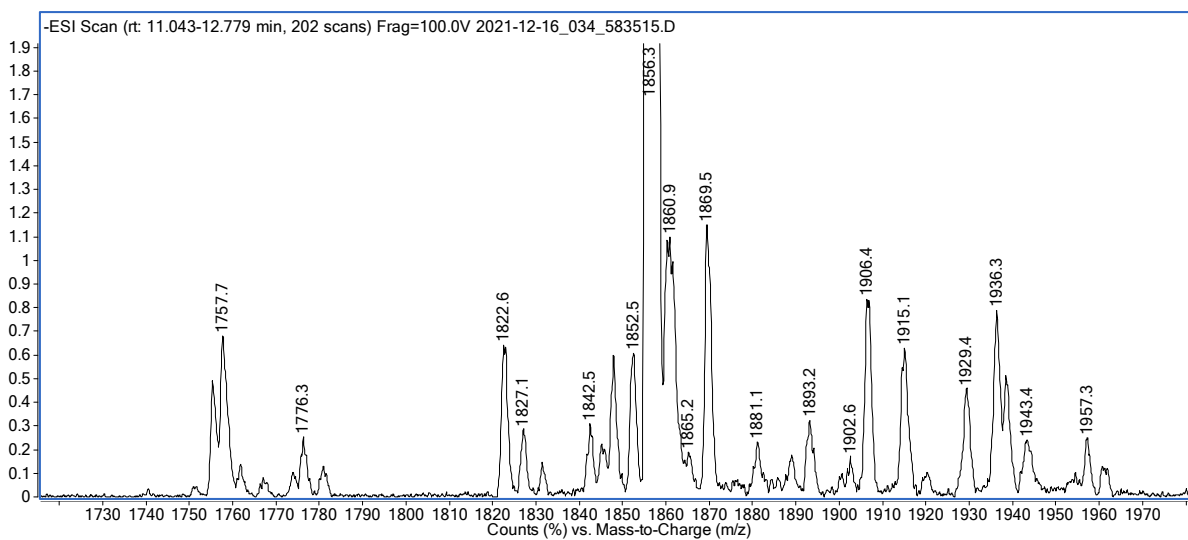


Figure S45. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 12

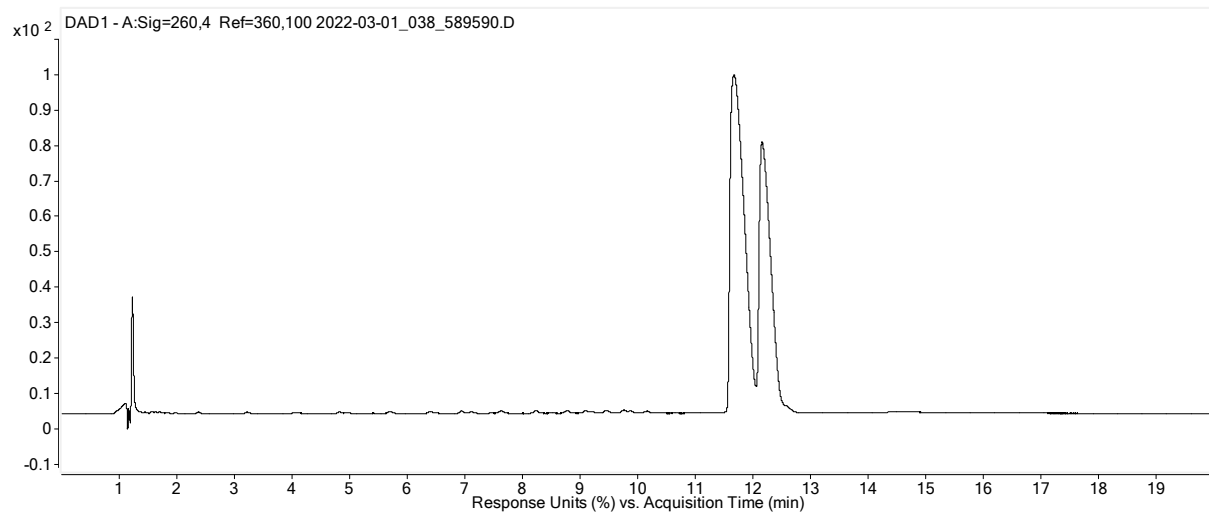


Figure S46. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 12

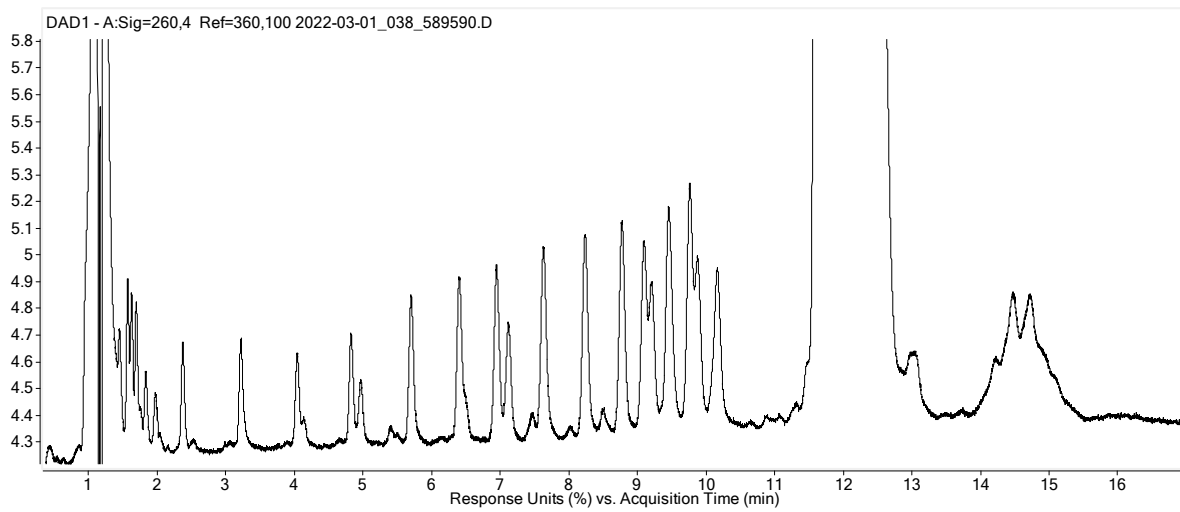


Figure S47. MS spectra of DMT-on crude ASO I from Batch 12

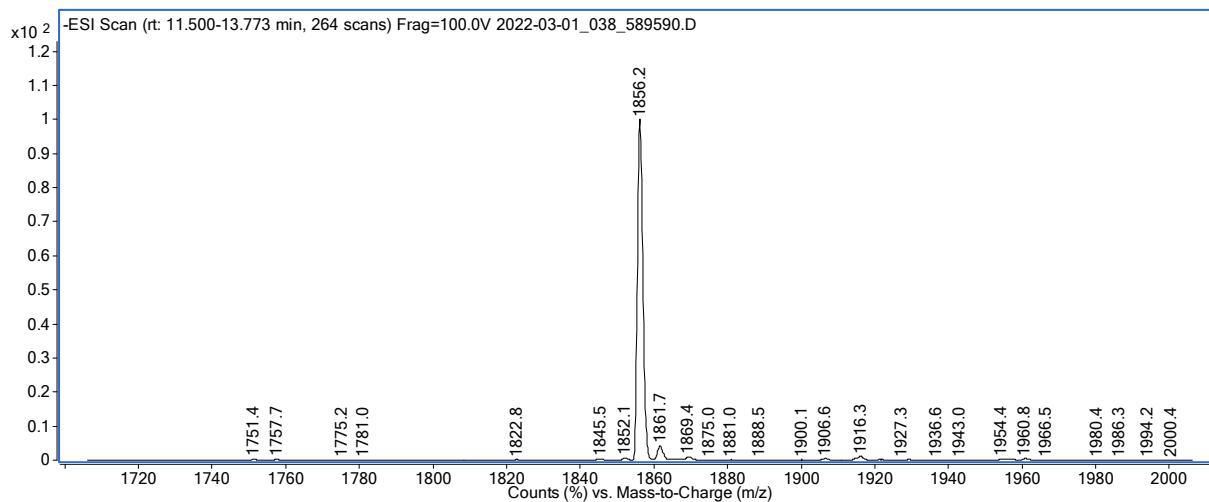


Figure S48. Zoomed in MS spectra of DMT-on crude ASO I from Batch 12

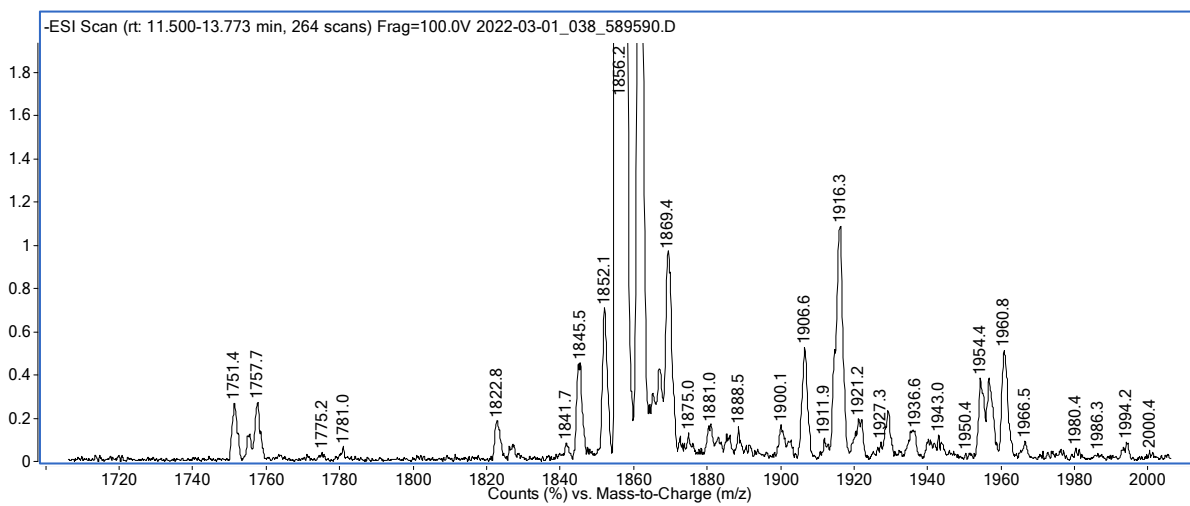


Figure S49. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 13

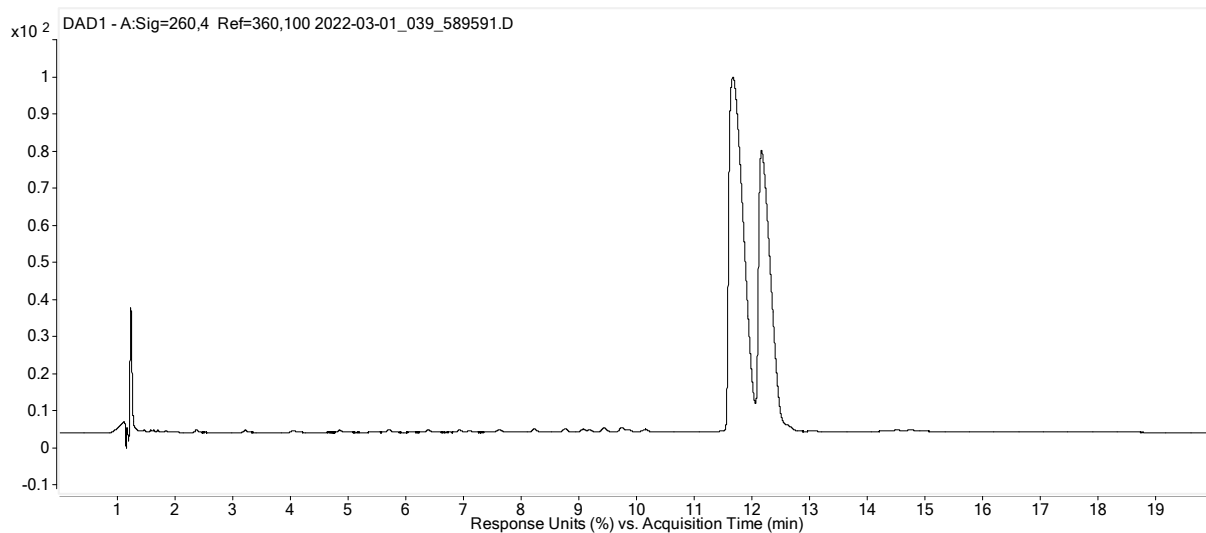


Figure S50. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 13

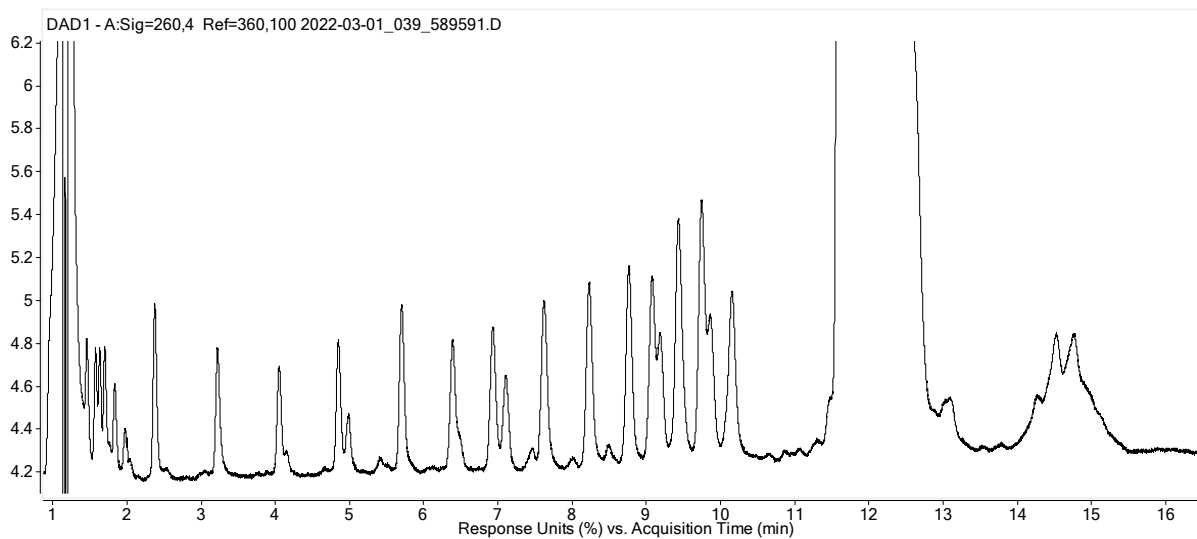


Figure S51. MS spectra of DMT-on crude ASO I from Batch 13

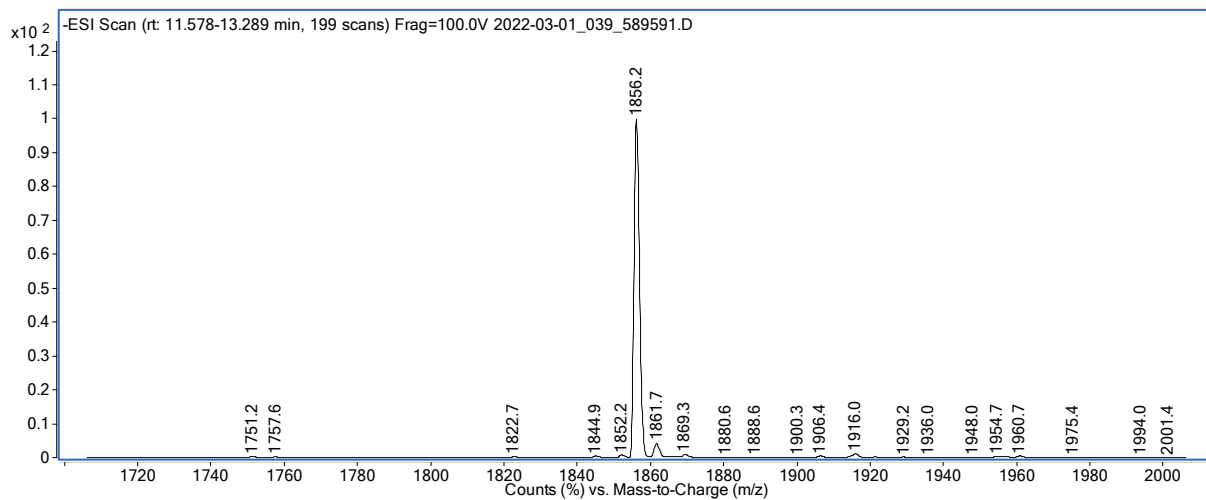


Figure S52. Zoomed in MS spectra of DMT-on crude ASO I from Batch 13

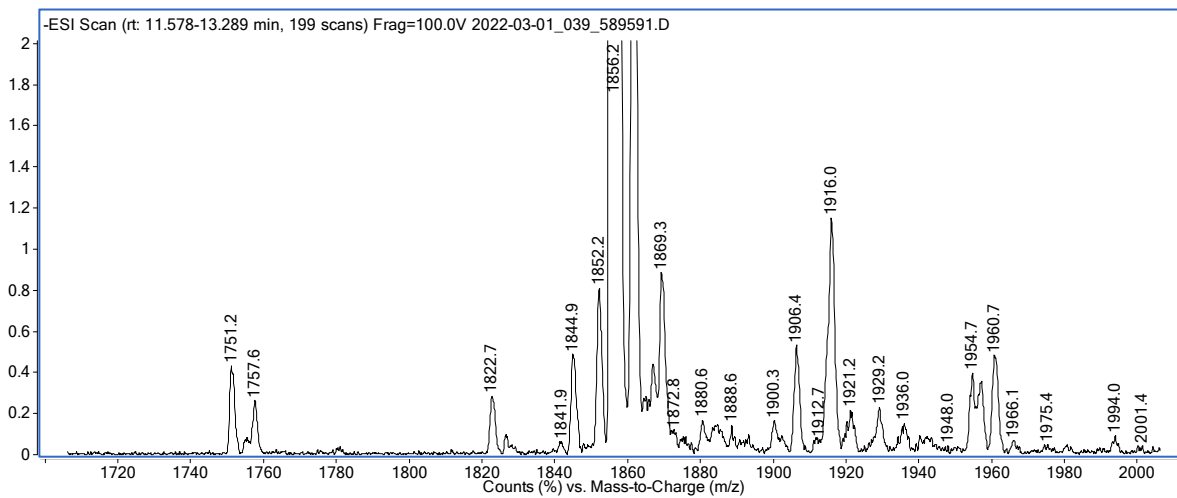


Figure S53. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 14

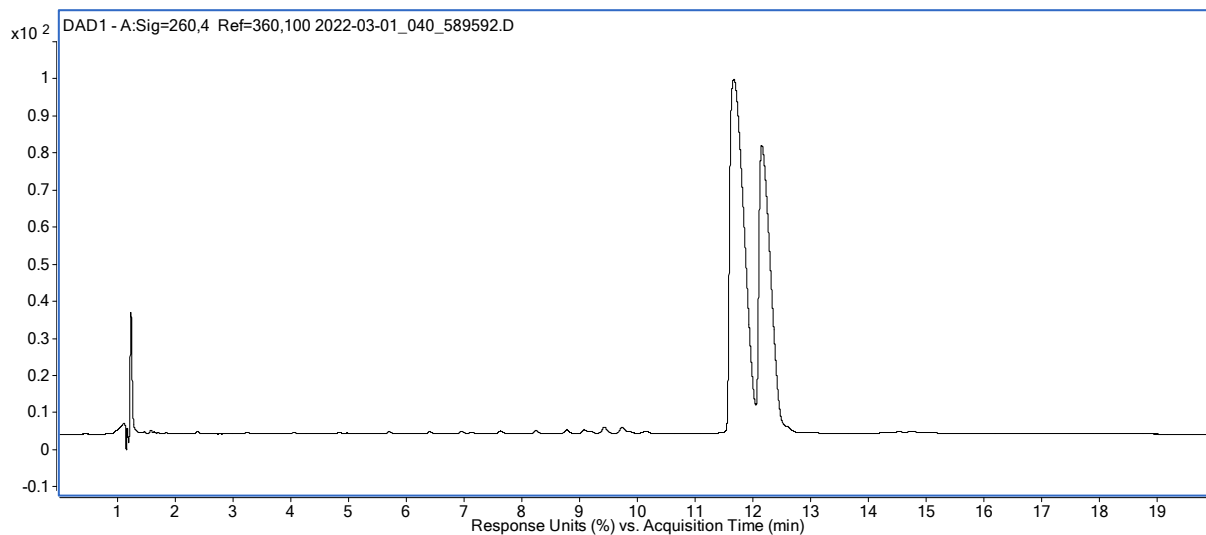


Figure S54. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 14

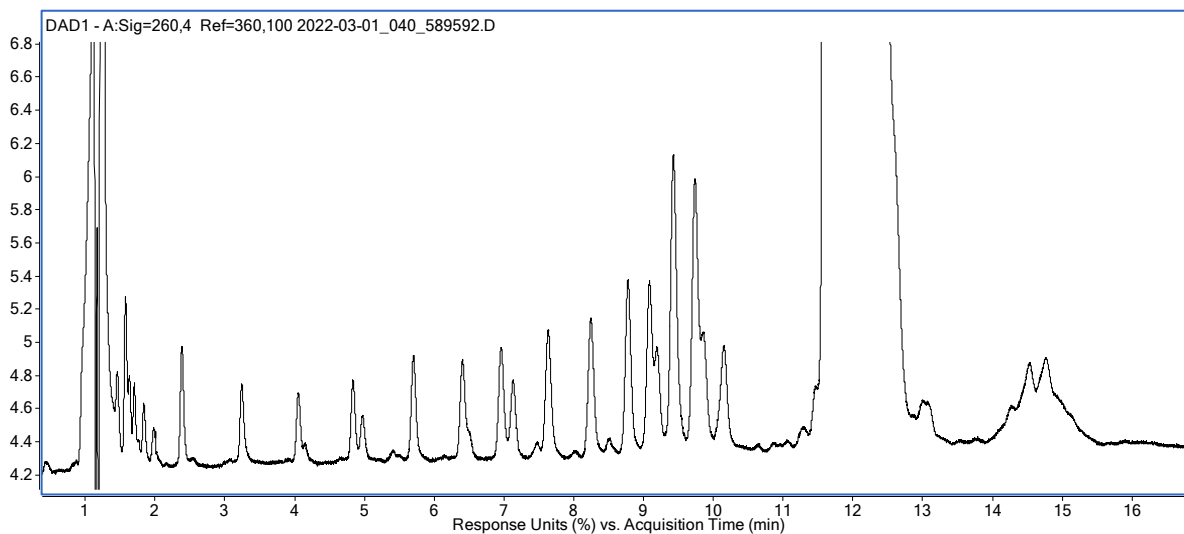


Figure S55. MS spectra of DMT-on crude ASO I from Batch 14

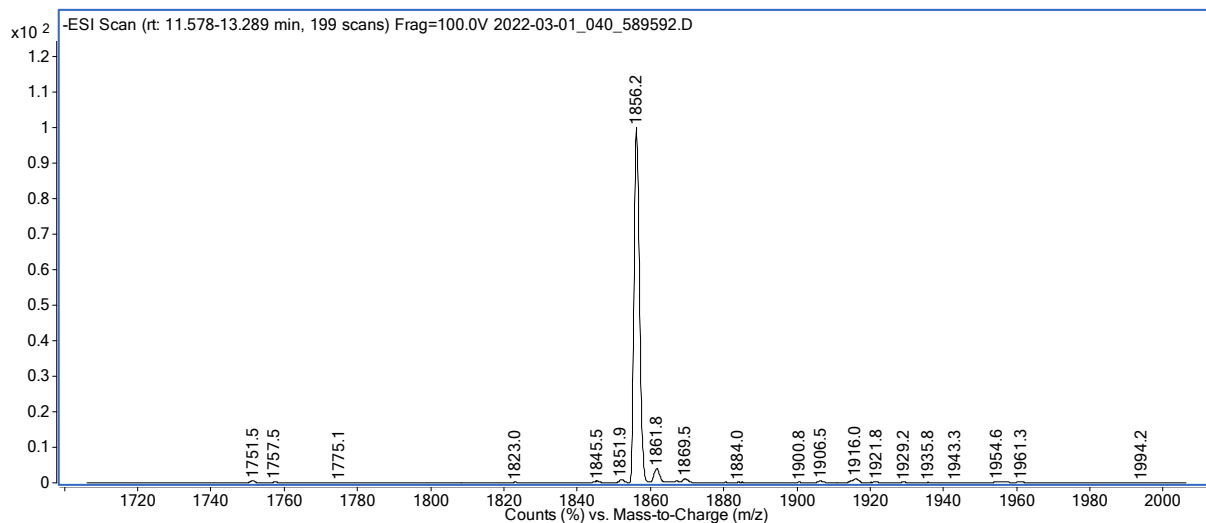


Figure S56. Zoomed in MS spectra of DMT-on crude ASO I from Batch 14

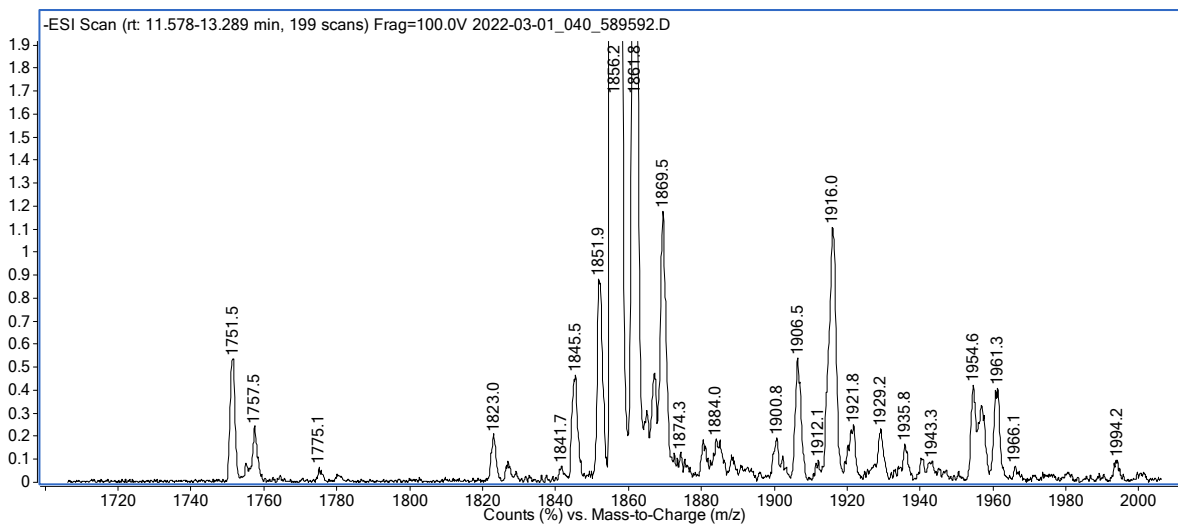


Figure S57. RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 15

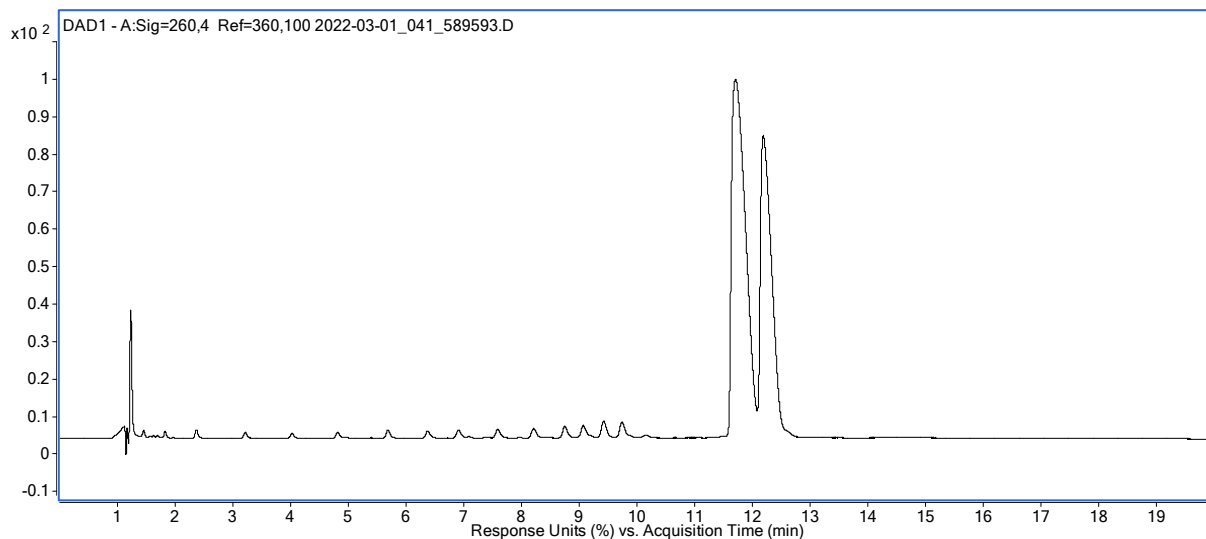


Figure S58. Zoomed in RP-UPLC UV chromatograms of DMT-on crude ASO I from Batch 15

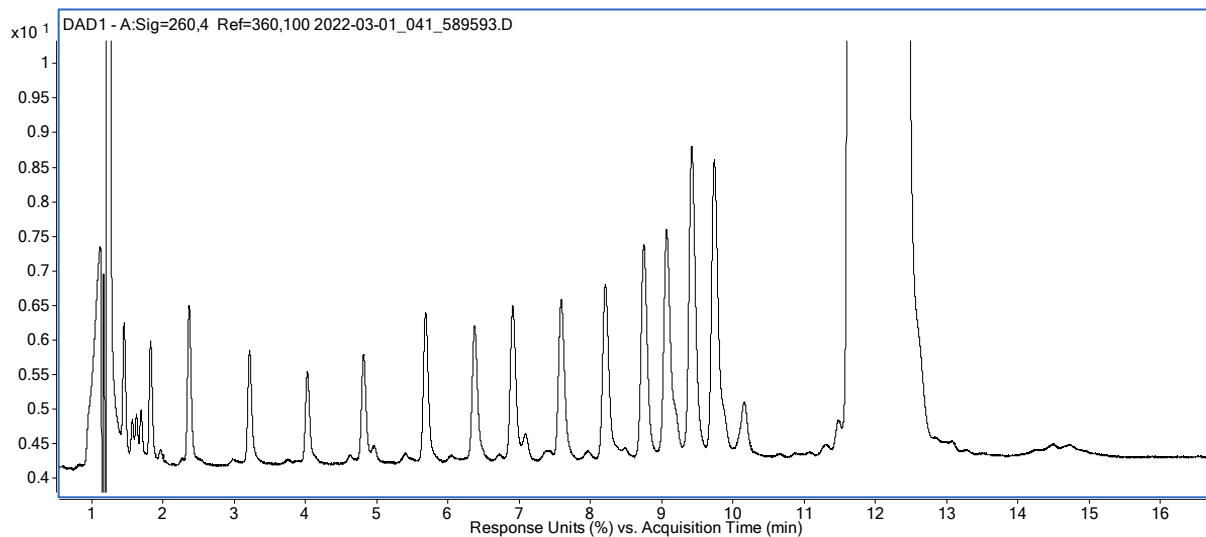


Figure S59. MS spectra of DMT-on crude ASO I from Batch 15

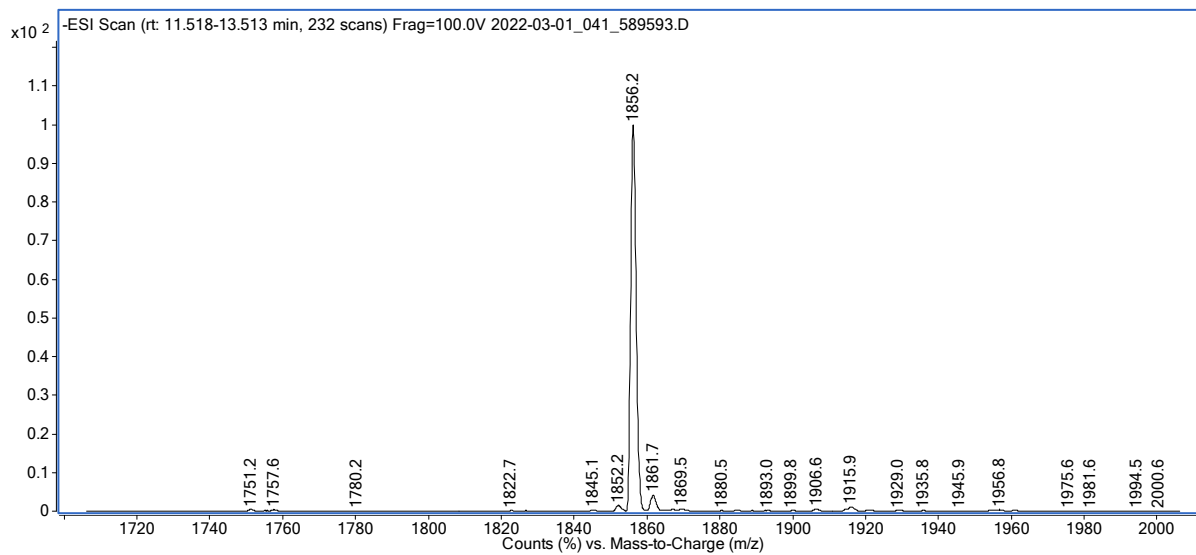


Figure S60. Zoomed in MS spectra of DMT-on crude ASO I from Batch 15

