Electronic Supplementary Information (ESI) for the article entitled:

Validation of the 2,3-dihydroxi-propionyl group in selenium speciation by chemical synthesis and LC-MS analyses

by

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SM – Table 1 HPLC instrumental parameters

Method	Column	Eluents	Gradient	Flow rate	Injection	Other
preparative scale RP-HPLC- UV	Agilent Zorbax XDB-C8 5 µm. 9.4x250 mm	A: H_2O B: ACN	0-5 min:50% B 5-25 min: ↑ 100% B 25-40 min: 100% B 40-41 min: ↓ 50%B 41-50 min: 50% B	2.0 ml/min	100 µl	detection at 220 nm
preparative scale SAX-HPLC- ICP-MS	Agilent Zorbax SAX 5 µm. 9.4x250 mm	A: 10 mmol pH=5.0 ammonium acetate B: 250 mmol pH=5.0 ammonium acetate	0-3 min: 0% B 3-20 min: ↑ 100% B 20-40 min: 100% B 40-42 min: ↓ 0% B 42-50 min: 0% B	5.0 ml/min	100 µl	flow rate divided to 4 ml/min and 1 ml/min. Only 1 ml/min entering ICP-MS
preparative scale RP-HPLC- ICP-MS	Agilent Zorbax XDB-C8 5 µm. 9.4x250 mm	A: 0.1 V/V % HCOOH in H_2O B: ACN	0-10 min: 3% B	3.0 ml/min	100 µl	flow rate divided to 2.4 ml/min and 0.6 ml/min. Only 0.6 ml/min entering ICP-MS
analytical scale RP-HPLC- ESI-QTOF-MS	Agilent Zorbax XDB-C18 3.5 µm. 2.1x50 mm	A: 0.1 V/V % HCOOH in H_2O B: 0.1 V/V % HCOOH in ACN	0-5 min:5% B 5-12 min: ↑ 95% B 12-14 min: 95% B 14-14.5 min: ↓ 5% B	350 µl/min	10 µl	
analytical scale SAX-HPLC- ICP-MS	Hamilton PRPX-100 10 µm. 4.1x250 mm	A: 10 mmol pH=5.0 ammonium acetate B: 250 mmol pH=5.0 ammonium acetate	0-5 min: 0% B 5-15 min: ↑ 100% B 15-35 min: 100% B 35-36 min: ↓ 0% B 36-42 min: 0% B	1.5 ml/min	100 µl	

SM – Table 2 Instrumental parameters of the ESI-QQQMS set-up.

Qtrap 3200 triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems)	
ESI source	Turbo V interface and Turbo ion Spray probe
Operation mode	Negative
Ion spray voltage. V	-4500
Curtain gas (nitrogen). psi	15
Ion source gas. psi	10
Turbo gas. spi	10
Desolvation temperature. °C	30
Collision activated dissociation gas. a. u.	10
Declustering potential. eV	55
Full scan recording range. m/z	100-1100
MS/MS recording range. m/z	50-1100

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SM – Table 3 Instrumental parameters of the HPLC-ESI-QTOFMS set-up.

6530 Accurate Mass QTOF LC-MS (Agilent)	
ESI source	Dual ESI (Agilent)
Operational mode	positive
Precursor ion isolation in MS/MS mode	medium (4 m/z)
Mass accuracy in MS mode	< 2 ppm
Mass resolution	> 10000
Detection frequency	4 GHz
Fragmentor voltage	150 V
Curtain voltage	65 V
Drying gas	13 L/min
Capillary voltage	800 V
Nebulizer pressure	40 psig
Gas temperature	325 ºC
Data analysis software	Mass Hunter Acquisition B.02.01(B211630) with SP3 Mass Hunter Qualitative Analysis B.03.01 (Build 3.1.346.0) with SP3

SM – Table 4 Mass accuracy information of the four synthesized selenium species (in bold) and their MS/MS fragments.

	measured	calculated	ppm difference
$[C_{16}H_{27}N_4O_8SSe]^+$	**563.0555**	**563.0562**	**-1.31**
$[C_8H_{13}O_4N_2SeS]^+$	312.9720	312.9761	-13.2
$[C_6H_{10}NO_5Se]^+$	255.9709	255.9719	-3.83
$[C_8H_{11}O_4N_2S]^+$	231.0438	231.0429	4.03
$[C_3H_6NO_2Se]^+$	167.9548	167.9558	-6.25
$[C_5H_8NO_3]^+$	130.0490	130.0498	-6.15
$[C_{13}H_{21}N_4O_8SSe]^+$	**475.0396**	**475.0400**	**-0.91**
$[C_{11}H_{18}N_3O_6SeS]^+$	400.0027	400.0075	-11.9
$[C_8H_{16}N_3O_5SeS]^+$	345.9925	345.9970	-13.0
$[C_5H_9N_2O_3SSe]^+$	256.9455	256.9499	-17.1
$[C_8H_{11}N_2O_4S]^+$	231.0398	231.0434	-15.8
$[C_3H_6NO_2Se]^+$	167.9532	167.9558	-15.8
$[C_5H_8NO_3]^+$	130.0480	130.0498	-13.5
$[C_{12}H_{21}N_2O_{10}Se_2]^+$	**512.9534**	**512.9530**	**0.78**
$[C_7H_{10}N_2O_5Se_2]^+$	361.9084	361.9046	10.5
$[C_6H_{10}NO_5Se_2]^+$	335.8921	335.8889	9.41
$[C_6H_8NO_4Se_2]^+$	317.8837	317.8784	16.7
$[C_6H_{10}SeNO_5]^+$	255.9738	255.9719	7.42
$[C_3H_6NO_2Se]^+$	167.9573	167.9558	8.93
$[C_9H_{17}N_2O_7Se_2]^+$	**424.9361**	**424.9368**	**-1.72**
$[C_6H_{10}NO_5Se_2]^+$	335.8910	335.8889	6.13
$[C_6H_8NO_4Se_2]^+$	317.8810	317.8784	8.24
$[C_6H_{10}SeNO_5]^+$	255.9721	255.9719	0.78
$[C_3H_6NO_2Se_2]^+$	247.8729	247.8740	-4.60
$[C_3H_6NO_2Se]^+$	167.9561	167.9558	1.79

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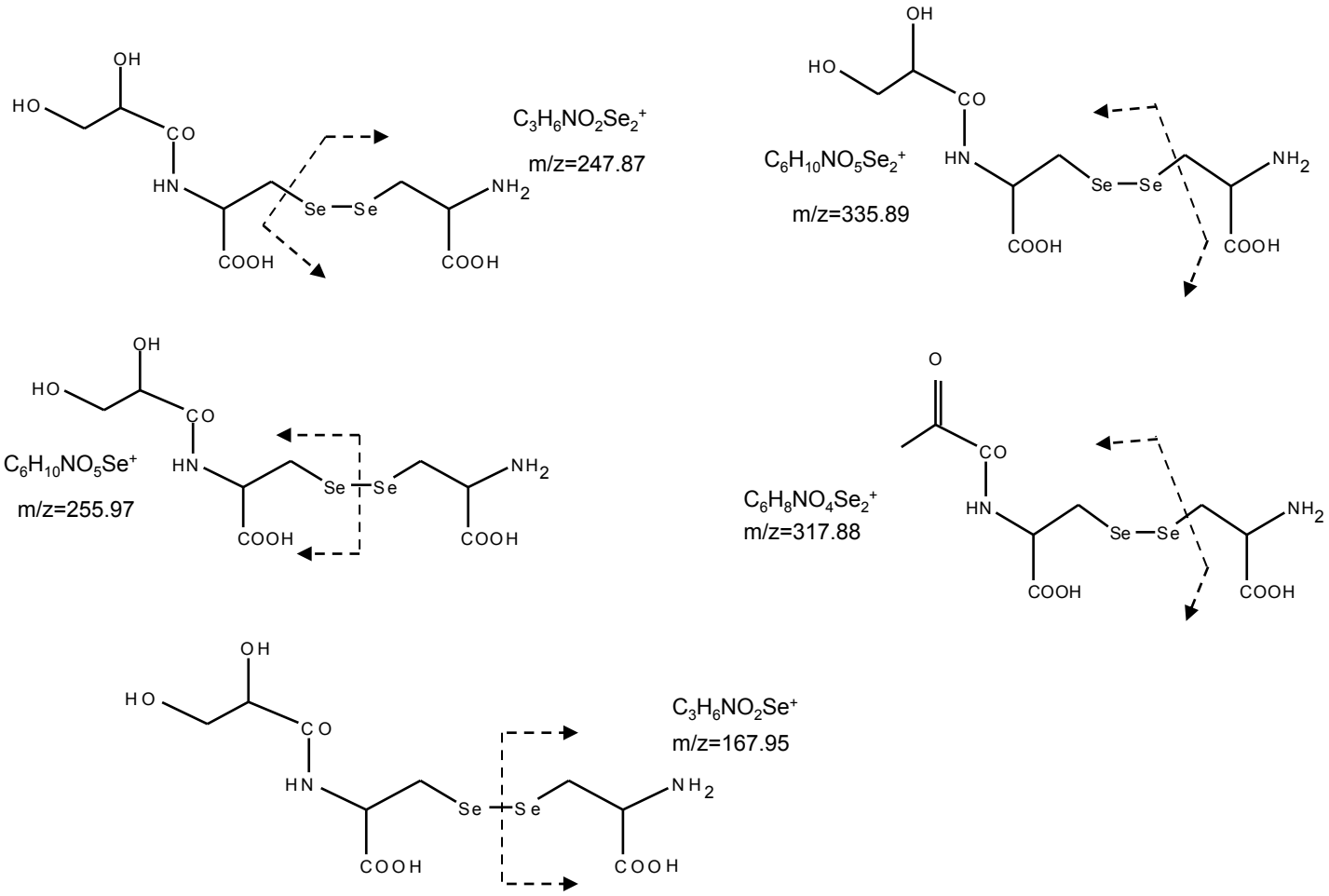
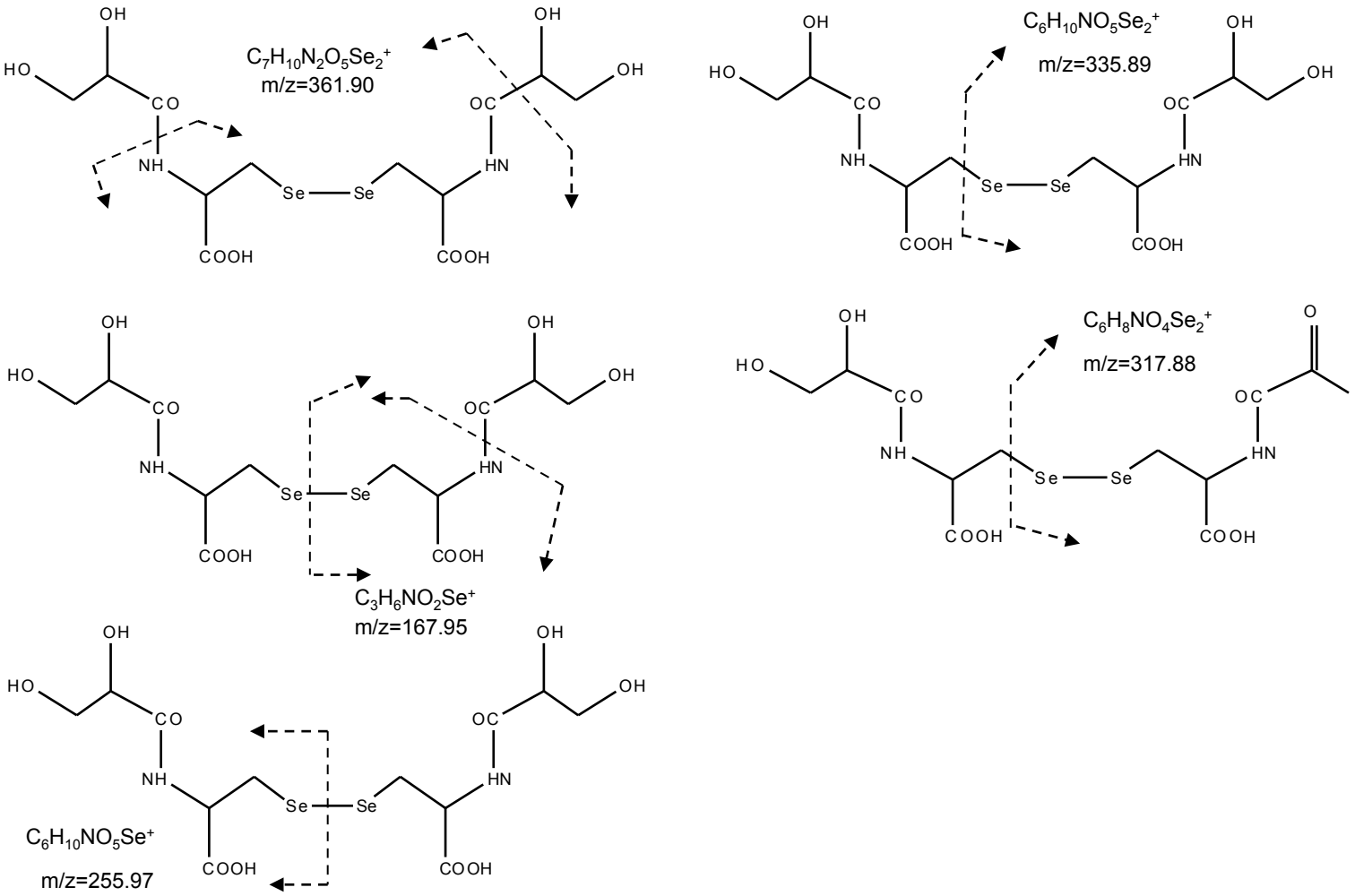
SM – Figure 1 Proposed fragmentation mechanisms of the conjugate of 2,3-DHP-Sec and Sec (m/z 424).



SM – Figure 2 Proposed fragmentation mechanisms of di-N-2,3-DHP-Sec (m/z 512).



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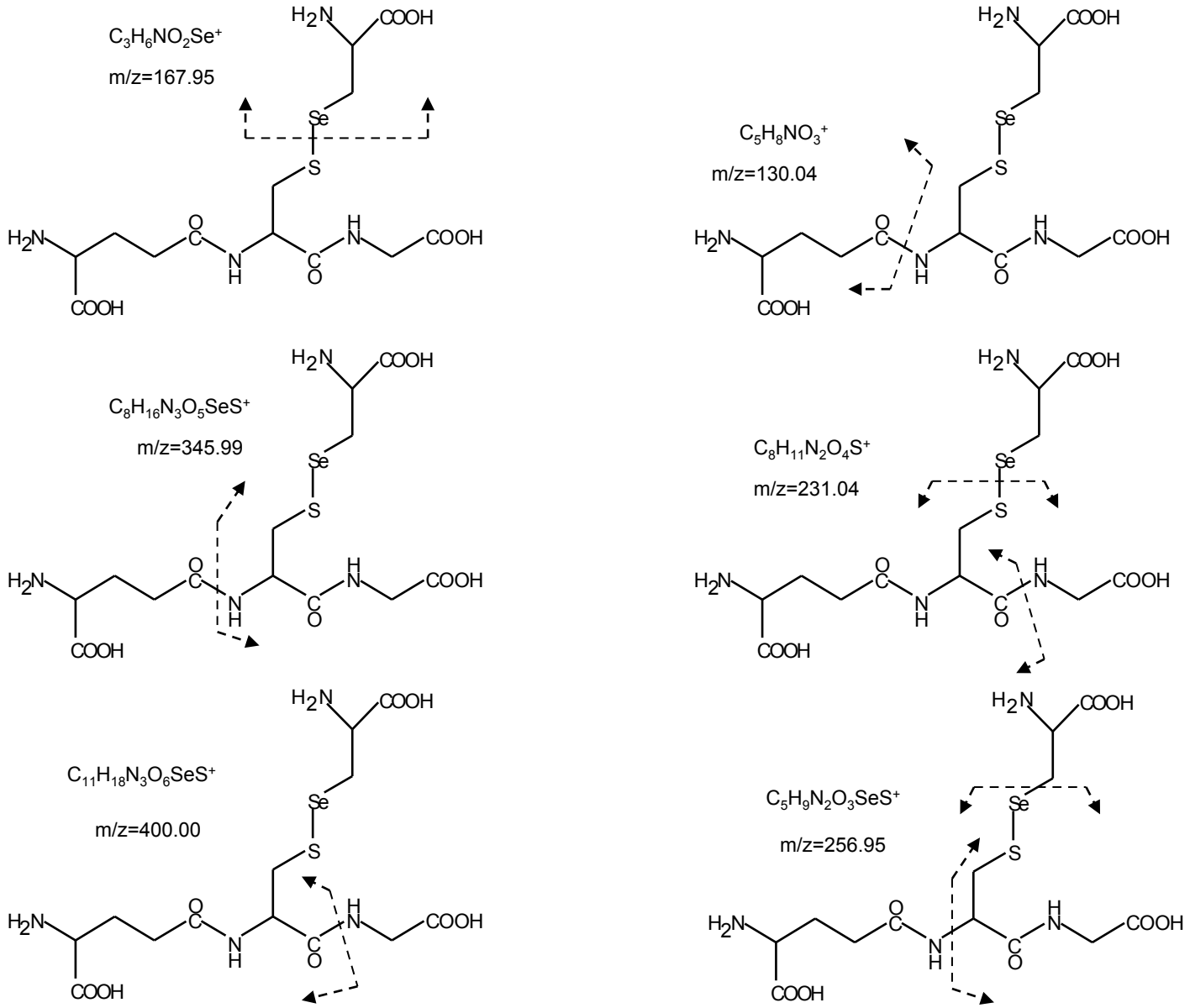
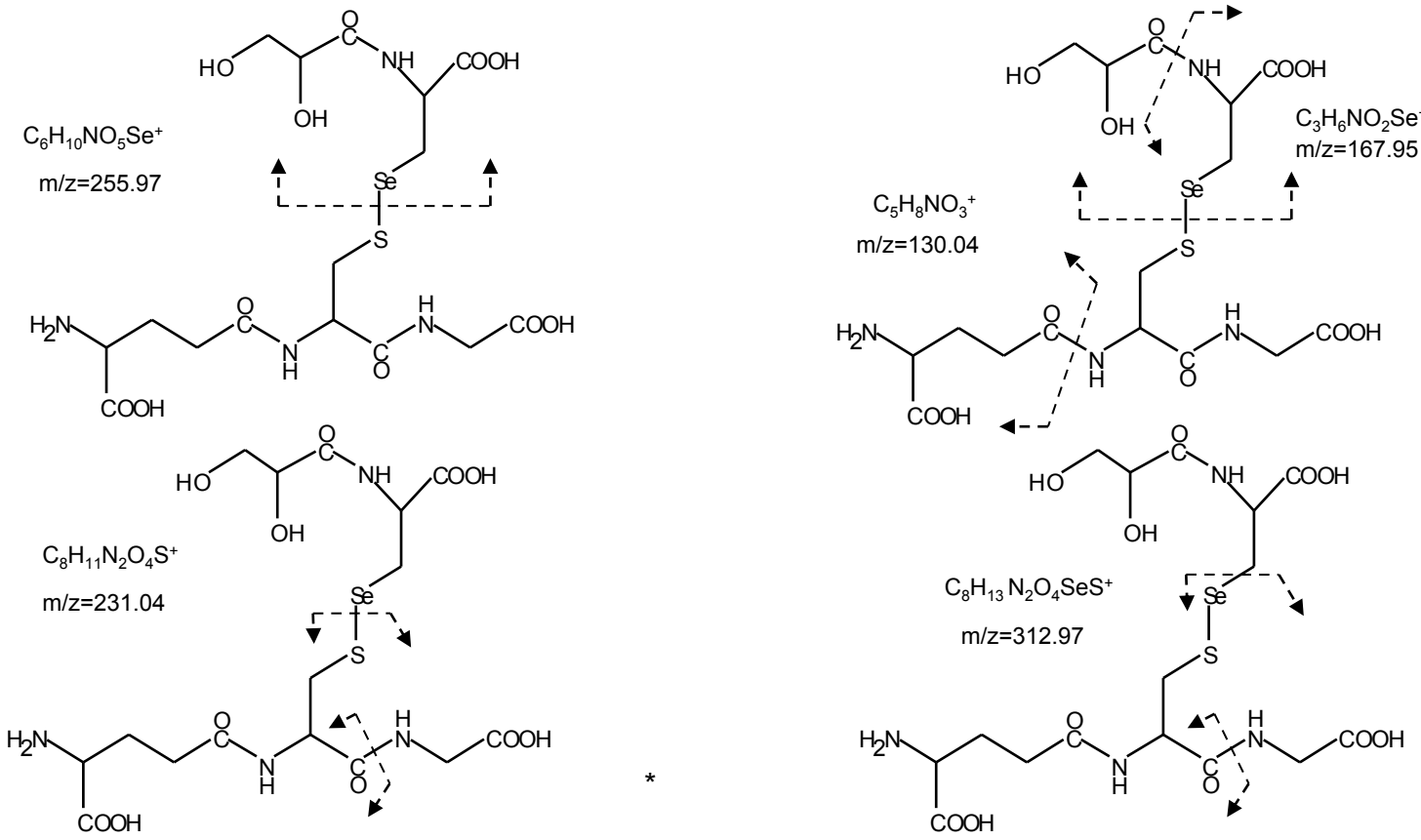
SM – Figure 3 Proposed fragmentation mechanisms of the conjugate of Sec and glutathione (m/z 475).



SM – Figure 4 Proposed fragmentation mechanisms of the conjugate of 2,3-DHP-Sec and glutathione (m/z 563).



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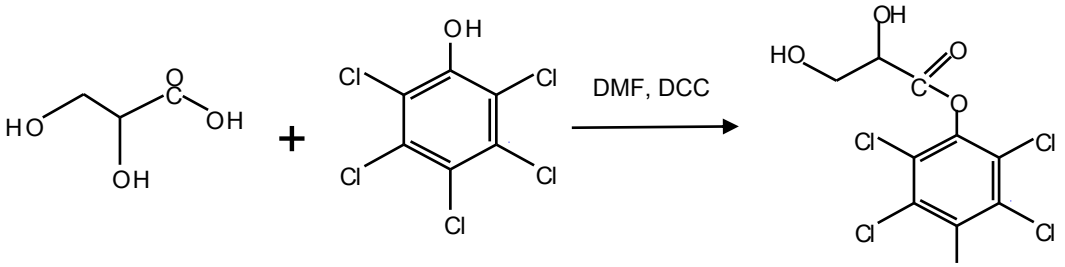
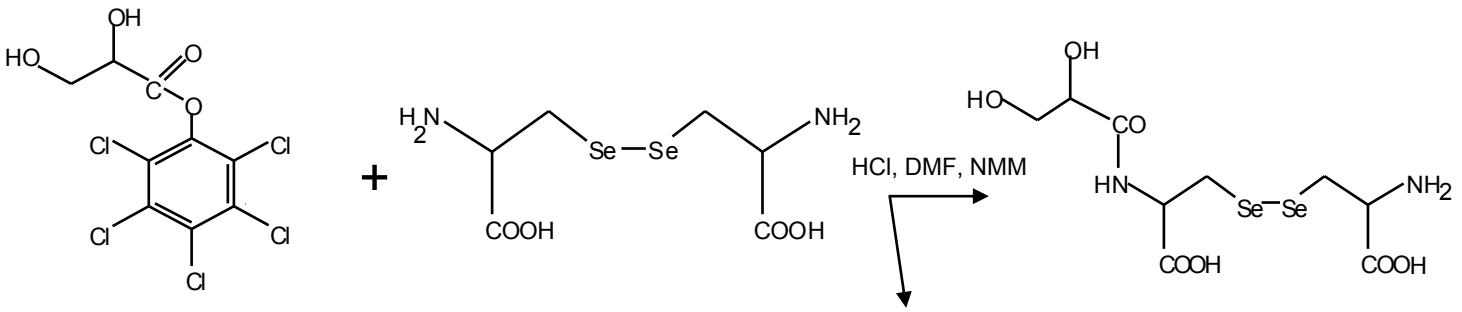
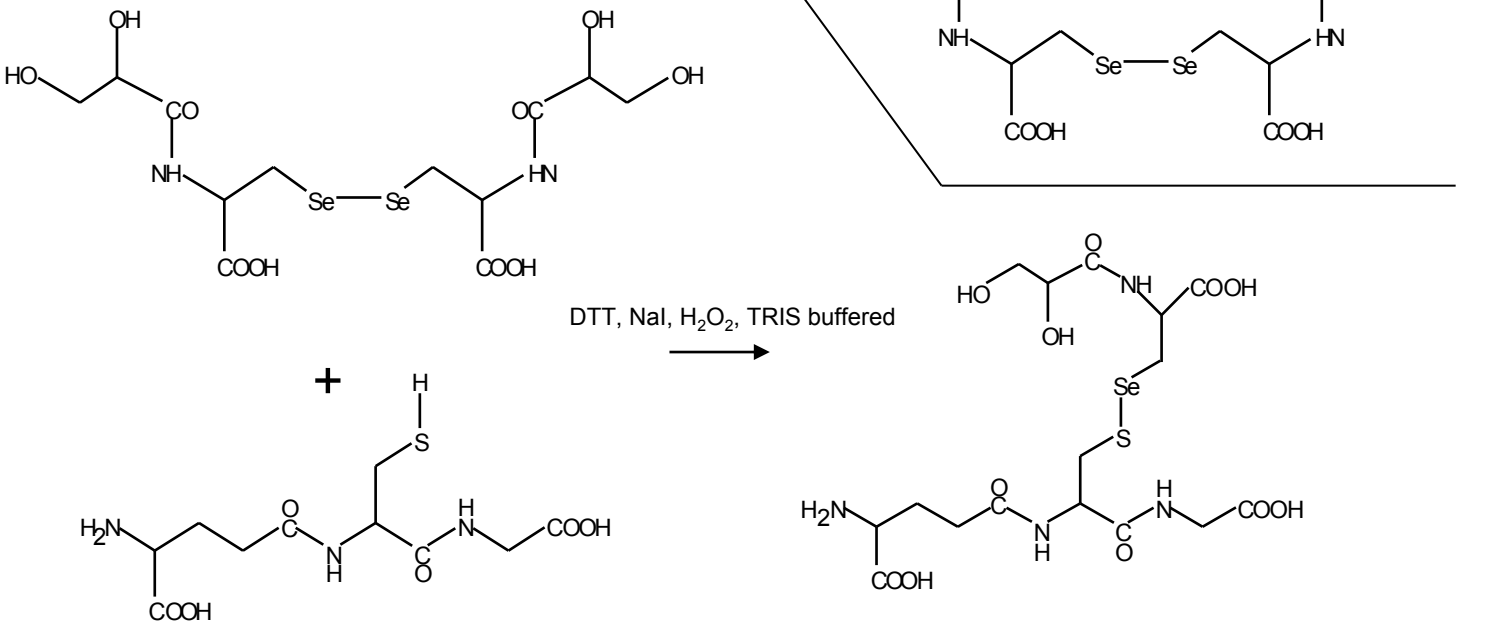
SM – Figure 5 Pathways of syntheses.



Activation of glyceric acid with pentachlorophenol

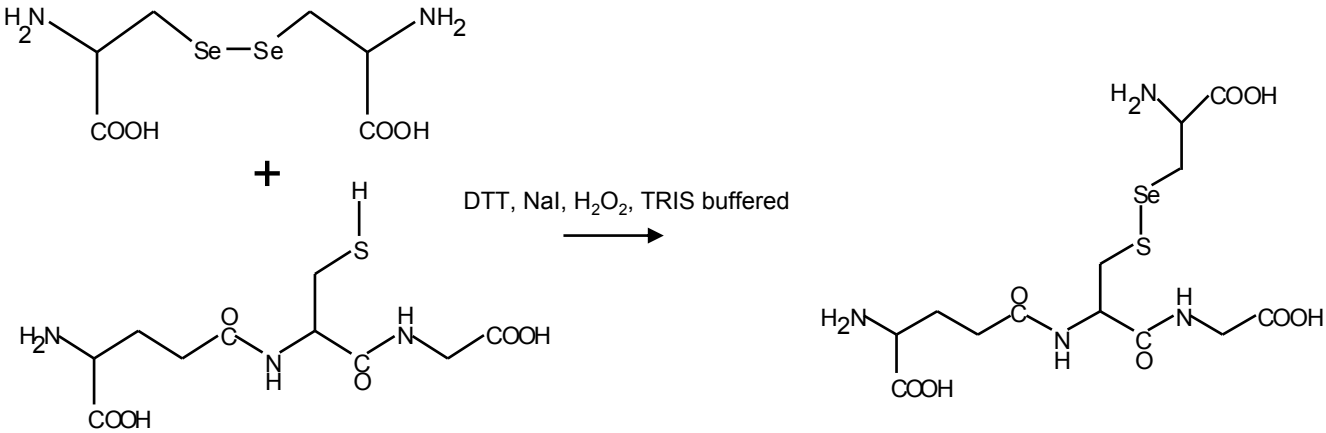


Coupling activated glyceric acid with selenocystine



Coupling reduced glutathione with di-N-2,3-DHP-Sec

Coupling reduced glutathione with selenocystine



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