Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004

# Supporting Information

## Mass spectrometric detection and characterization of metabolites of gemini surfactants used as gene delivery Vectors

Wei Jin[1], Randy Purves[1, 2], Ed Krol[1], Ildiko Badea[1] and Anas El-Aneed[1]*

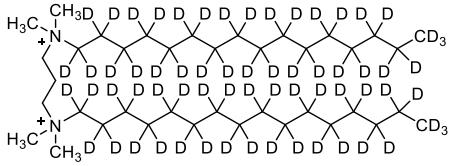
[1]Drug Design & Discovery Group, College of Pharmacy and Nutrition, University of Saskatchewan, 107 Wiggins Road, Saskatoon, SK, Canada, S7N 5E5.

[2]Centre for Veterinary Drug Residues, Canadian Food Inspection Agency, 116 Veterinary Rd, Saskatoon, SK, Canada, S7N 2R3.

*Corresponding author. E-mail address: anas.el-aneed@usask.ca (A. El-Aneed)

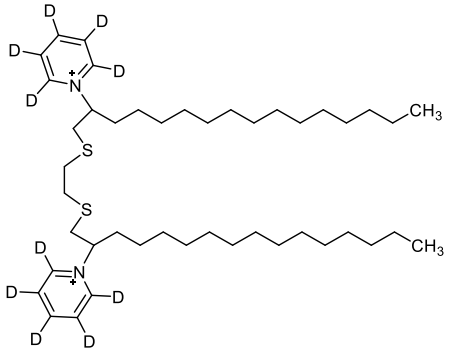
Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004

**a** **16-3-16-$D_{66}$**



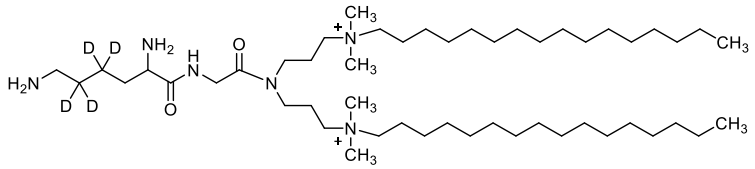
**$[M]^{2+}$** ***m/z*** **323.51 ($C_{39}H_{18}D_{66}N_2^{2+}$)**

**b** **16(Py)-S-2-S-16(Py)-$D_{10}$**



**$[M]^{2+}$** ***m/z*** **354.31 ($C_{44}H_{68}D_{10}N_2S_2^{2+}$)**

**c** **16-7N(GK)-16-$D_4$**



**$[M]^{2+}$** ***m/z*** **413.43 ($C_{50}H_{102}D_4N_6O_2^{2+}$)**

Figure S1, The structures of deuterated gemini surfactants, a) 16-3-16-$D_{66}$, b) 16(Py)-S-2-S-16(Py)-$D_{10}$, and c) 16-7N(GK)-16-$D_4$

Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004

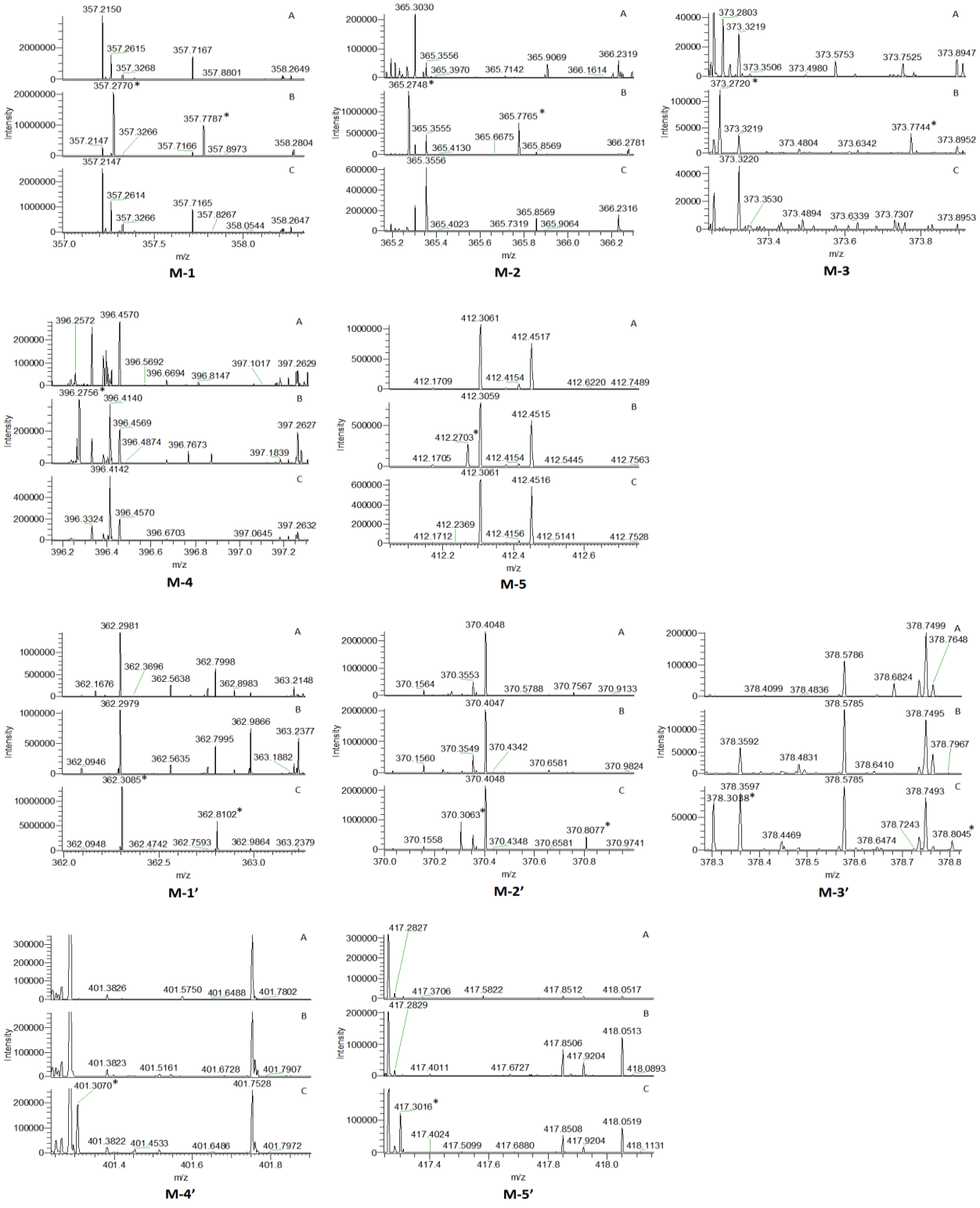



Figure S2. MS spectra of the metabolites of 16(Py)-S-2-S-16(Py) and 16(Py)-S-2-S-16(Py)-$D_{10}$ based on accurate mass measurement at 5 h of treatment. (* denotes the peak of the metabolite. panel A- control, panel B- cells

treated with 16(Py)-S-2-S-16(Py) nanoparticles, and panel C- cells treated with 16(Py)-S-2-S-16(Py)-$D_{10}$ nanoparticles. Zoomed in all three panels to show the peaks of the metabolites).

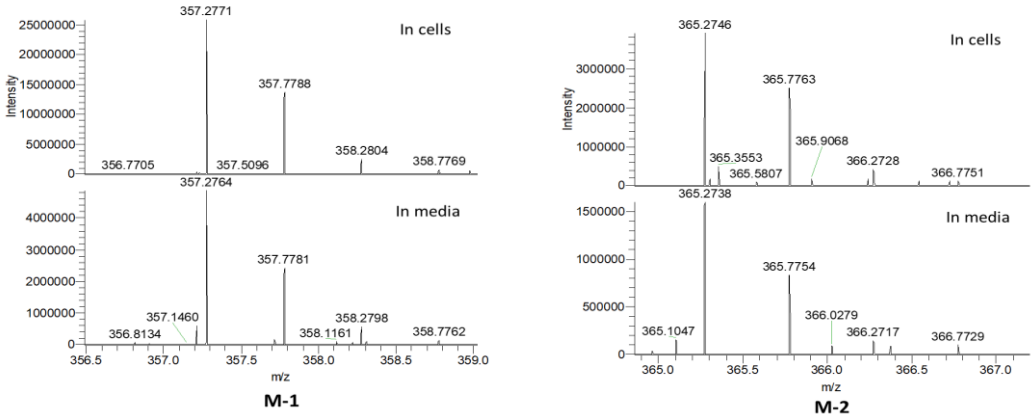



Figure S3. MS spectra of metabolite M-1 (*m/z* 357) and M-2 (*m/z* 365) of 16(Py)-S-2-S-16(Py) produced in PAM 212 cells and media at 10 h of treatment. The ion intensity of M-1 and M-2 generated by natural oxidation of 16(Py)-S-2-S-16(Py) in media is lower compared with that within cells. Ion count was 18% in the media for M-1 in comparison to treated cells while it was 41% for M-2. This indicates that oxidation metabolism occurred in cells (i.e, biological system).

Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004

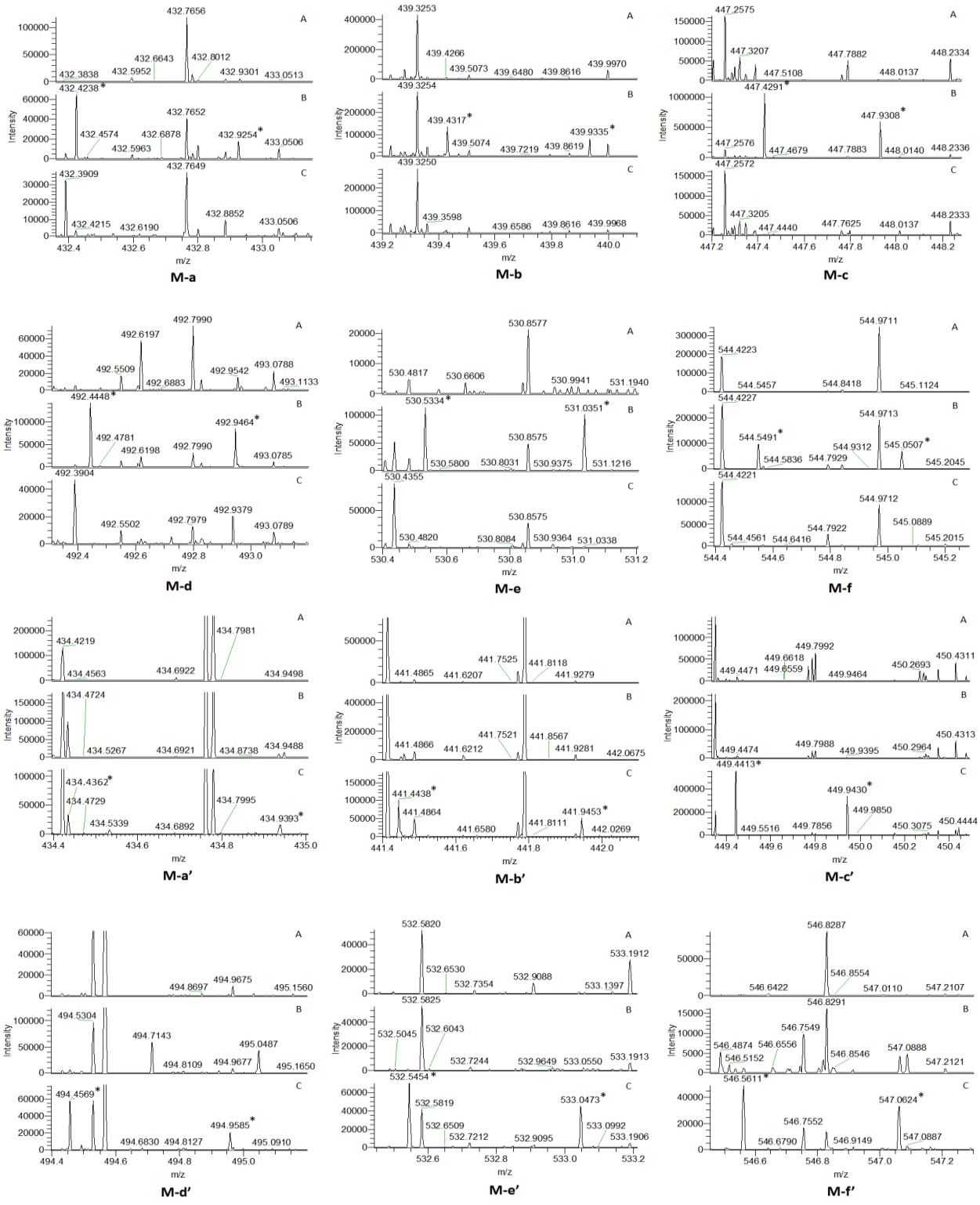
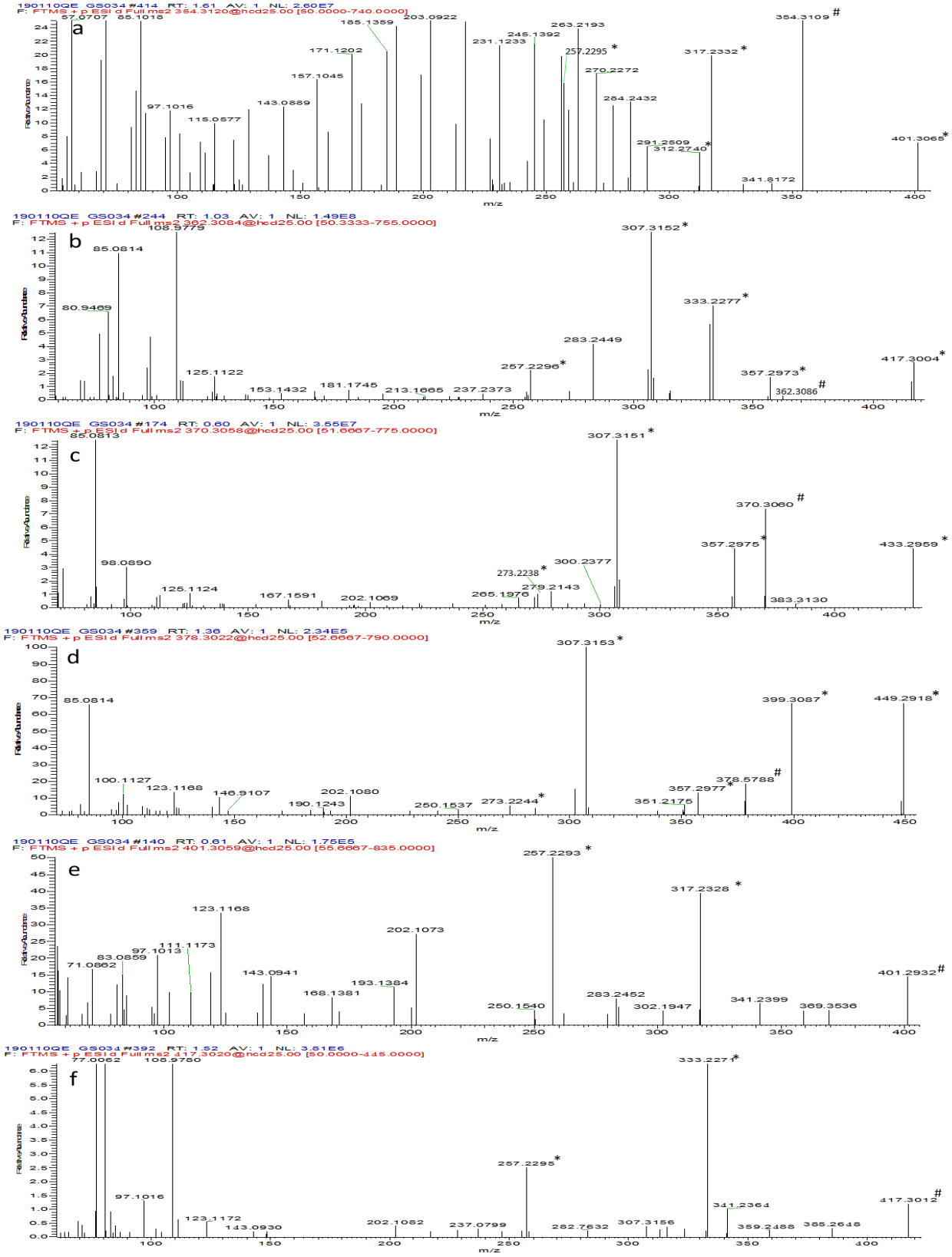



Figure S4. MS spectra of the metabolites of 16-7N(GK)-16 and 16-7N(GK)-16-$D_4$ based on accurate mass measurement at 5 h treatment. (* denotes the peaks of the identified metabolites. panel A- control, panel B-

Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004

cells treated with 16-7N(GK)-16 nanoparticles, and panel C- cells treated with 16-7N(GK)-16-$D_4$ nanoparticles. Zoomed in to show the peaks of the metabolites).

Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004



Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004

Figure S5. The MS/MS spectra of 16(Py)-S-2-S-16(Py)-$D_{10}$ and its metabolites: a) 16(Py)-S-2-S-16(Py)-$D_{10}$, b) M-1', c) M-2', d) M-3', e) M-4', and f) M-5' (# denotes the parent ion and * denotes the diagnostic product ion).

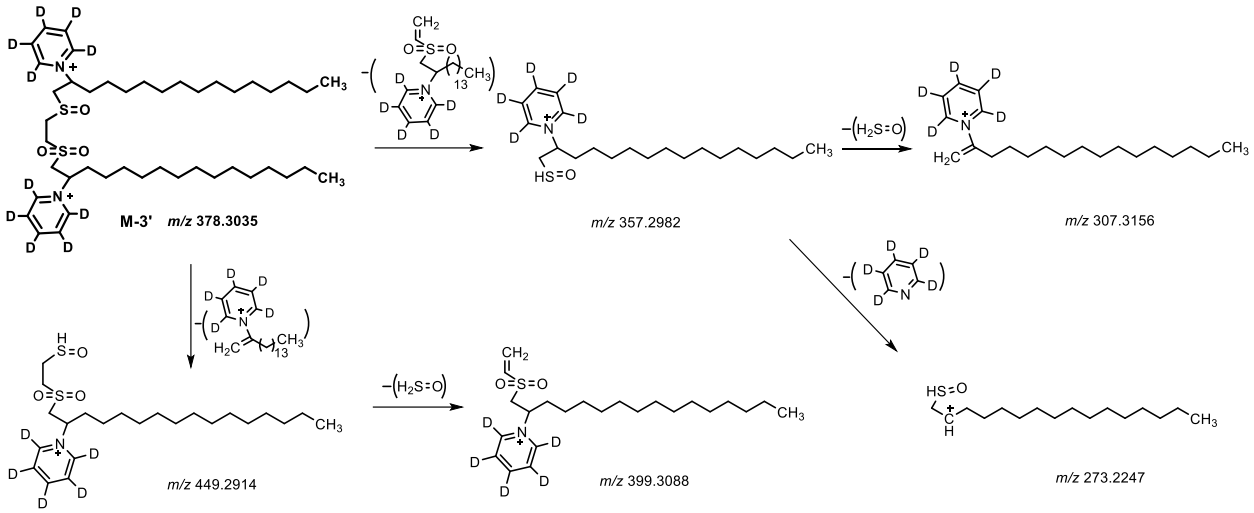



Figure S6. The proposed structures of major product ions of metabolite M-3' of 16(Py)-S-2-S-16(Py)-$D_{10}$,

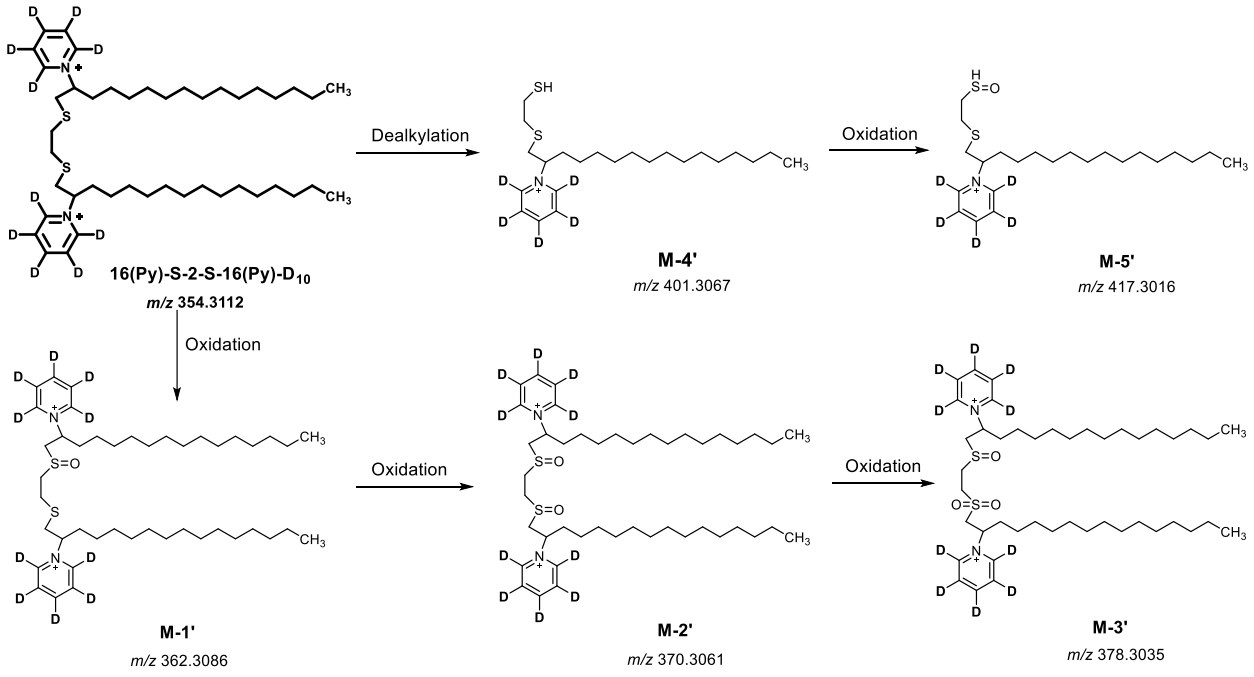
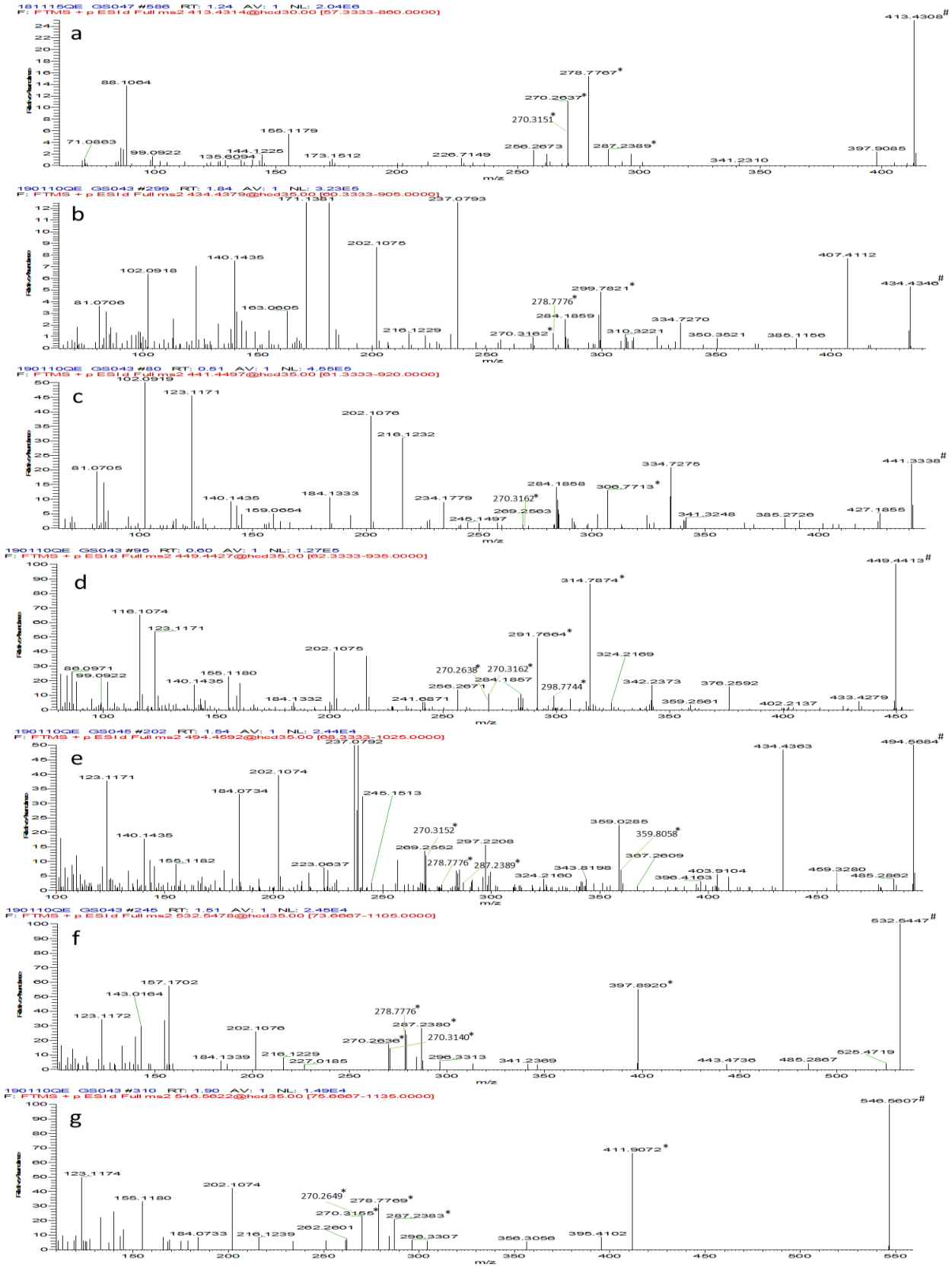



Figure S7. The metabolites of 16(Py)-S-2-S-16(Py)-$D_{10}$ and its proposed metabolic pathway.

Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004

Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004



Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004

Figure S8. The MS/MS spectra of 16-7N(GK)-16-$D_4$ and its metabolites: a) 16-7N(GK)-16-$D_4$, b) M-a', c) M-b', d) M-c', e) M-d', f) M-e', and g) M-f'. (# denotes the parent ions and * denotes the diagnostic product ions).

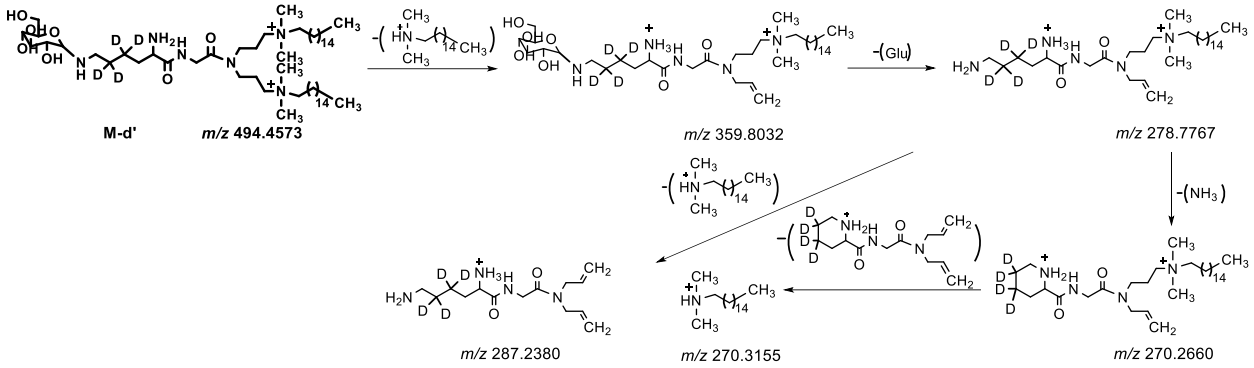
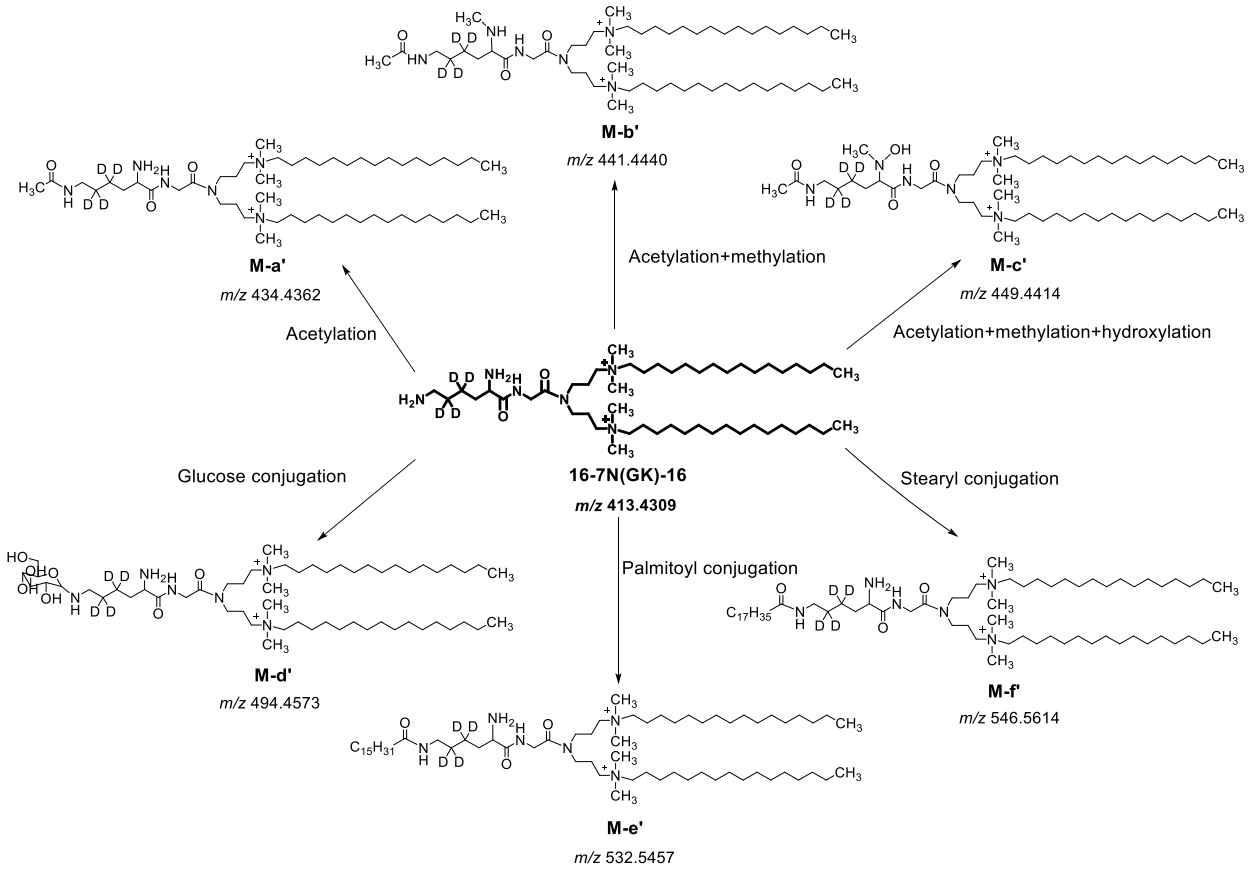



Figure S9. The proposed structures of major product ions of the metabolite M-d' of 16-7N(GK)-16-$D_4$



Supporting information to the original publication titled: Mass Spectrometric Detection and Characterization of Metabolites of Gemini Surfactants Used as Gene Delivery Vectors. J. Am. Soc. Mass Spectrom. 2020, 31, 2, 366-378. doi.org/10.1021/jasms.9b00004

Figure S10. The metabolites of 16-7N(GK)-16-$D_4$ and its proposed metabolic pathway.

Table S1. Potential metabolites based on accurate mass measurements for the gemini surfactants 16(Py)-S-2-S-16(Py)-$D_{10}$ and 16-7N(GK)-16-$D_4$.

| **Name** | **Molecular Formula** | **Exact Mass (*m/z*)** | **Observed Mass (*m/z*)** | **Mass Accuracy (ppm)** | **Metabolic Reactions** |
|---|---|---|---|---|---|
| 16(Py)-S-2-S-16(Py)-$D_{10}$ | $C_{44}H_{68}D_{10}N_2S_2^{2+}$ | 354.3112 | 354.3115 | 0.8 | NA |
| M-1’ | $C_{44}H_{68}D_{10}N_2OS_2^{2+}$ | 362.3086 | 362.3085 | 0.3 | Oxidation |
| M-2’ | $C_{44}H_{68}D_{10}N_2O_2S_2^{2+}$ | 370.3061 | 370.3063 | 0.5 | Oxidation |
| M-3’ | $C_{44}H_{68}D_{10}N_2O_3S_2^{2+}$ | 378.3035 | 378.3038 | 0.8 | Oxidation |
| M-4’ | $C_{23}H_{37}D_5NS_2^{+}$ | 401.3067 | 401.3070 | 0.7 | Dealkylation |
| M-5’ | $C_{23}H_{37}D_5NOS_2^{+}$ | 417.3016 | 417.3016 | 0.0 | Dealkylation, Oxidation |
| 16-7N(GK)-16-$D_4$ | $C_{50}H_{102}D_4N_6O_2^{2+}$ | 413.4309 | 413.4307 | 0.5 | NA |
| M-a’ | $C_{52}H_{104}D_4N_6O_3^{2+}$ | 434.4362 | 434.4362 | 0.0 | Acetylation |
| M-b’ | $C_{53}H_{106}D_4N_6O_3^{2+}$ | 441.4440 | 441.4438 | 0.5 | Acetylation, Methylation |
| M-c’ | $C_{53}H_{106}D_4N_6O_4^{2+}$ | 449.4414 | 449.4413 | 0.2 | Acetylation, Methylation, Hydroxylation |
| M-d’ | $C_{56}H_{112}D_4N_6O_7^{2+}$ | 494.4573 | 494.4569 | 0.8 | Glucose conjugation |
| M-e’ | $C_{66}H_{132}D_4N_6O_3^{2+}$ | 532.5457 | 532.5454 | 0.6 | Palmitoyl conjugation |
| M-f’ | $C_{68}H_{136}D_4N_6O_3^{2+}$ | 546.5614 | 546.5611 | 0.5 | Stearyl conjugation |