Supporting Online Material for:

Enzymatic basis of ribosomal peptide prenylation in cyanobacteria

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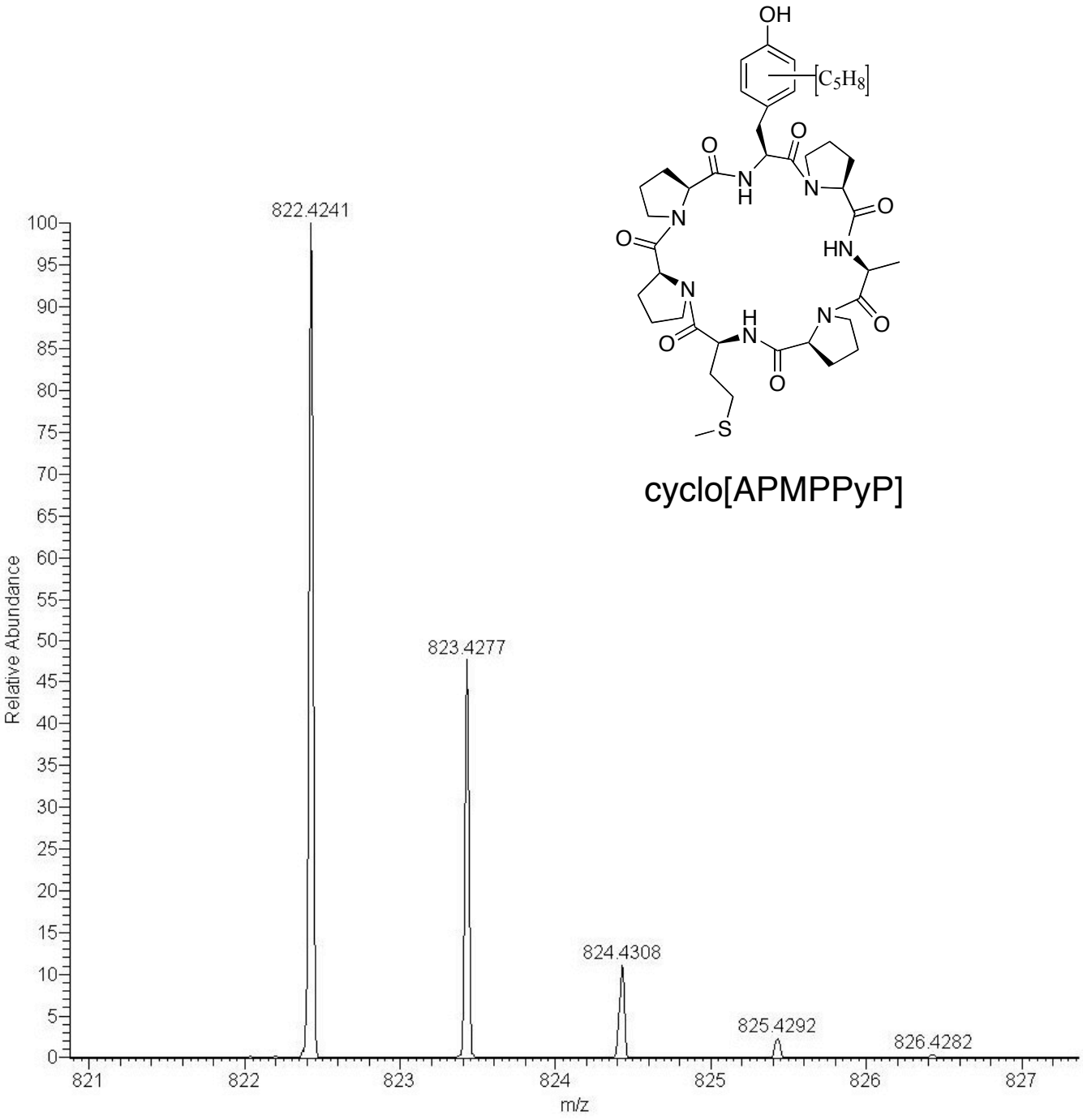
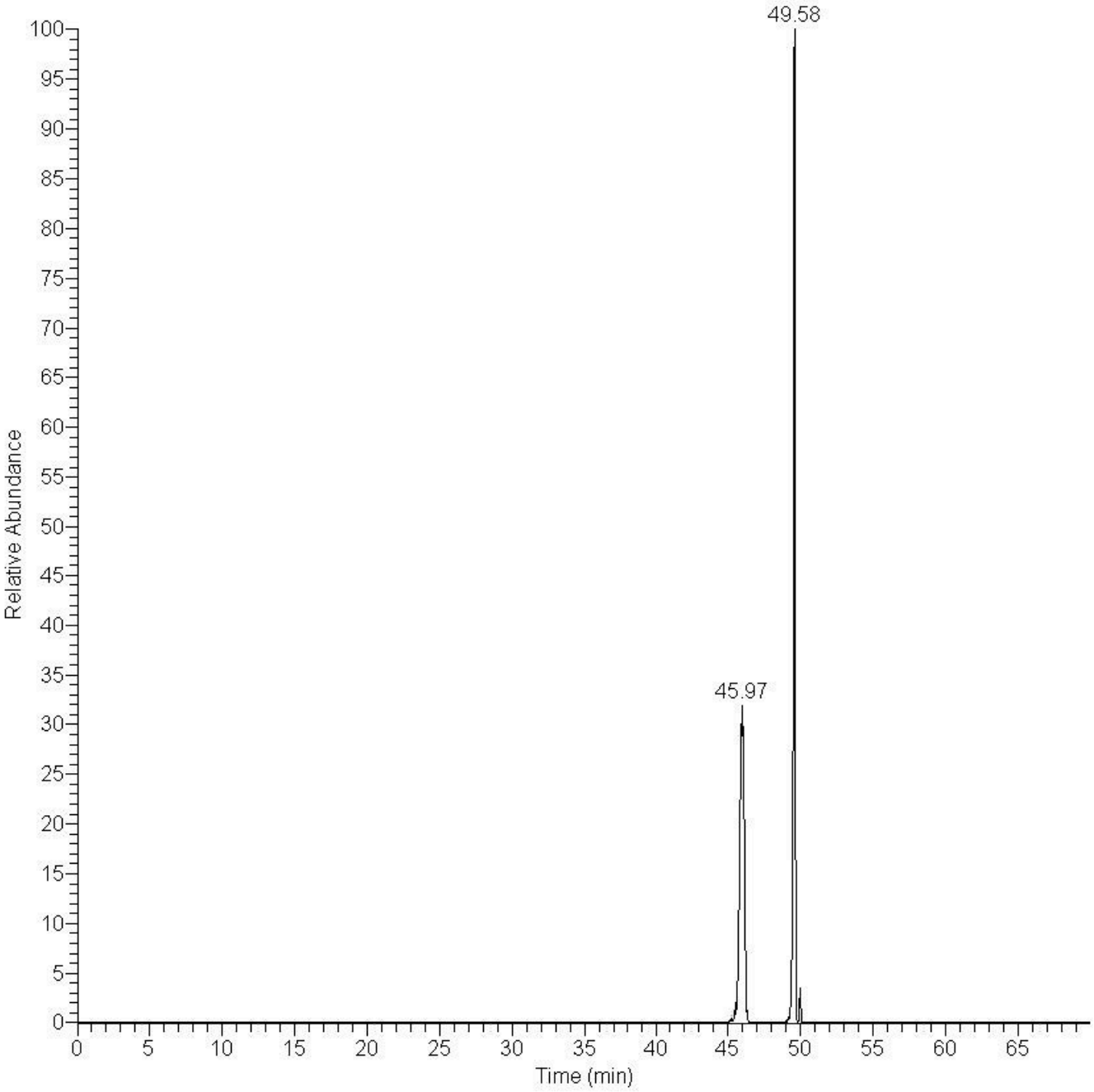
Figure S1. LC-FT-ICR and MS-MS, or ESI-MS characterization of products of reactions containing: (a-d) cyclo[APMPPYP] (e-h) APMPPYP (i-l) cyclo[APMPPYPAPMPPP] (m-p), (q-t) cyclo[APMPPYPAPMPPYP] (u-x) cyclo[KKPYILP] (y-bb) cyclo[KPYILP] (cc-ff) KPYILP (gg-hh) boc-L-Tyr (ii) N-acetyl-L-Tyr. Note that prenylated tyrosine is denoted by a lower-case 'y', while unmodified tyrosine is denoted by an upper-case 'Y'

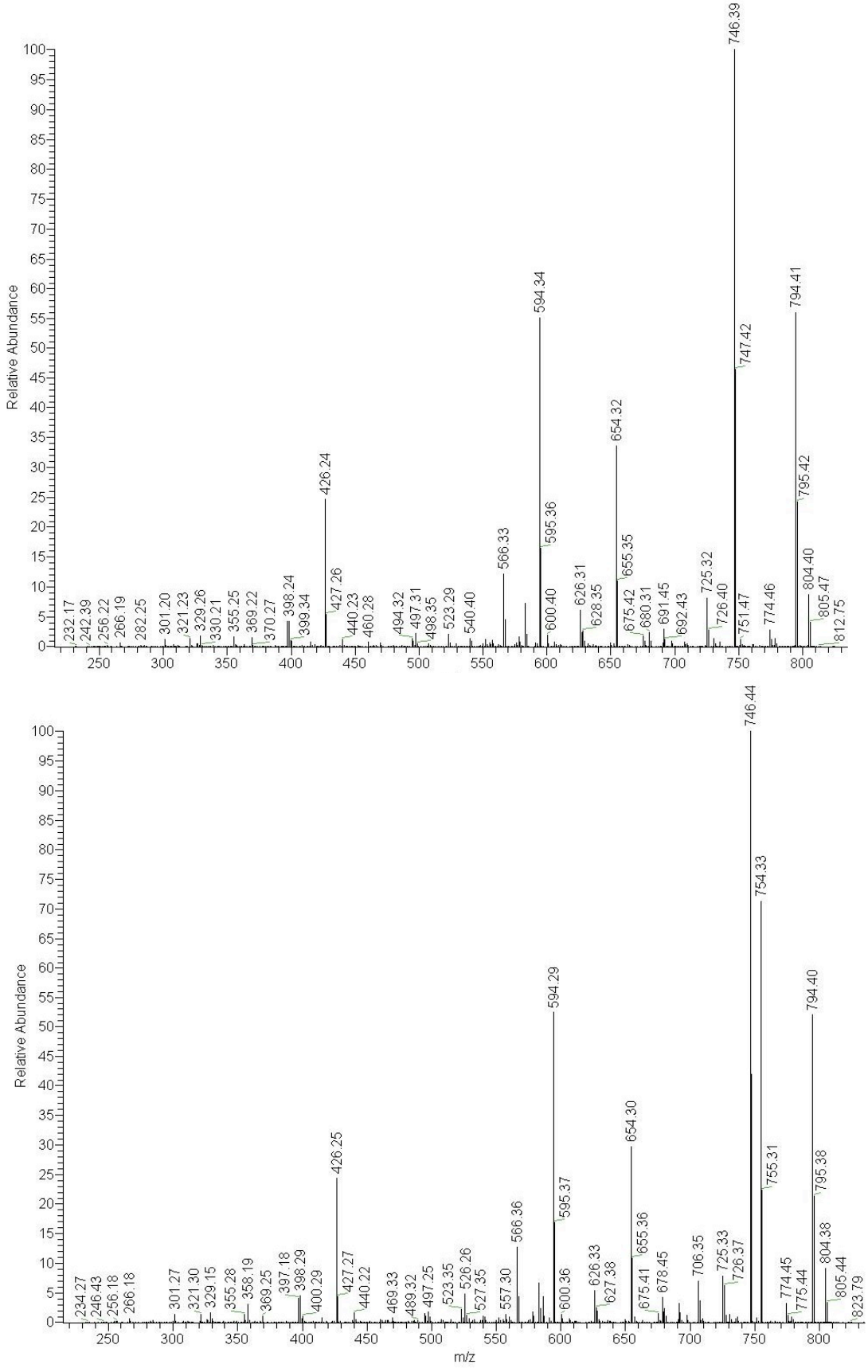
(a) FT-ICR data showing ion whose mass (+/- 2 ppm) corresponds to prenylated cyclo[APMPPYP]



(b) LC-FT-ICR chromatogram selected for mass of prenylated cyclo[APMPPYP]



(c) MS-MS spectra for early (top) and late (bottom) eluting products of the reaction of LynF with cyclo[APMPPYP] (see chromatogram on previous page)

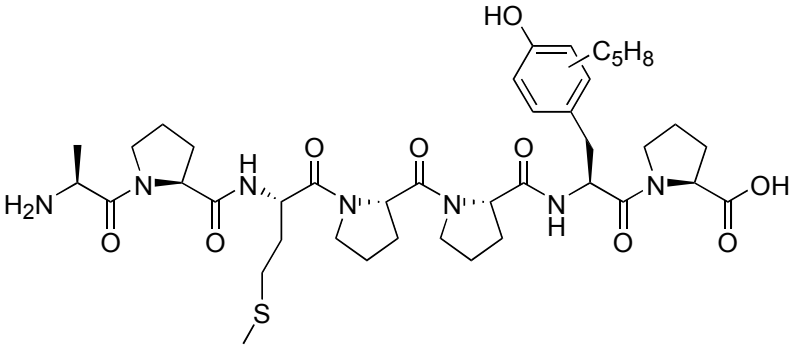


(d) MS-MS assignments for prenylated cyclo[APMPPYP] early (top) and late (bottom) eluting isomers

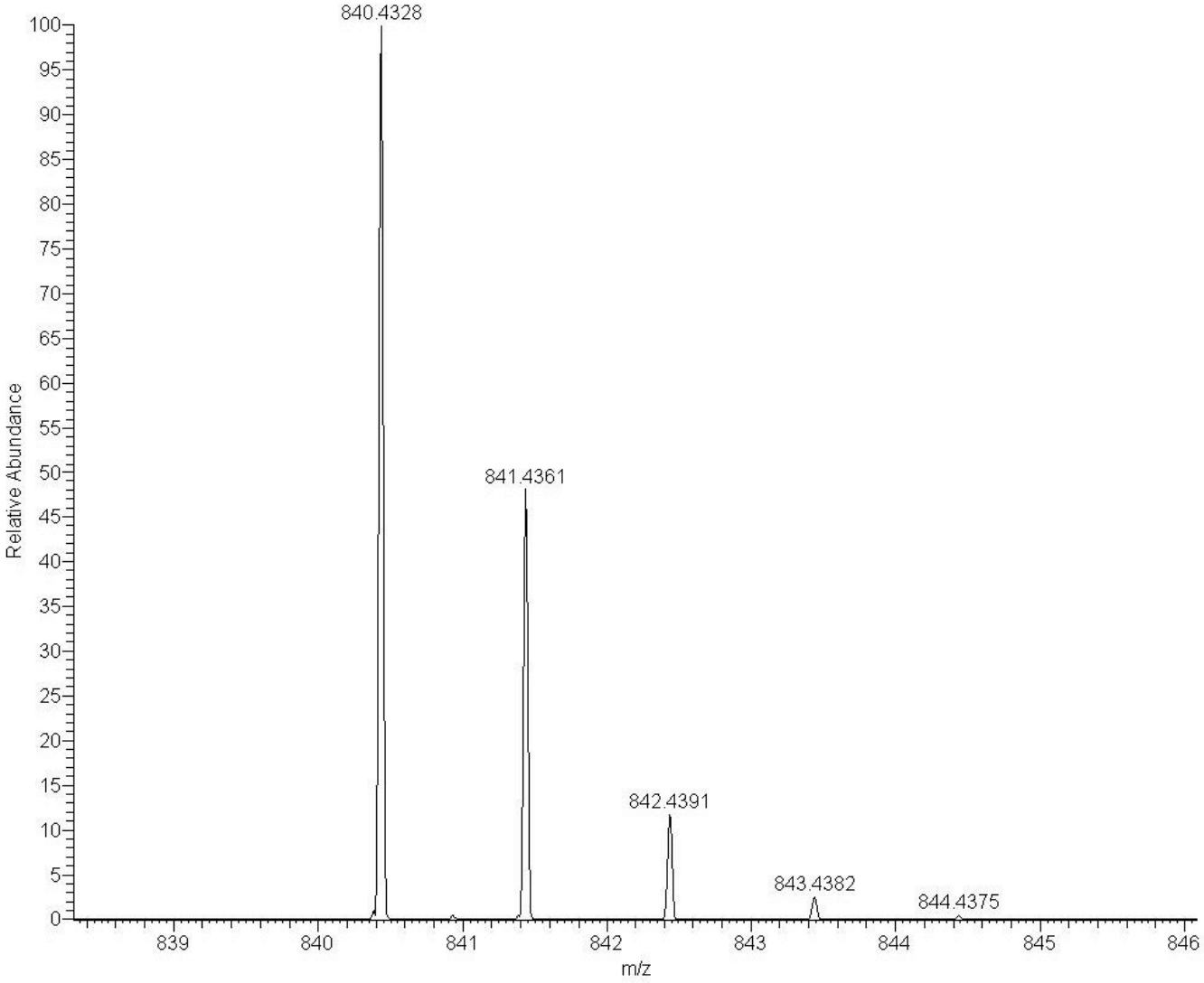
cyclo[APMPPYP] 1st peak (C-prenyl)					
y- and b- cleavages (+1)	Expected	Observed	y- and b- cleavages (+1)	Expected	Observed
AP	169.10	-	yP	329.19	329.26
APM	300.14	-	yPA	400.22	400.29
APMP	397.19	397.16	yPAP	497.28	497.31
APMPP	494.24	494.32	yPAPM	628.32	628.35
APMPPy	725.37	725.32	yPAPMP	725.37	725.32
PM	229.10	-	PA	169.10	-
PMP	326.15	-	PAP	266.15	266.19
PMPP	423.21	-	PAPM	397.19	-
PMPPy	654.33	654.32	PAPMP	494.24	494.32
PMPPyP	751.39	751.47	PAPMPP	591.30	591.35
MP	229.10	-			
MPP	326.15	-	Miscellaneous Ions		
MPPy	557.28	557.43	-H2O	804.41	804.40
MPPyP	654.33	654.32	-Acylium (C=O)	794.42	794.41
MPPyPA	725.37	725.32	-Methyl sulfide	774.42	774.46
PP	263.18	-	-Met side-chain	746.39	746.39
PPy	426.24	426.24	PyPAP y- a- cleavage	566.33	566.33
PPyP	523.29	523.29			
PPyPA	594.33	594.34			
PPyPAP	691.38	691.45			
Py	329.19	329.26			
PyP	426.24	426.24			
PyPA	497.28	497.31			
PyPAP	594.33	594.34			
PyPAPM	725.37	725.32			

cyclo[APMPPYP] 2nd peak (O-prenyl)					
y- and b- cleavages (+1)	Expected	Observed	y- and b- cleavages (+1)	Expected	Observed
AP	169.10	-	yP	329.19	329.15
APM	300.14	-	yPA	400.22	400.29
APMP	397.19	397.18	yPAP	497.28	497.25
APMPP	494.24	494.28	yPAPM	628.32	628.34
APMPPy	725.37	725.33	yPAPMP	725.37	725.33
PM	229.10	-	PA	169.10	-
PMP	326.15	326.19	PAP	266.15	266.18
PMPP	423.21	-	PAPM	397.19	397.18
PMPPy	654.33	654.30	PAPMP	494.24	494.28
PMPPyP	751.39	751.35	PAPMPP	591.30	591.34
MP	229.10	-			
MPP	326.15	326.19	Miscellaneous Ions		
MPPy	557.28	557.30	-H2O	804.41	804.38
MPPyP	654.33	654.30	-Acylium (C=O)	794.42	794.40
MPPyPA	725.37	725.33	-Methyl sulfanyl radical	774.42	774.45
PP	263.18	-	-Prenyl	754.35	754.33
PPy	426.24	426.25	-Met side-chain	746.39	746.44
PPyP	523.29	523.34	PyPAP y- a- cleavage	566.33	566.36
PPyPA	594.33	594.29	PPyPA -Prenyl	526.27	526.26
PPyPAP	691.38	691.44	PPy, PyP -Prenyl	358.18	358.19
Py	329.19	329.15			
PyP	426.24	426.25			
PyPA	497.28	497.25			
PyPAP	594.33	594.29			
PyPAPM	725.37	725.33			

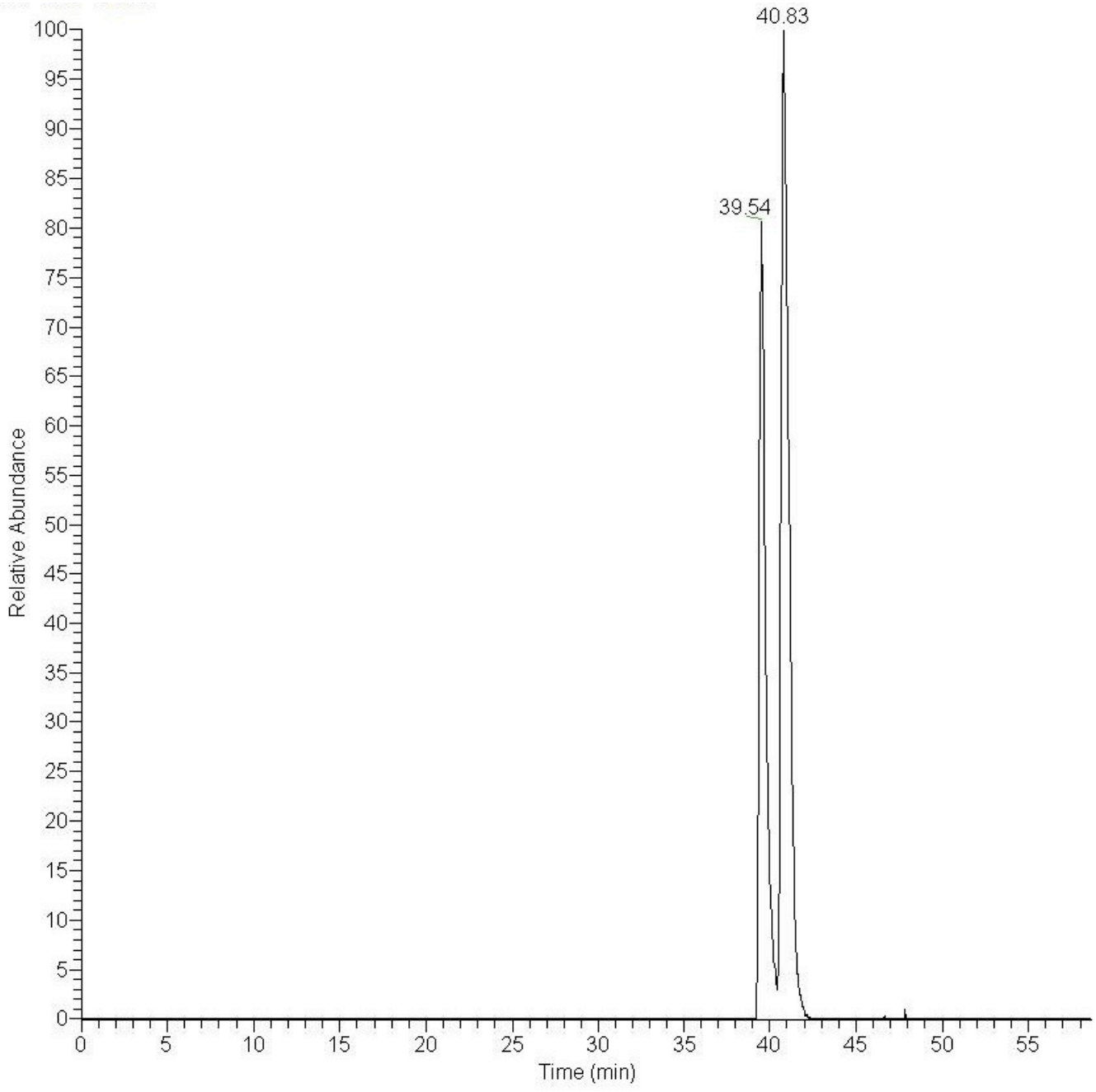
(e) FT-ICR data showing ion whose mass (+/- 2 ppm) corresponds to prenylated APMPPYP



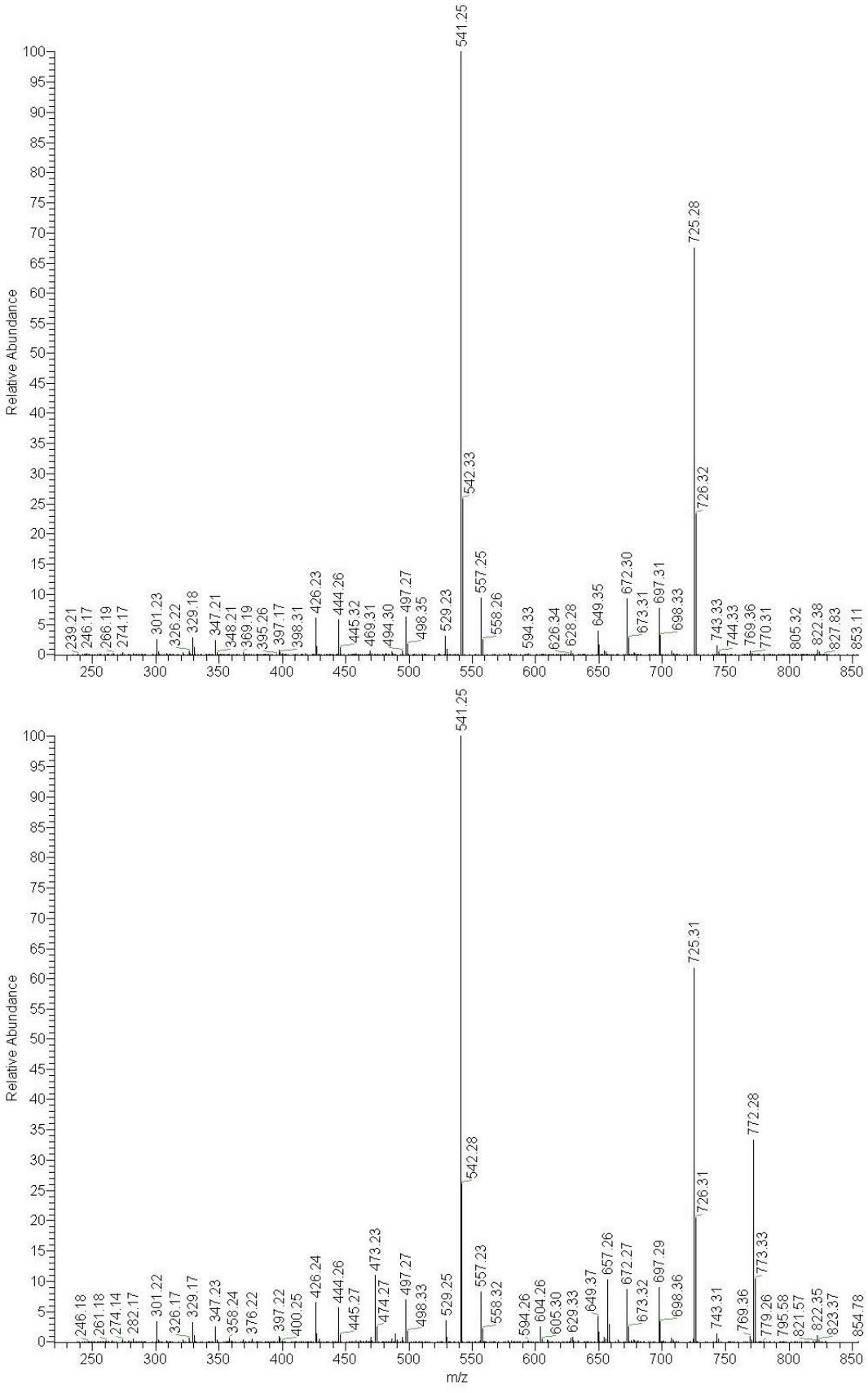
APMPPyP



(f) LC-FT-ICR chromatogram selected for mass of prenylated APMPPYP



(g) MS-MS spectra for early (top) and late (bottom) eluting products of the reaction of LynF with APMPPYP (see chromatogram on previous page)

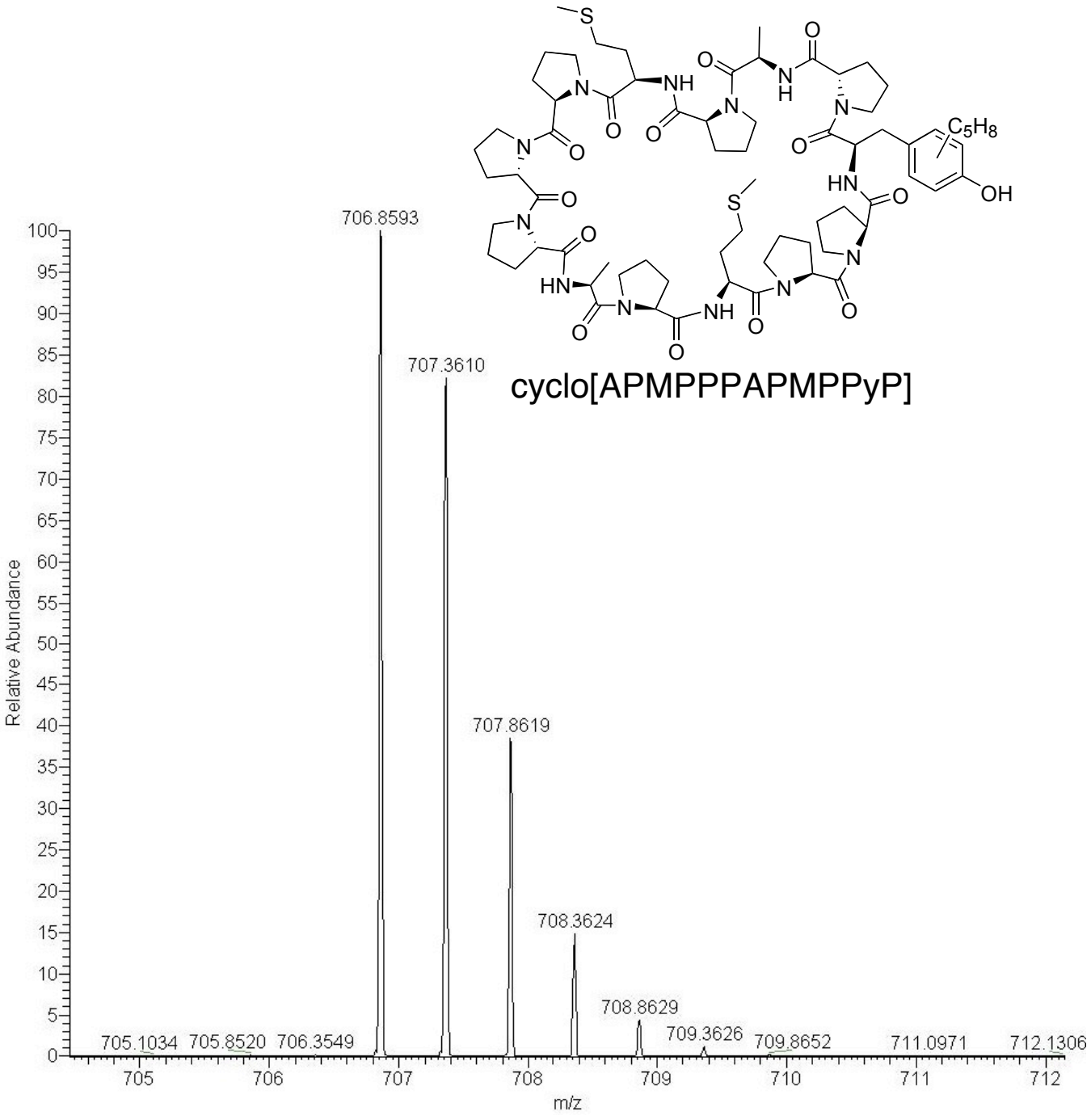


(h) MS-MS assignments for prenylated APMPPYP early (top) and late (bottom) eluting isomers

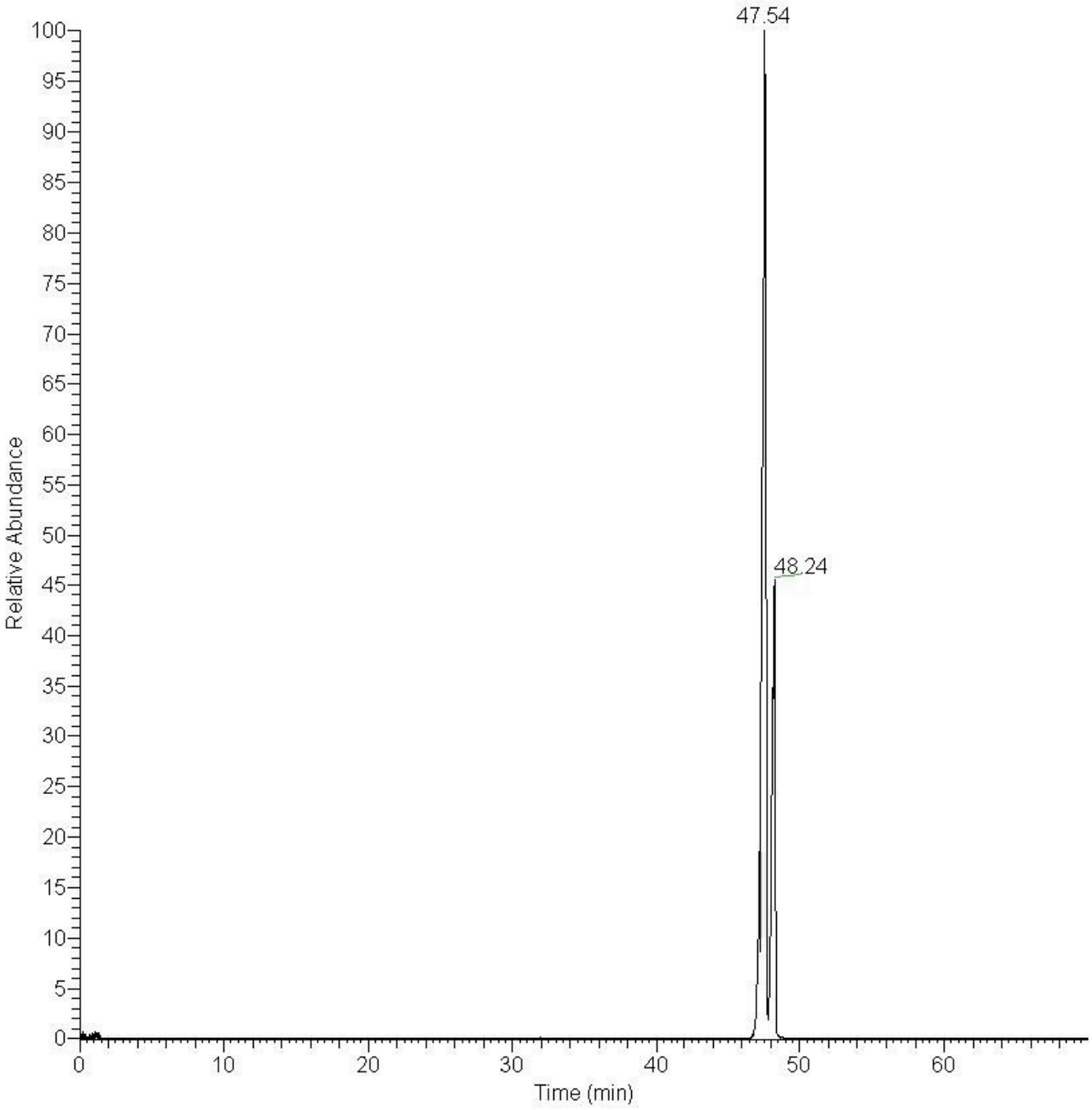
APMPPYP 1st peak (C-prenyl)		
b-ions (+1)	**Expected**	**Observed**
AP	169.10	-
APM	300.14	-
APMP	397.19	397.22
APMPP	494.24	494.33
APMPPy	725.37	725.31
y-ions (+1)		
PMPPyP	769.39	769.36
MPPyP	672.34	672.27
PPyP	541.30	541.25
PyP	444.25	444.26
Py	347.19	347.23
y- b- cleavages (+1)		
Py	329.19	329.17
PPy	426.24	426.24
MPPy	557.28	557.25
y- a- cleavages (+1)		
Py	301.19	301.22
Miscellaneous Ions		
APMPPy a-cleavage +1	697.37	697.31

APMPPYP 2nd peak (O-prenyl)		
b-ions (+1)	**Expected**	**Observed**
AP	169.10	-
APM	300.14	-
APMP	397.19	397.22
APMPP	494.24	-
APMPPy	725.37	725.31
y-ions (+1)		
PMPPyP	769.39	769.36
MPPyP	672.34	672.27
PPyP	541.30	541.25
PyP	444.25	444.26
Py	347.19	347.23
y- b- cleavages (+1)		
Py	329.19	329.17
PPy	426.24	426.24
MPPy	557.28	557.23
y- a- cleavages (+1)		
Py	301.19	301.22
Miscellaneous Ions		
All -prenyl	772.37	772.28
APMPPy a-cleavage +1	697.37	697.29
APMPPy -prenyl	657.31	657.26
PPyP -prenyl	473.24	473.23

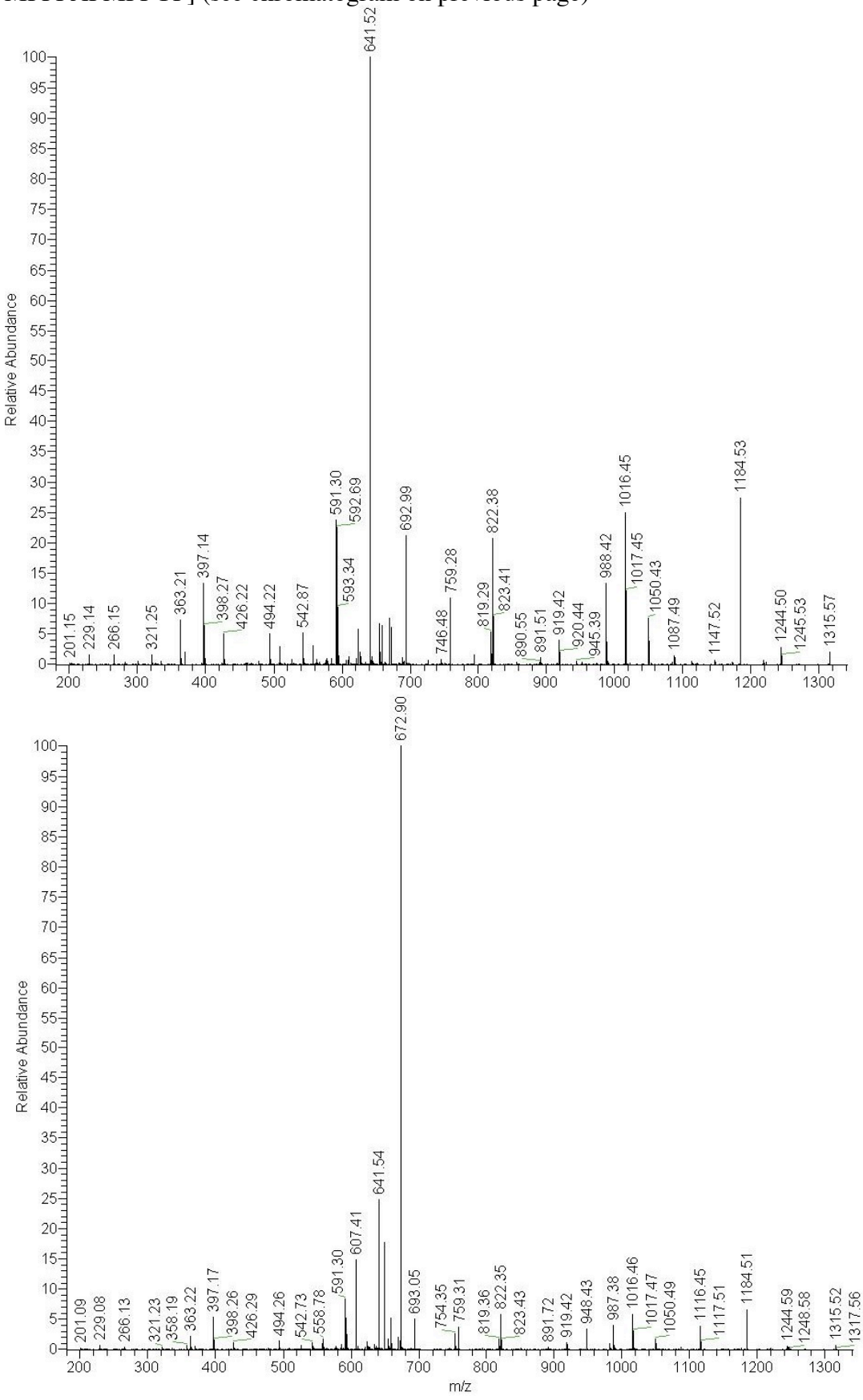
(i) FT-ICR data showing ion whose mass (+/- 2 ppm) corresponds to prenylated cyclo[APMPPPAPMPPYP]



(j) LC-FT-ICR chromatogram selected for mass of prenylated cyclo[APMPPPAPMPPYP]



(k) MS-MS spectra for early (top) and late (bottom) eluting products of the reaction of LynF with cyclo[APMPPPAPMPPYP] (see chromatogram on previous page)



(l) MS-MS assignments for prenylated cyclo[APMPPPAPMPPYP] early (this page) and late (next page) eluting isomers

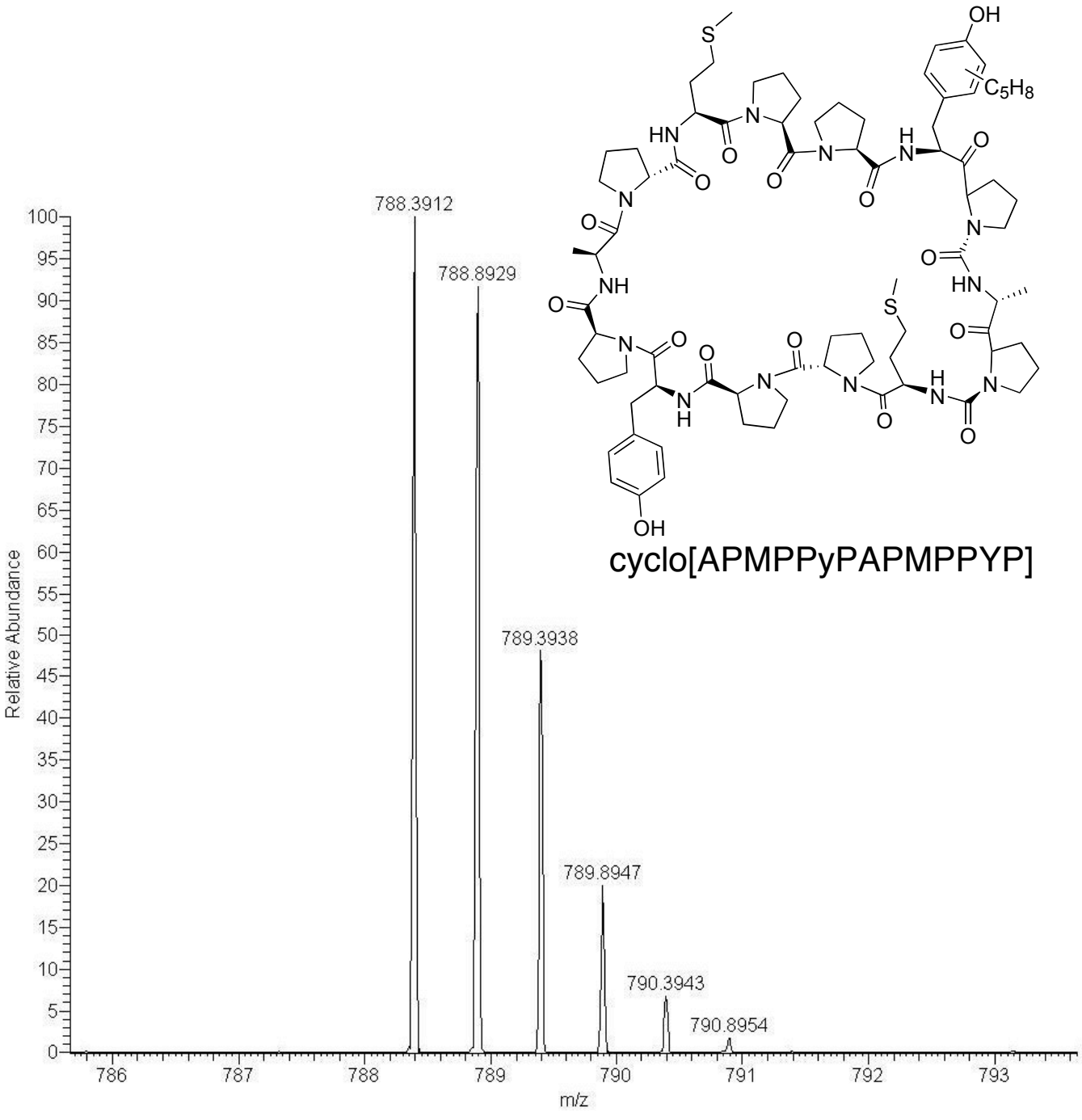
cyclo[APMPPPAPMPPYP] 1st peak (C-prenyl)								
y- b- cleavages (+1)	Expected	Observed	y- b- cleavages (+1)	Expected	Observed	y- b- cleavages (+1)	Expected	Observed
APM	300.14	300.22	MPPPAPMPPy	1147.57	1147.52	PAPMPPy	822.42	822.38
APMP	397.19	397.14	MPPPAPMPPyP	1244.62	1244.51	PAPMPPyP	919.47	919.42
APMPP	494.24	494.22	MPPPAPMPPyPA	1315.66	1315.57	PAPMPPyPAP	1087.56	1087.49
APMPPP	591.30	591.3	PPPA	363.20	363.21	PAPMPPyPAPM	1218.60	1218.53
APMPPPAP	759.39	759.28	PPPAP	460.26	460.28	PAPMPPyPAPMP	1315.66	1315.57
APMPPPAPM	890.43	890.54	PPPAPM	591.30	591.3	APM	300.14	300.22
APMPPPAPMP	987.48	987.42	PPPAPMP	688.35	688.39	APMP	397.19	397.14
APMPPPAPMPP	1084.53	1084.5	PPPAPMPP	785.40	785.47	APMPP	494.24	494.22
APMPPPAPMPPy	1315.66	1315.57	PPPAPMPPy	1016.53	1016.45	APMPPy	725.37	725.38
PM	229.10	229.14	PPPAPMPPyP	1113.58	1113.52	APMPPyP	822.42	822.38
PMP	326.15	326.1	PPPAPMPPyPA	1184.62	1184.52	APMPPyPAPMP	1218.60	1218.53
PMPP	423.21	423.22	PPA	266.15	266.15	APMPPyPAPMPP	1315.66	1315.57
PMPPP	520.26	520.28	PPAP	363.20	363.21	PM	229.10	229.14
PMPPPA	591.30	591.3	PPAPM	494.24	494.22	PMP	326.15	326.1
PMPPPAP	688.35	688.39	PPAPMP	591.30	591.3	PMPP	423.21	423.22
PMPPPAPM	819.39	819.3	PPAPMPP	688.35	688.39	PMPPy	654.33	654.31
PMPPPAPMP	916.44	916.46	PPAPMPPy	919.47	919.42	PMPPyP	751.38	751.41
PMPPPAPMPPy	1244.62	1244.51	PPAPMPPyP	1016.53	1016.45	PMPPyPA	822.42	822.38
MP	229.10	229.14	PPAPMPPyPA	1087.56	1087.49	PMPPyPAP	919.47	919.42
MPP	326.15	326.1	PPAPMPPyPAP	1184.62	1184.52	PMPPyPAPM	1050.51	1050.43
MPPP	423.21	423.22	PPAPMPPyPAPM	1315.66	1315.57	PMPPyPAPMP	1147.57	1147.52
MPPPA	494.24	494.22	PAP	266.15	266.15	PMPPyPAPMPP	1244.62	1244.51
MPPPAP	591.30	591.3	PAPM	397.19	397.14	MP	229.10	229.14
MPPPAPMP	819.39	819.3	PAPMP	494.24	494.22	MPP	326.15	326.1
MPPPAPMPP	916.44	916.46	PAPMPP	591.30	591.3	MPPy	557.28	557.36

y- b- cleavages (+1)	Expected	Observed	y- b- cleavages (+1)	Expected	Observed	Miscellaneous Ions	Expected	Observed
MPPyP	654.33	654.31	yPAPMPP	822.42	822.38	All, -acylium, +2	692.86	692.99
MPPyPA	725.37	725.38	yPAPMPPP	919.47	919.42	PPPAPMPPY, y- a- cleavage +1	988.53	988.42
MPPyPAP	822.42	822.38	yPAPMPPPAP	1087.56	1087.49	PAPM -Met sidechain	321.16	321.25
MPPyPAPM	953.46	953.48	yPAPMPPPAPM	1218.60	1184.52			
MPPyPAPMP	1050.51	1050.43	yPAPMPPPAPMP	1315.66	1315.57			
MPPyPAPMPP	1147.57	1147.52	PAP	266.15	266.15			
MPPyPAPMPPP	1244.62	1244.51	PAPM	397.19	397.14			
MPPyPAPMPPPA	1315.66	1315.57	PAPMP	494.24	494.22			
PPy	426.24	426.22	PAPMPP	591.30	591.3			
PPyPA	594.33	594.33	PAPMPPP	688.35	688.39			
PPyPAPM	822.42	822.38	PAPMPPPA	759.39	759.28			
PPyPAPMP	919.47	919.42	PAPMPPPAP	856.44	856.33			
PPyPAPMPP	1016.53	1016.45	PAPMPPPAPM	987.48	987.42			
PPyPAPMPPP	1113.58	1113.52	PAPMPPPAPMP	1084.53	1084.5			
PPyPAPMPPPA	1184.62	1184.52						
PyP	426.24	426.22	y- b- cleavages (+2)					
PyPAP	594.33	594.33	PAPMPPPAP	428.72	428.83			
PyPAPM	725.37	725.38	PyPAPMPPP	508.77	508.83			
PyPAPMP	822.42	822.38	PMPPyPAPM	525.76	525.76			
PyPAPMPP	919.47	919.42	APMPPPAPMPP	542.77	542.87			
PyPAPMPPP	1016.53	1016.45	PPAPMPPyPAP	592.82	592.69			
PyPAPMPPPA	1087.56	1087.49	PAPMPPyPAPM	609.81	609.84			
PyPAPMPPPAP	1184.62	1184.52	PMPPyPAPMPP	622.81	623.00			
PyPAPMPPPAPM	1315.66	1315.57	PPyPAPMPPPAP	641.34	641.52			
yPAPMP	725.37	725.38	PAPMPPyPAPMP	658.34	658.31			

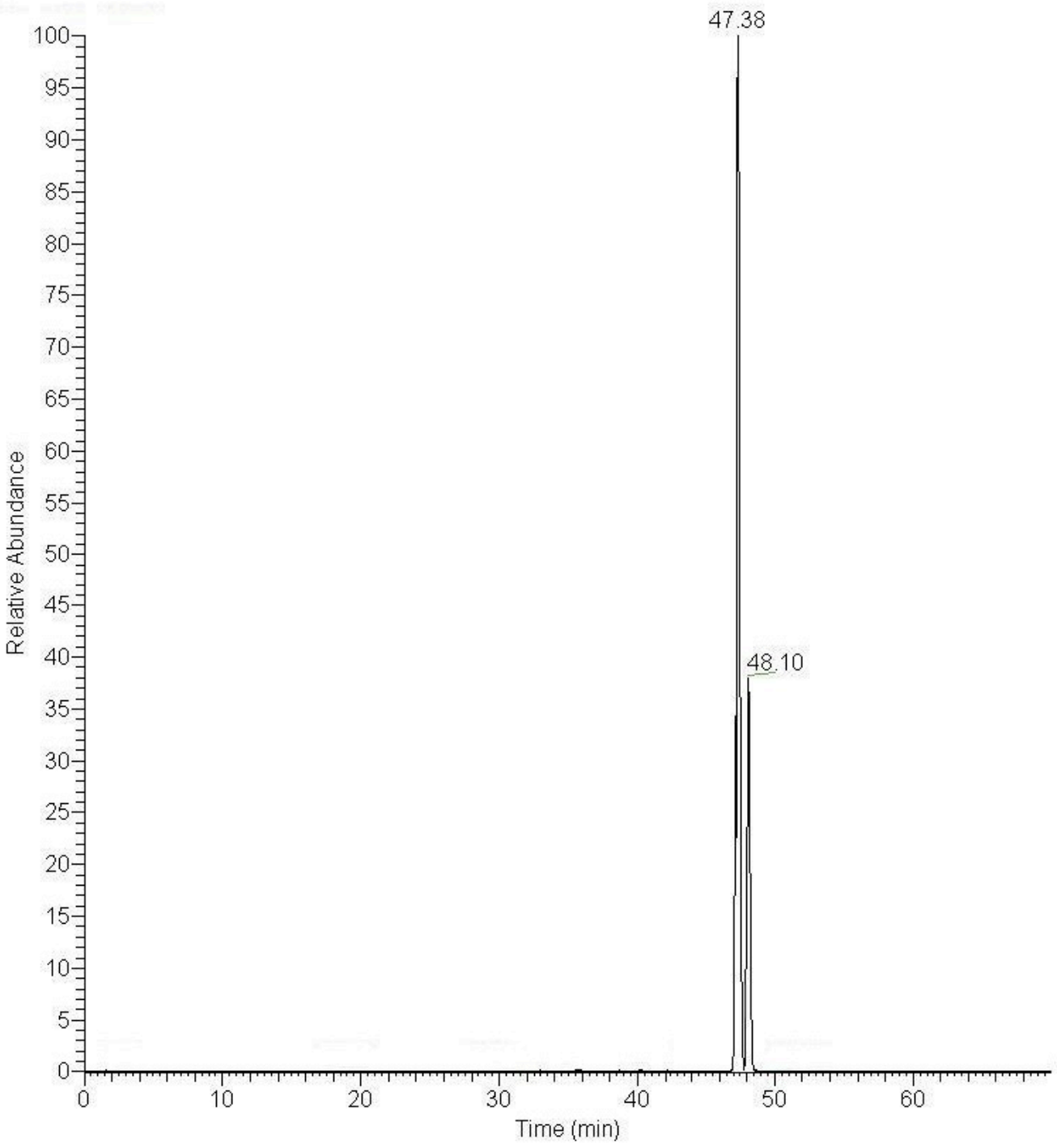
cyclo[APMPPPAPMPPYP] 2nd peak (O-prenyl)								
y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**
APMP	397.19	397.17	**MPPPAPMPPyP**	1244.62	1244.59	**PAPMPPyPA**	990.51	990.48
APMPP	494.24	494.26	**MPPPAPMPPyPA**	1315.66	1315.52	**PAPMPPyPAP**	1087.56	1087.50
APMPPP	591.30	591.30	**PPPA**	363.20	363.22	**PAPMPPyPAPM**	1218.60	1218.69
APMPPPAP	759.39	759.31	**PPPAP**	460.26	460.42	**PAPMPPyPAPMP**	1315.66	1315.52
APMPPPAPM	890.43	890.45	**PPPAPM**	591.30	591.30	**APMP**	397.19	397.17
APMPPPAPMP	987.48	987.38	**PPPAPMP**	688.35	688.32	**APMPP**	494.24	494.26
APMPPPAPMPP	1084.53	1084.64	**PPPAPMPP**	785.40	785.52	**APMPPy**	725.37	725.32
APMPPPAPMPPy	1315.66	1315.52	**PPPAPMPPy**	1016.53	1016.46	**APMPPyP**	822.42	822.35
PM	229.10	229.08	**PPPAPMPPyPA**	1184.62	1184.51	**APMPPyPAP**	990.51	990.48
PMP	326.15	326.18	**PPA**	266.15	266.13	**APMPPyPAPM**	1121.55	1121.48
PMPP	423.21	432.19	**PPAP**	363.20	363.22	**APMPPyPAPMP**	1218.60	1218.69
PMPPPA	591.30	591.30	**PPAPM**	494.24	494.26	**APMPPyPAPMPP**	1315.66	1315.52
PMPPPAP	688.35	688.32	**PPAPMP**	591.30	591.30	**PM**	229.10	229.08
PMPPPAPM	819.39	819.36	**PPAPMPP**	688.35	688.32	**PMP**	326.15	326.18
PMPPPAPMP	916.44	916.43	**PPAPMPPy**	919.47	919.42	**PMPP**	423.21	423.19
PMPPPAPMPPy	1244.62	1244.59	**PPAPMPPyP**	1016.53	1016.46	**PMPPyPA**	822.42	822.35
MP	229.10	229.08	**PPAPMPPyPA**	1087.56	1087.50	**PMPPyPAP**	919.47	919.42
MPP	326.15	326.18	**PPAPMPPyPAP**	1184.62	1184.51	**PMPPyPAPM**	1050.51	1050.49
MPPP	423.21	423.19	**PPAPMPPyPAPM**	1315.66	1315.52	**PMPPyPAPMP**	1147.57	1147.44
MPPPA	494.24	494.26	**PAP**	266.15	266.13	**PMPPyPAPMPP**	1244.62	1244.59
MPPPAP	591.30	591.30	**PAPM**	397.19	397.17	**MP**	229.10	229.08
MPPPAPM	722.34	722.36	**PAPMP**	494.24	494.26	**MPP**	326.15	326.18
MPPPAPMP	819.39	819.36	**PAPMPP**	591.30	591.30	**MPPy**	557.28	557.53
MPPPAPMPP	916.44	916.43	**PAPMPPy**	822.42	822.35	**MPPyPA**	725.37	725.42
MPPPAPMPPy	1147.57	1147.44	**PAPMPPyP**	919.47	919.42	**MPPyPAP**	822.42	822.35

y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**	**Miscellaneous Ions**	**Expected**	**Observed**
MPPyPAPMP	1050.51	1050.49	**yPAPMPPPAPM**	1218.60	1218.69	**+1, -Prenyl**	1344.65	1344.53
MPPyPAPMPP	1147.57	1147.44	**yPAPMPPPAPMP**	1315.66	1315.52	**APMPPPAPMPPy -Prenyl**	1315.66	1315.52
MPPyPAPMPPP	1244.62	1244.59	**PAP**	266.15	266.13			
MPPyPAPMPPPA	1315.66	1315.52	**PAPM**	397.19	397.17			
PPy	426.24	426.29	**PAPMP**	494.24	494.26			
PPyP	523.29	523.34	**PAPMPP**	591.30	591.30			
PPyPA	594.33	594.35	**PAPMPPP**	688.35	688.32			
PPyPAPM	822.42	822.35	**PAPMPPPA**	759.39	759.31			
PPyPAPMP	919.47	919.42	**PAPMPPPAP**	856.44	856.39			
PPyPAPMPP	1016.53	1016.46	**PAPMPPPAPM**	987.48	987.38			
PPyPAPMPPPA	1184.62	1184.51	**PAPMPPPAPMP**	1084.53	1084.64			
PyP	426.24	426.29						
PyPAP	594.33	594.35	**y- b- cleavages (+2)**					
PyPAPM	725.37	725.42	**PMPPPAPMPP**	507.25	507.41			
PyPAPMP	822.42	822.35	**APMPPPAPMPP**	542.77	542.73			
PyPAPMPP	919.47	919.42	**PPPAPMPPyPA**	592.81	592.62			
PyPAPMPPP	1016.53	1016.46	**PPyPAPMPPPAP**	641.34	641.54			
PyPAPMPPPA	1087.56	1087.50						
PyPAPMPPPAP	1184.62	1184.51	**Miscellaneous Ions**					
PyPAPMPPPAPM	1315.66	1315.52	**PPPAPMPPyPA, -Prenyl, +2**	607.30	607.41			
yPAPMP	725.37	725.42	**+2 -Prenyl, -Methyl Sulfide**	648.83	648.90			
yPAPMPP	822.42	822.35	**+2, -Prenyl, -Acylium**	658.83	658.68			
yPAPMPPP	919.47	919.42	**+2, -Prenyl**	672.82	672.90			
yPAPMPPPA	990.51	990.48	**PPPAPMPPy -Prenyl**	948.46	948.43			
yPAPMPPPAP	1087.56	1087.50	**PPPAPMPPyPA -Prenyl**	1116.55	1116.45			

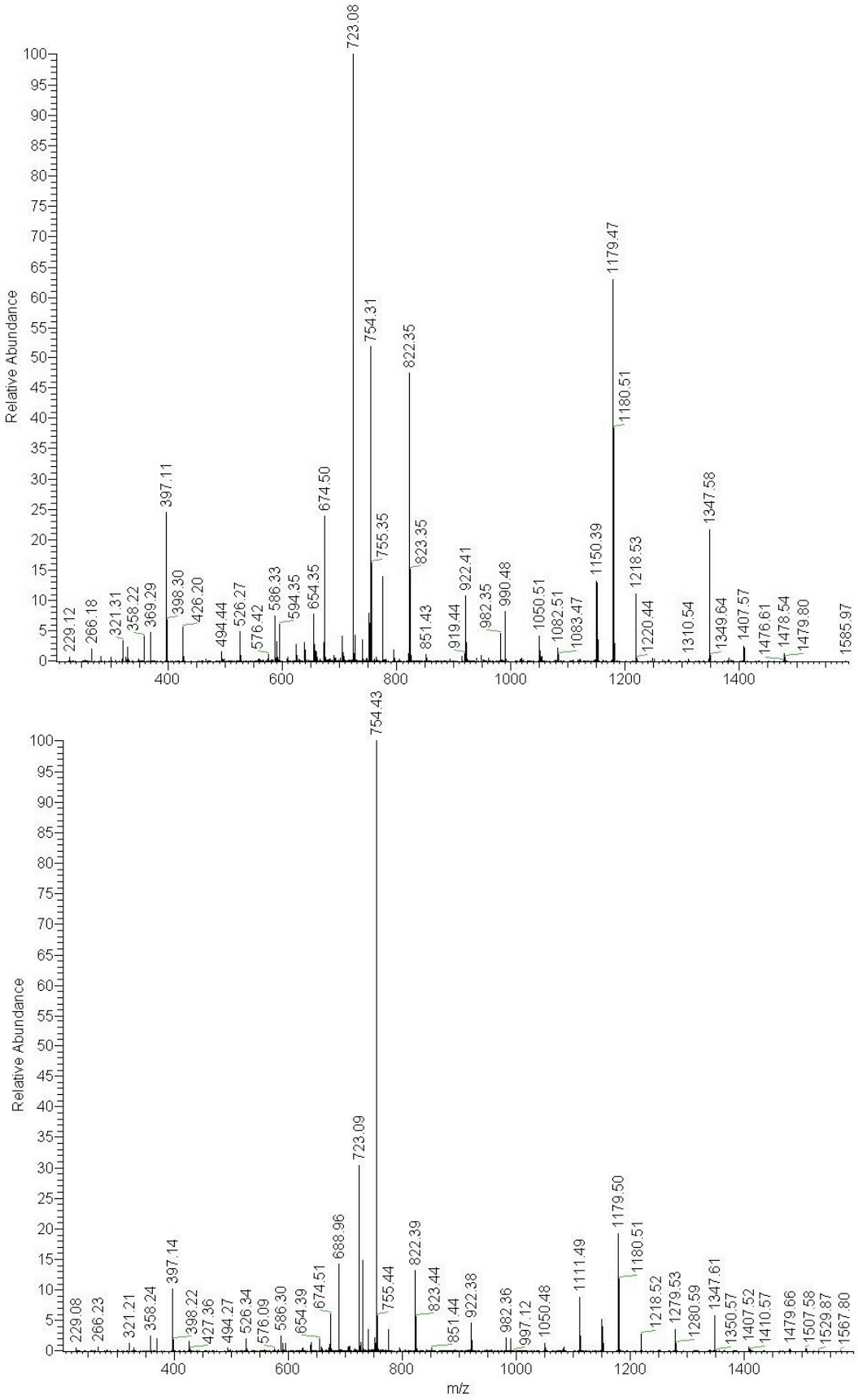
(m) FT-ICR data showing ion whose mass (+/- 2 ppm) corresponds to singly prenylated cyclo[APMPPYPAPMPPYP]



(n) LC-FT-ICR chromatogram selected for mass of singly prenylated cyclo[APMPPYPAPMPPYP]



(o) MS-MS spectra for early (top) and late (bottom) eluting singly prenylated products of the reaction of LynF with cyclo[APMPPYPAPMPPYP] (see chromatogram on previous page)



(p) MS-MS assignments for singly prenylated cyclo[APMPPYPAPMPPYP] early (this page) and late (next page) eluting isomers

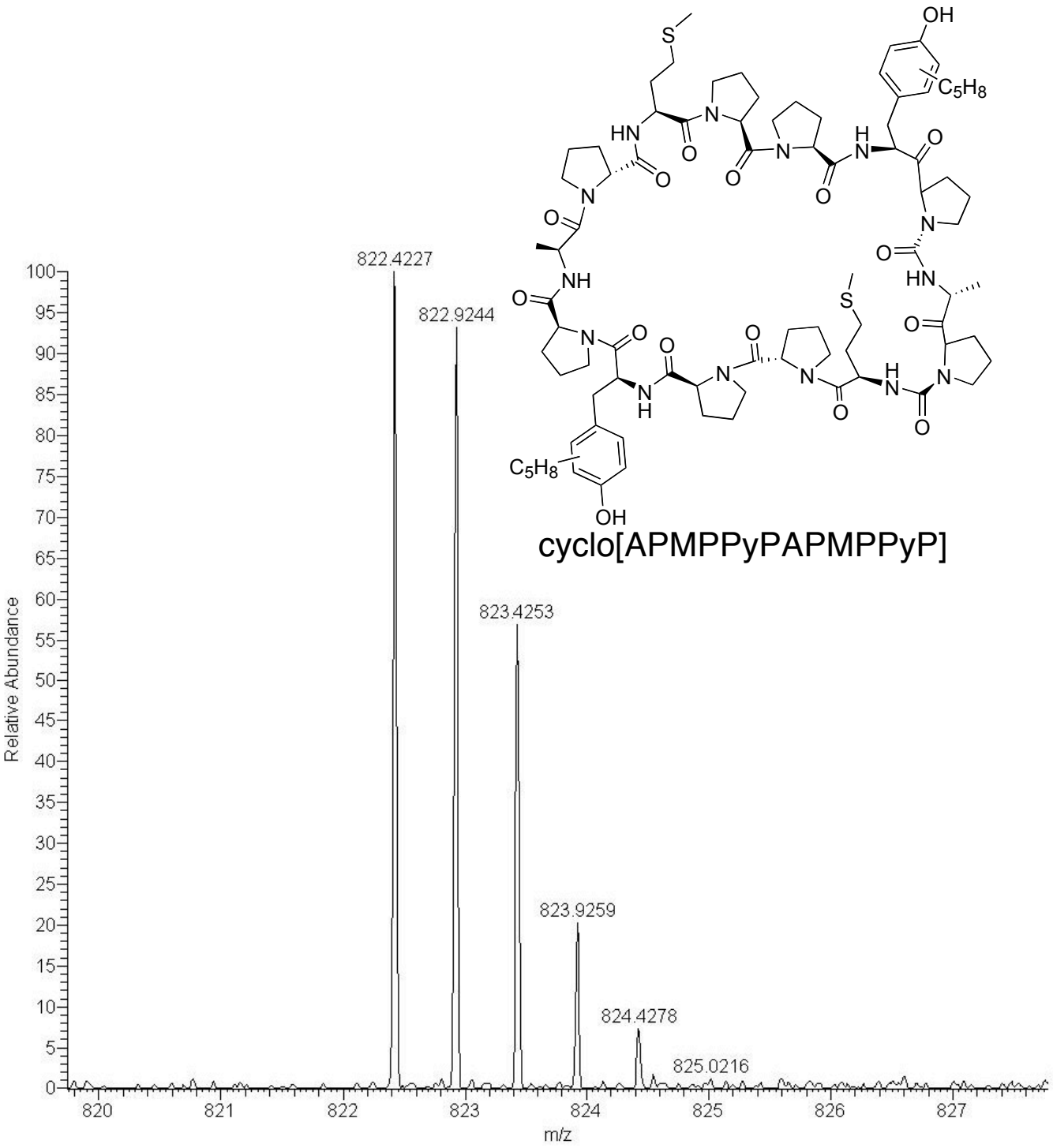
cyclo[APMPPYPAPMPPYP] 1st peak (C-prenyl)								
y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**
APMP	397.19	397.11	**MPPYPAPMPPyPA**	1478.72	1478.54	**PAPMPPyP**	919.47	919.44
APMPP	494.24	494.44	**PPY**	358.18	358.22	**PAPMPPyPA**	990.51	990.48
APMPPYP	754.36	754.31	**PPYPA**	526.27	526.27	**PAPMPPyPAPM**	1218.60	1218.53
APMPPYPAP	922.45	922.41	**PPYPAP**	623.32	623.26	**APMP**	397.19	397.11
APMPPYPAPMP	1150.54	1150.39	**PPYPAPM**	754.36	754.31	**APMPP**	494.24	494.44
APMPPYPAPMPP	1247.60	1247.45	**PPYPAPMP**	851.41	851.43	**APMPPyP**	822.42	822.35
APMPPYPAPMPPy	1478.72	1478.54	**PPYPAPMPP**	948.47	948.45	**APMPPyPA**	893.46	893.51
PM	229.10	229.12	**PPYPAPMPPy**	1179.59	1179.47	**APMPPyPAP**	990.51	990.48
PMP	326.15	326.25	**PPYPAPMPPyPA**	1347.68	1347.58	**APMPPyPAPM**	1121.55	1121.39
PMPPY	586.27	586.33	**PYP**	358.18	358.22	**APMPPyPAPMP**	1218.60	1218.53
PMPPYP	683.32	683.39	**PYPAP**	526.27	526.27	**APMPPyPAPMPPY**	1478.72	1478.54
PMPPYPA	754.36	754.31	**PYPAPMP**	754.36	754.31	**PM**	229.10	229.12
PMPPYPAP	851.41	851.43	**PYPAPMPP**	851.41	851.43	**PMP**	326.15	326.25
PMPPYPAPM	982.45	982.35	**PYPAPMPPy**	1082.54	1082.51	**PMPPy**	654.33	654.35
PMPPYPAPMP	1079.51	1079.44	**PYPAPMPPyP**	1179.59	1179.47	**PMPPyPA**	822.42	822.35
PMPPYPAPMPPy	1407.68	1407.57	**PYPAPMPPyPAP**	1347.68	1347.58	**PMPPyPAP**	919.47	919.44
MP	229.10	229.12	**PYPAPMPPyPAPM**	1478.72	1478.54	**PMPPyPAPM**	1050.51	1050.51
MPP	326.15	326.25	**YPAPMPP**	754.36	754.31	**PMPPyPAPMP**	1147.57	1147.53
MPPYP	586.27	586.33	**YPAPMPPy**	985.48	985.32	**PMPPyPAPMPPY**	1407.68	1407.57
MPPYPAP	754.36	754.31	**YPAPMPPyP**	1082.54	1082.51	**MP**	229.10	229.12
MPPYPAPMP	982.45	982.35	**YPAPMPPyPAPMP**	1478.72	1478.54	**MPP**	326.15	326.25
MPPYPAPMPP	1079.51	1079.44	**PAP**	266.15	266.18	**MPPyP**	654.33	654.35
MPPYPAPMPPy	1310.63	1310.53	**PAPM**	397.19	397.11	**MPPyPAP**	822.42	822.35
MPPYPAPMPPyP	1407.68	1407.57	**PAPMP**	494.24	494.44	**MPPyPAPM**	953.46	953.57
MPPYPAPMPPyPA	1478.72	1478.54	**PAPMPPy**	822.42	822.35	**MPPyPAPMP**	1050.51	1050.51

y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**
MPPyPAPMPP	1147.57	1147.33	**PAPMP**	494.24	494.44
MPPyPAPMPPY	1310.63	1310.54	**PAPMPPY**	754.36	754.31
MPPyPAPMPPYP	1407.68	1407.57	**PAPMPPYP**	851.41	851.43
MPPyPAPMPPYPA	1478.72	1478.54	**PAPMPPYPA**	922.45	922.41
PPy	426.24	426.20	**PAPMPPYPAP**	1019.50	1019.39
PPyPA	594.33	594.35	**PAPMPPYPAPM**	1150.54	1150.39
PPyPAPM	822.42	822.35	**PAPMPPYPAPMP**	1247.60	1247.45
PPyPAPMP	919.47	919.44			
PPyPAPMPP	1016.53	1016.55	**y- b- cleavages (+2)**		
PPyPAPMPPY	1179.59	1179.47	**PPYPAPMPPyPAP**	722.87	723.08
PPyPAPMPPYPA	1347.68	1347.58	**PMPPYPAPMPPy**	704.35	704.36
PyP	426.24	426.20	**PyPAPMPPYPAP**	674.34	674.50
PyPAP	594.33	594.35	**PPyPAPMPPYP**	638.82	638.82
PyPAPM	725.37	725.31	**PAPMPPYPAPMP**	624.30	624.46
PyPAPMP	822.42	822.35			
PyPAPMPP	919.47	919.44	**Miscelaneous Ions**		
PyPAPMPPY	1082.54	1082.51	**PAPM y- a- cleavage**	369.20	329.29
PyPAPMPPYP	1179.59	1179.47	**PAPM -Met sidechain**	321.16	321.31
PyPAPMPPYPAP	1347.68	1347.58			
PyPAPMPPYPAPM	1478.72	1478.54			
yPAPMPP	822.42	822.35			
yPAPMPPYP	1082.54	1082.51			
yPAPMPPYPAPMP	1478.72	1478.54			
PAP	266.15	266.18			
PAPM	397.19	397.11			

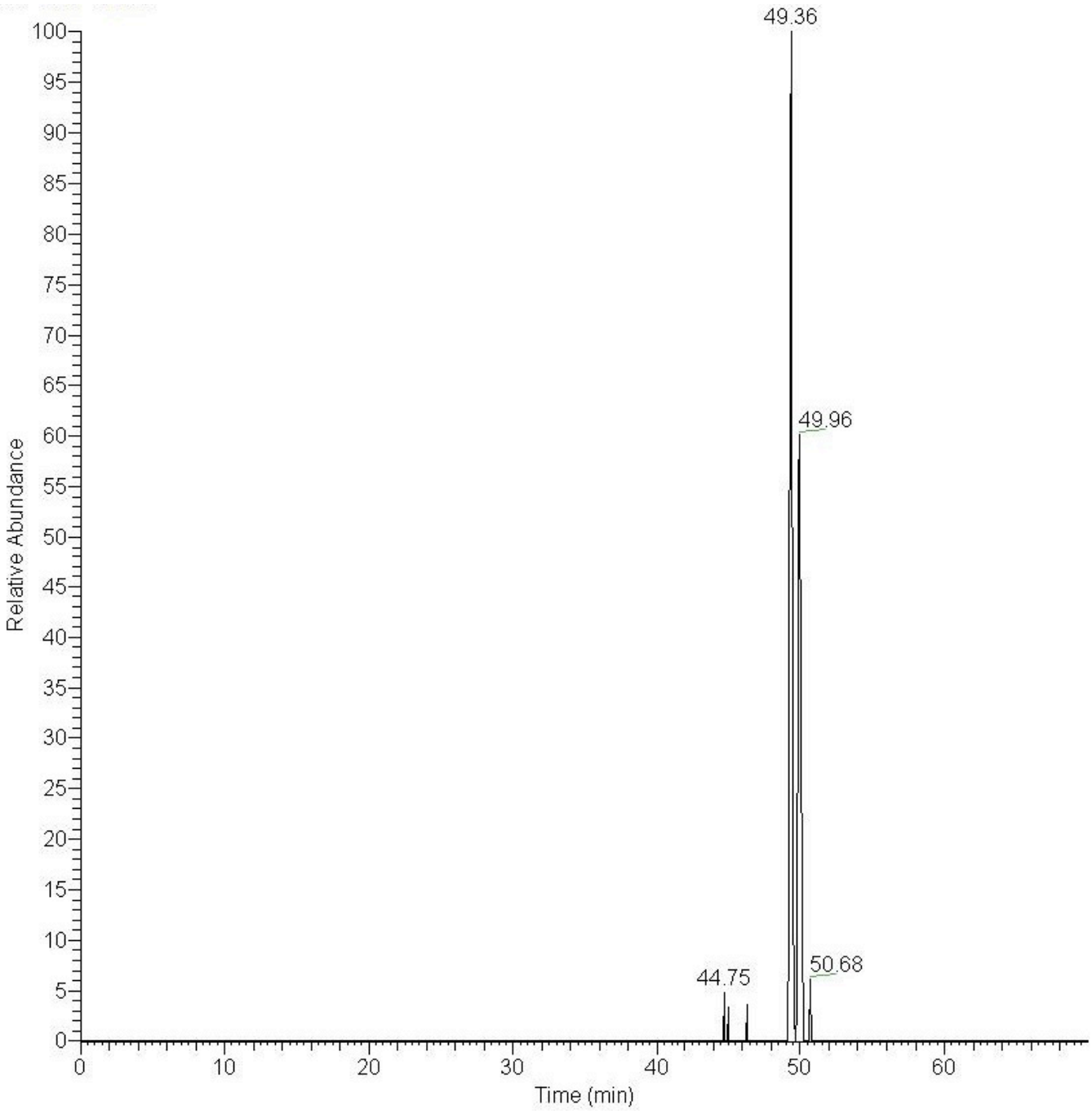
cyclo[APMPPYPAPMPPYP] 2nd peak (O-prenyl)								
y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**
APM	300.14	299.94	**PPYP**	455.23	455.40	**YPAPMPPyPAPM**	1,381.67	1,381.54
APMP	397.19	397.14	**PPYPA**	526.27	526.33	**YPAPMPPyPAPMP**	1,478.72	1,478.47
APMPP	494.24	494.28	**PPYPAP**	623.32	623.43	**PAP**	266.15	266.23
APMPPYP	754.36	754.43	**PPYPAPM**	754.36	754.43	**PAPM**	397.19	397.14
APMPPYPAP	922.45	922.38	**PPYPAPMP**	851.41	851.42	**PAPMP**	494.24	494.28
APMPPYPAPMP	1,150.54	1,150.45	**PPYPAPMPP**	948.47	948.54	**PAPMPPy**	822.42	822.39
APMPPYPAPMPP	1,247.60	1,247.60	**PPYPAPMPPy**	1,179.59	1,179.49	**PAPMPPyPA**	990.51	990.48
APMPPYPAPMPPy	1,478.72	1,478.47	**PPYPAPMPPyP**	1,276.64	1,276.48	**PAPMPPyPAPM**	1,218.60	1,218.51
PM	229.10	229.08	**PPYPAPMPPyPA**	1,347.68	1,347.60	**APM**	300.14	299.94
PMPPY	586.27	586.30	**PYP**	358.18	358.23	**APMP**	397.19	397.14
PMPPYP	683.32	683.13	**PYPA**	429.21	429.36	**APMPP**	494.24	494.28
PMPPYPA	754.36	754.43	**PYPAP**	526.27	526.33	**APMPPyP**	822.42	822.39
PMPPYPAP	851.41	851.42	**PYPAPMP**	754.36	754.43	**APMPPyPAP**	990.51	990.48
PMPPYPAPM	982.45	982.37	**PYPAPMPP**	851.41	851.42	**APMPPyPAPM**	1,121.55	1,121.51
PMPPYPAPMP	1,079.51	1,079.40	**PYPAPMPPy**	1,082.54	1,082.45	**APMPPyPAPMP**	1,218.60	1,218.51
PMPPYPAPMPPy	1,407.68	1,407.52	**PYPAPMPPyP**	1,179.59	1,179.49	**APMPPyPAPMPP**	1,315.66	1,315.55
MP	229.10	229.08	**PYPAPMPPyPA**	1,250.63	1,250.73	**APMPPyPAPMPPY**	1,478.72	1,478.47
MPPYP	586.27	586.30	**PYPAPMPPyPAP**	1,347.68	1,347.60	**PM**	229.10	229.08
MPPYPAP	754.36	754.43	**PYPAPMPPyPAPM**	1,478.72	1,478.47	**PMPPy**	654.33	654.38
MPPYPAPMP	982.45	982.37	**YPA**	332.16	332.38	**PMPPyPA**	822.42	822.39
MPPYPAPMPP	1,079.51	1,079.40	**YPAP**	429.21	429.36	**PMPPyPAPM**	1,050.51	1,050.47
MPPYPAPMPPy	1,310.63	1,310.66	**YPAPMPP**	754.36	754.43	**PMPPyPAPMP**	1,147.57	1,147.46
MPPYPAPMPPyP	1,407.68	1,407.60	**YPAPMPPyP**	1,082.54	1,082.45	**PMPPyPAPMPPY**	1,407.68	1,407.52
MPPYPAPMPPyPA	1,478.72	1,478.47	**YPAPMPPyPA**	1,153.57	1,153.43	**MP**	229.10	229.08
PPY	358.18	358.23	**YPAPMPPyPAP**	1,250.63	1,250.73	**MPPy**	557.28	557.92

y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**
MPPyP	654.33	654.38	**PAP**	266.15	266.23
MPPyPAP	822.42	822.39	**PAPM**	397.19	397.14
MPPyPAPMP	1,050.51	1,050.47	**PAPMP**	494.24	494.28
MPPyPAPMPP	1,147.57	1,147.46	**PAPMPPY**	754.36	754.43
MPPyPAPMPPY	1,310.63	1,310.66	**PAPMPPYP**	851.41	851.42
MPPyPAPMPPYP	1,407.68	1,407.52	**PAPMPPYPA**	922.45	922.38
MPPyPAPMPPYPA	1,478.72	1,478.47	**PAPMPPYPAPM**	1,150.54	1,150.45
PPy	426.24	426.23	**PAPMPPYPAPMP**	1,247.60	1,247.60
PPyPA	594.33	594.29			
PPyPAPM	822.42	822.39	**y- b- cleavages (+2)**		
PPyPAPMPPY	1,179.59	1,179.49	**MPPYPAPMPPyPA**	739.86	739.82
PPyPAPMPPYPA	1,347.68	1,347.60	**PMPPYPAPMPPy**	704.34	704.32
PyP	426.24	426.23	**MPPYPAPMPP**	540.26	540.27
PyPAP	594.33	594.29			
PyPAPMP	822.42	822.39	**Miscellaneous Ions**		
PyPAPMPPY	1,082.54	1,082.45	**PyPAPMPPYPAP +2**	674.34	674.51
PyPAPMPPYP	1,179.59	1,179.49	**PPyPAPMPPYPAP -prenyl +2**	688.84	688.96
PyPAPMPPYPA	1,250.63	1,250.73	**PPyPAPMPPYPAP +2**	722.87	723.08
PyPAPMPPYPAP	1,347.68	1,347.60	**PYPAPMPPYP -prenyl +1**	1,111.53	1,111.48
PyPAPMPPYPAPM	1,478.72	1,478.47	**APMPPYPAPMP -prenyl +1**	1,150.54	1,150.45
yPAPMPP	822.42	822.39	**PPYPAPMPPyPAP -prenyl +1**	1,279.62	1,279.54
yPAPMPPYP	1,082.54	1,082.45			
yPAPMPPYPAP	1,250.63	1,250.73			
yPAPMPPYPAPM	1,381.67	1,381.54			
yPAPMPPYPAPMP	1,478.72	1,478.47			

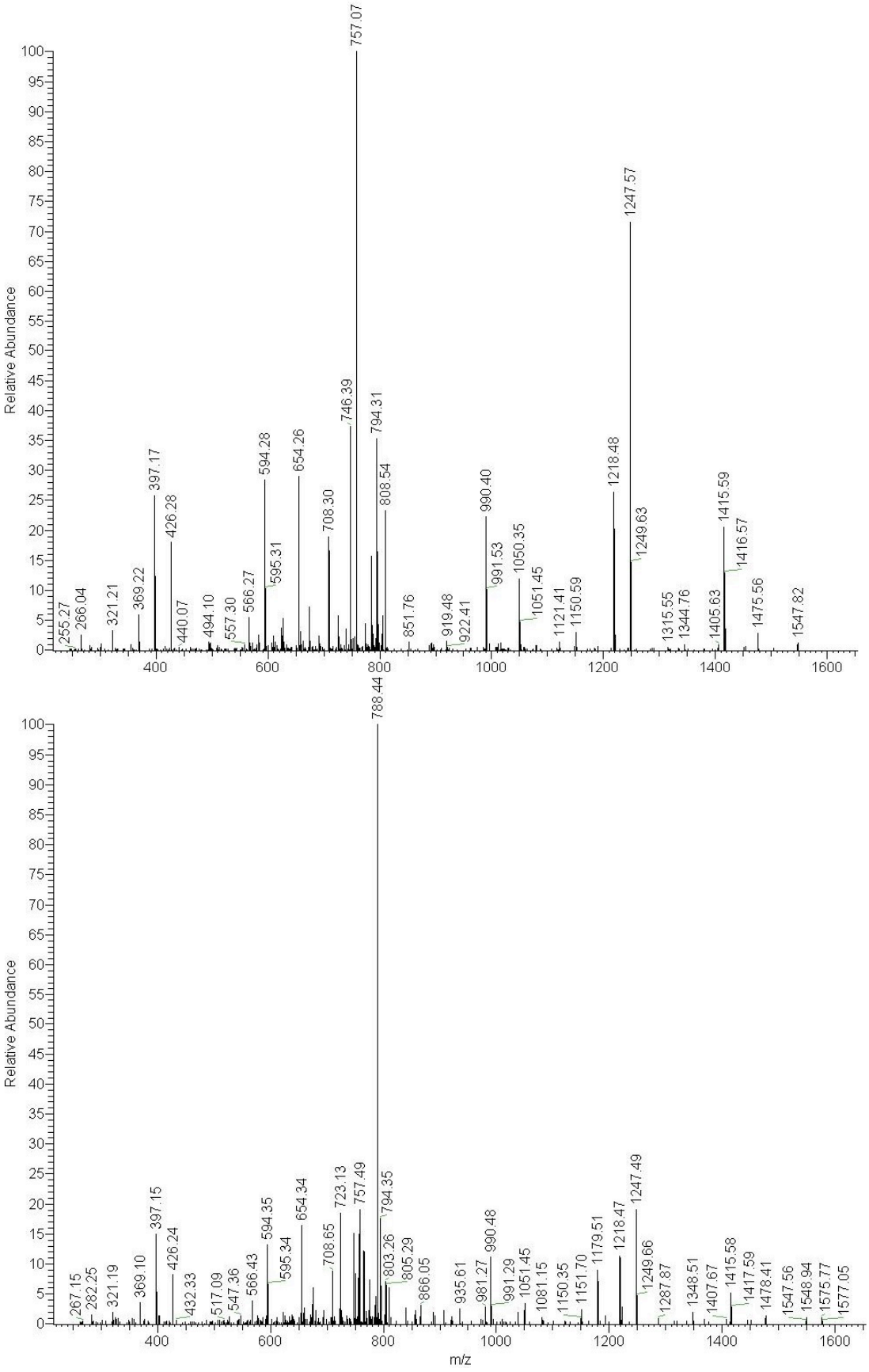
(q) FT-ICR data showing ion whose mass (+/- 2ppm) corresponds to doubly prenylated cyclo[APMPPYPAPMPPYP]



(r) LC-FT-ICR chromatogram selected for mass of doubly prenylated cyclo[APMPPYPAPMPPYP]



(s) MS-MS spectra for early (top) and late (bottom) eluting doubly prenylated products of the reaction of LynF with cyclo[APMPPYPAPMPPYP] (see chromatogram on previous page)

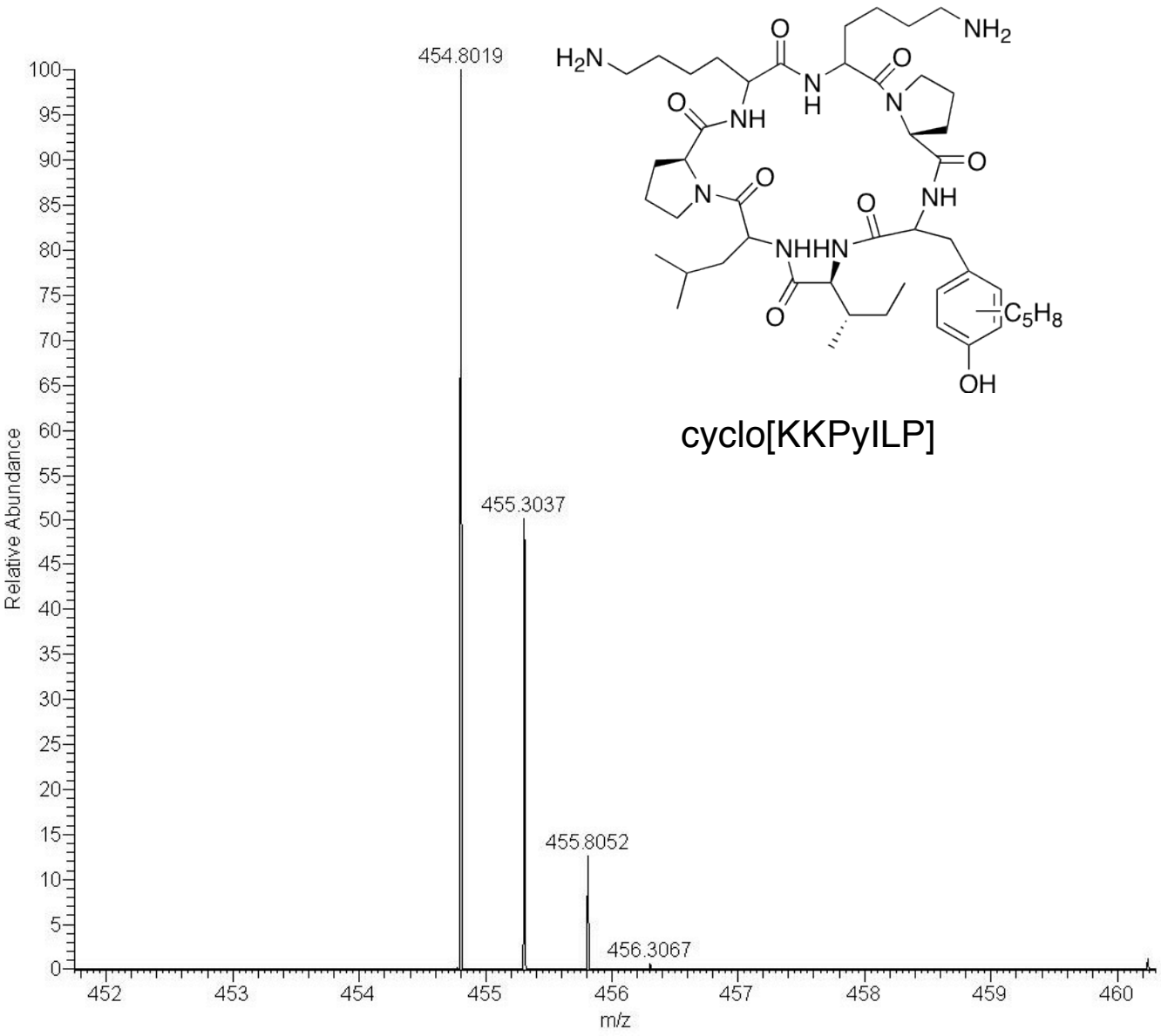


(t) MS-MS assignments for doubly prenylated cyclo[APMPPYPAPMPPYP] early (top) and late (bottom) eluting isomers

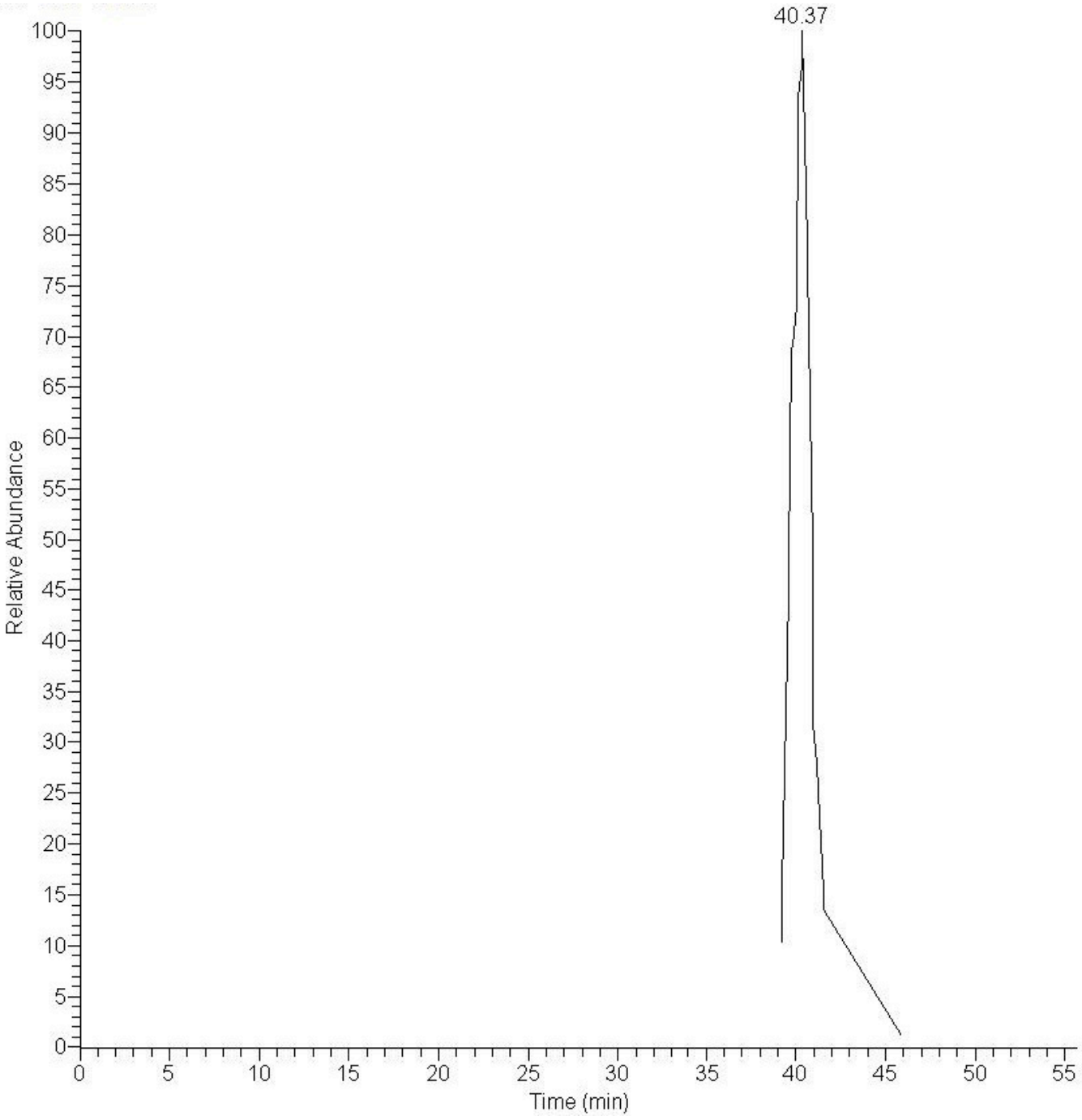
cyclo[APMPPYPAPMPPYP] 1st peak								
y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**
APMP	397.19	397.17	**PPyPAPMPP**	1016.53	1016.45	**PAPMPPyPAPMP**	1315.66	1315.55
APMPPy	725.37	725.40	**PPyPAPMPPy**	1247.66	1247.57			
APMPPyPAP	990.51	990.40	**PPyPAPMPPyP**	1344.71	1344.76	**y- b- cleavage (+2)**		
APMPPyPAPM	1121.55	1121.41	**PPyPAPMPPyPA**	1415.75	1415.59	**PPyPAPMPPyPAP**	756.90	757.07
APMPPyPAPMP	1218.60	1218.48	**PyP**	426.24	426.28	**PyPAPMPPyPAP**	708.38	708.30
APMPPyPAPMPP	1315.66	1315.55	**PyPA**	497.27	497.35	**PPyPAPMPPyP**	672.35	672.83
APMPPyPAPMPPy	1546.79	1546.40	**PyPAP**	594.33	594.28	**APMPPyPAPMPP**	657.83	658.47
PMP	326.15	326.18	**PyPAPM**	725.37	725.40	**PyPAPMPPyP**	623.83	624.14
PMPP	423.21	423.13	**PyPAPMPP**	919.47	919.48	**APMPPyPAPMP**	609.30	609.98
PMPPy	654.33	654.26	**PyPAPMPPy**	1150.61	1150.59			
PMPPyPAP	919.47	919.48	**PyPAPMPPyP**	1247.66	1247.57	**Miscellaneous Ions**		
PMPPyPAPM	1050.51	1050.35	**PyPAPMPPyPAP**	1415.75	1415.59	**PAPM -Met sidechain**	321.16	321.21
PMPPyPAPMP	1147.57	1147.56	**PyPAPMPPyPAPM**	1546.79	1546.40	**PAPM y- a- cleavage**	369.20	369.22
PMPPyPAPMPPy	1475.75	1475.56	**yPAP**	497.27	497.35	**PyPAP y- a- cleavage**	566.33	566.27
MPP	326.15	326.18	**yPAPMP**	725.37	725.40	**PMPPyPA -Met sidechain**	746.39	746.39
MPPy	557.28	557.30	**yPAPMPPyP**	1150.61	1150.59	**APMPPyP y- a- cleavage**	794.43	794.31
MPPyP	654.33	654.26	**yPAPMPPyPA**	1221.64	1221.67			
MPPyPA	725.37	725.40	**yPAPMPPyPAPMP**	1546.79	1546.40			
MPPyPAPMP	1050.51	1050.35	**PAP**	266.15	266.04			
MPPyPAPMPP	1147.57	1147.56	**PAPM**	397.19	397.17			
MPPyPAPMPPyP	1475.75	1475.56	**PAPMP**	494.24	494.10			
PPy	426.24	426.28	**PAPMPPyP**	919.47	919.48			
PPyPA	594.33	594.28	**PAPMPPyPA**	990.51	990.40			
PPyPAP	691.38	691.16	**PAPMPPyPAP**	1087.56	1087.74			
PPyPAPMP	919.47	919.48	**PAPMPPyPAPM**	1218.60	1218.48			

cyclo[APMPPYPAPMPPYP] 2nd peak								
y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**	**Miscellaneous Ions**	**Expected**	**Observed**
APMP	397.19	397.17	**PyPAP**	594.33	594.34	**PPyPAPMPPyPAP -prenyl, +2**	722.87	723.13
APMPPy	725.37	725.38	**PyPAPM**	725.37	725.38	**All, -prenyl, +2**	788.39	788.45
APMPPyPAP	990.51	990.52	**PyPAPMPP**	919.47	919.49	**PPyPAPMPPy, -prenyl +1**	1179.59	1179.53
APMPPyPAPM	1121.55	1121.56	**PyPAPMPPy**	1150.61	1150.46	**PPyPAPMPPyPA, -prenyl +1**	1347.68	1347.65
APMPPyPAPMP	1218.60	1218.43	**PyPAPMPPyP**	1247.66	1247.55	**PyPAPMPPyPAP -prenyl +2**	674.34	674.55
APMPPyPAPMPP	1315.66	1315.68	**PyPAPMPPyPAP**	1415.75	1415.75			
PMPP	423.21	423.26	**yPAPMP**	725.37	725.38			
PMPPy	654.33	654.38	**yPAPMPPyP**	1150.61	1150.46			
PMPPyPAP	919.47	919.49	**PAP**	266.15	266.10			
PMPPyPAPM	1050.51	1050.51	**PAPM**	397.19	397.17			
PMPPyPAPMPPy	1475.75	1475.66	**PAPMPPyP**	919.47	919.49			
MPPy	557.28	557.38	**PAPMPPyPA**	990.51	990.52			
MPPyP	654.33	654.38	**PAPMPPyPAPM**	1218.60	1218.43			
MPPyPA	725.37	725.38	**PAPMPPyPAPMP**	1315.66	1315.68			
MPPyPAPMP	1050.51	1050.51						
MPPyPAPMPPyP	1475.75	1475.66	**y- b- cleavages (+2)**					
PPy	426.24	426.28	**PyPAPMPPyPAP**	708.38	708.66			
PPyP	523.29	523.43	**PPyPAPMPPyPAP**	756.90	756.79			
PPyPA	594.33	594.34						
PPyPAP	691.38	691.37	**Miscellaneous Ions**					
PPyPAPMP	919.47	919.49	**PAPM -Met sidechain**	321.16	321.20			
PPyPAPMPP	1016.53	1016.41	**PAPM y- a- cleavage**	369.20	369.11			
PPyPAPMPPy	1247.66	1247.55	**PyPAP y- a- cleavage**	566.33	566.41			
PPyPAPMPPyPA	1415.75	1415.75	**PMPPyPA -Met sidechain**	746.39	746.40			
PyP	426.24	426.28	**APMPPyP y- a- cleavage**	794.43	794.55			

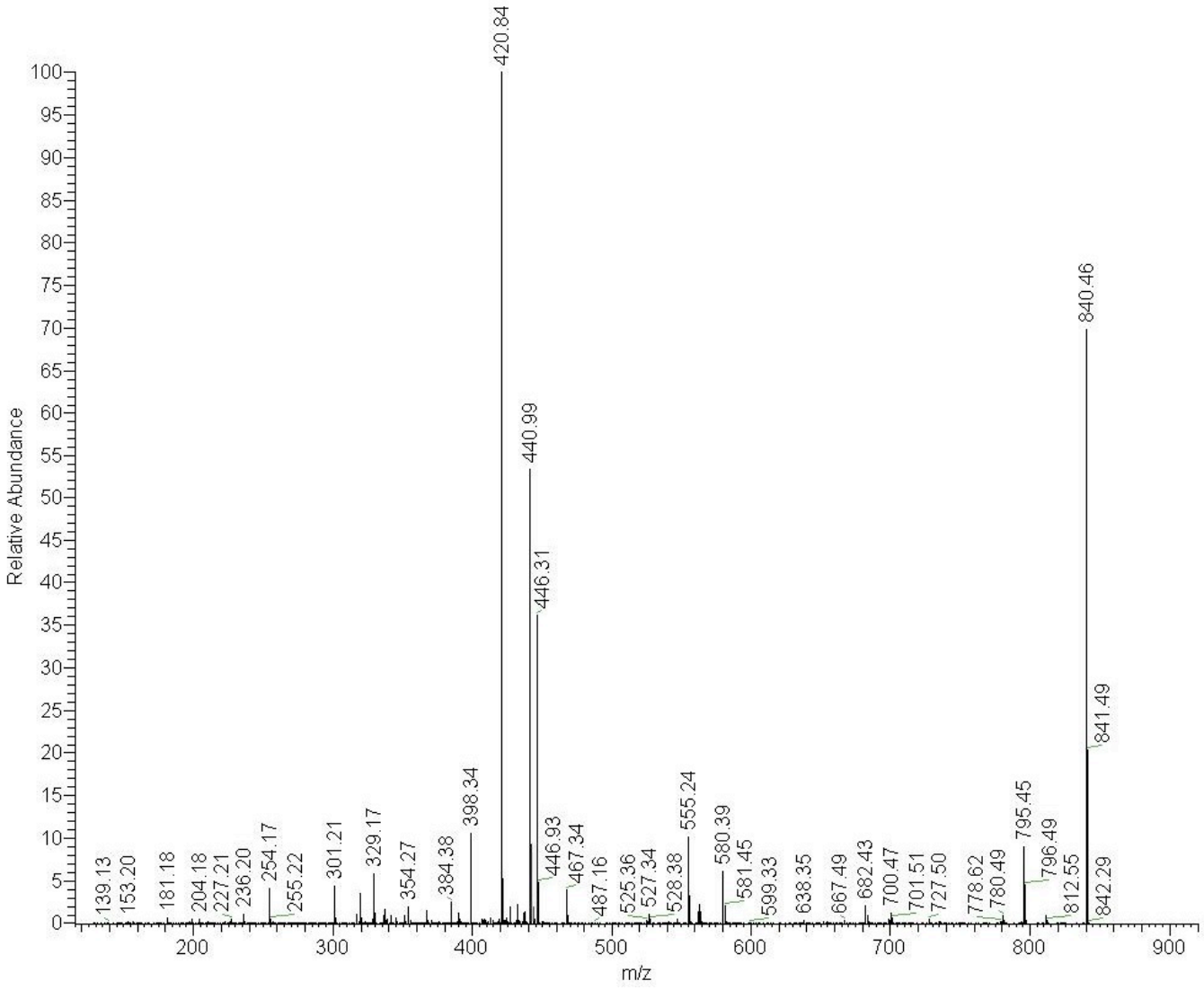
(u) FT-ICR data showing ion whose mass (+/- 2 ppm) corresponds to prenylated cyclo[KKPYILP]



(v) LC-FT-ICR chromatogram selected for mass of prenylated cyclo[KKPYILP]; in this case alone was only a single broad chromatographic peak observed, most likely indicating that C- and O-prenylated isomers were not well separated



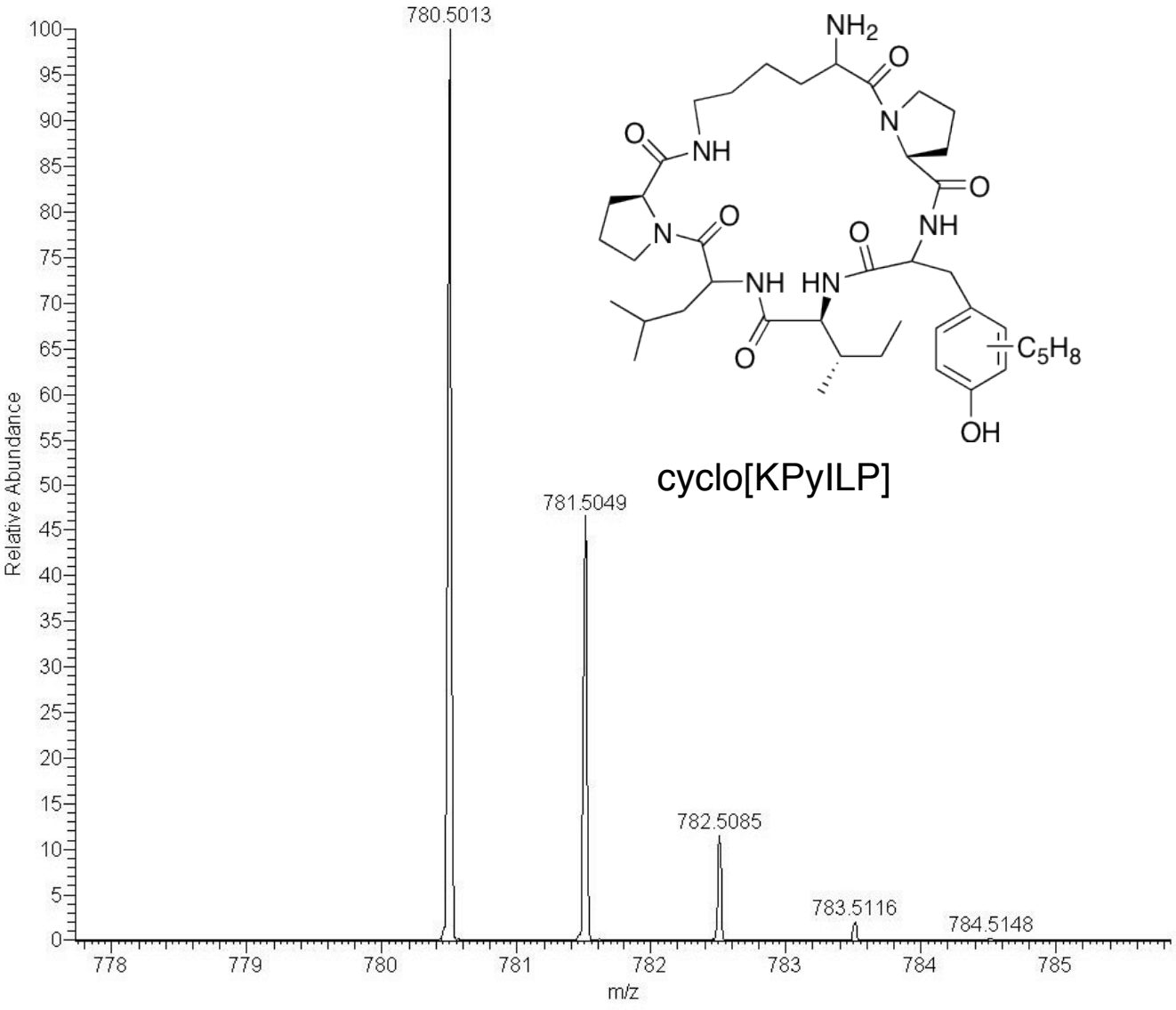
(w) MS-MS spectrum for product of reaction of LynF with cyclo[KKPYILP]



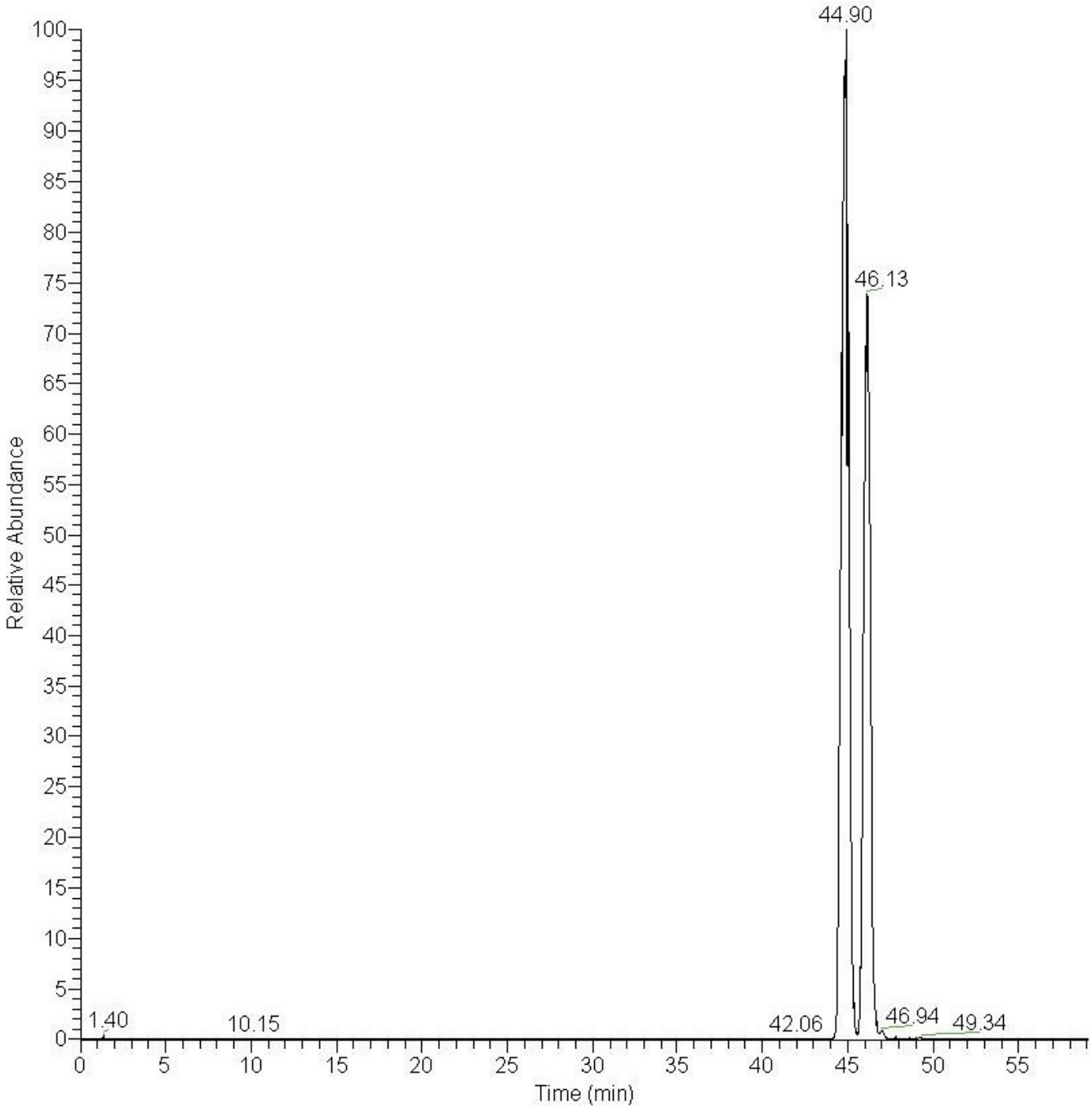
(x) MS-MS assignments for prenylated cyclo[KKPYILP] single peak; assignments consistent with presence of O-prenylated compound

cyclo[KKPYILP] single peak					
y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+1)**	**Expected**	**Observed**
KK	257.20	-	**ILPKKP**	677.47	-
KKP	354.25	354.27	**LP**	211.14	-
KKPy	585.37	-	**LPK**	339.24	339.28
KKPyI	698.46	698.44	**LPKK**	467.33	467.34
KKPyIL	811.54	811.53	**LPKKP**	564.39	-
KP	226.16	-	**LPKKPy**	795.51	795.45
KPy	457.28	-	**PK**	226.16	-
KPyI	570.36	-	**PKK**	354.25	354.27
KPyIL	683.45	-	**PKKP**	451.30	-
KPyILP	780.50	780.49	**PKKPy**	682.43	682.43
Py	329.18	329.17	**PKKPyI**	795.51	795.45
PyI	442.27	442.16			
PyIL	555.35	555.24	**y- b- cleavages (+2)**		
PyILP	652.40	-	**KKPyI**	349.74	349.83
PyILPK	780.50	780.49	**PKKPyI**	398.26	398.35
yI	345.22	345.23	**PKKPy**	341.72	341.87
yIL	458.30	-			
yILP	555.35	555.24	**Miscellaneous Ions**		
yILPK	683.45	-	**All -prenyl +1**	840.54	840.46
yILPKK	811.54	811.53	**All -prenyl +2**	420.77	420.85
IL	227.18	227.21	**All -NH3, +2**	446.29	446.31
ILP	324.23	-	**All -acylium, +2**	440.81	440.98
ILPK	452.32	-	**Py y- a- cleavage +1**	301.19	301.21
ILPKK	580.42	580.39			

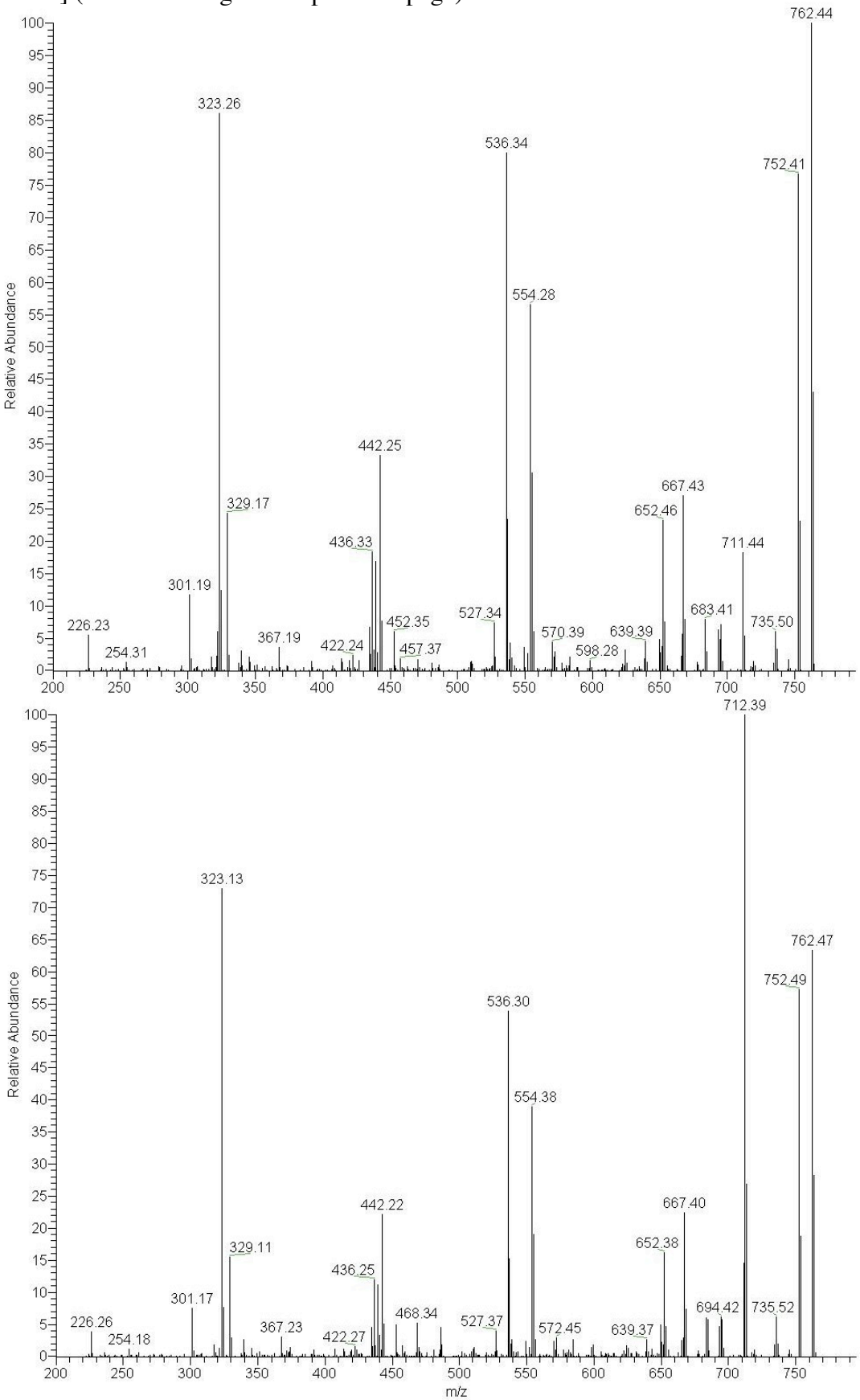
(y) FT-ICR data showing ion whose mass (+/- 2 ppm) corresponds to prenylated cyclo[KPYILP]



(z) LC-FT-ICR chromatogram selected for mass of prenylated cyclo[KPYILP]



(aa) MS-MS spectra for early (top) and late (bottom) eluting products of reaction of LynF with cyclo[KPYILP] (see chromatogram on previous page)

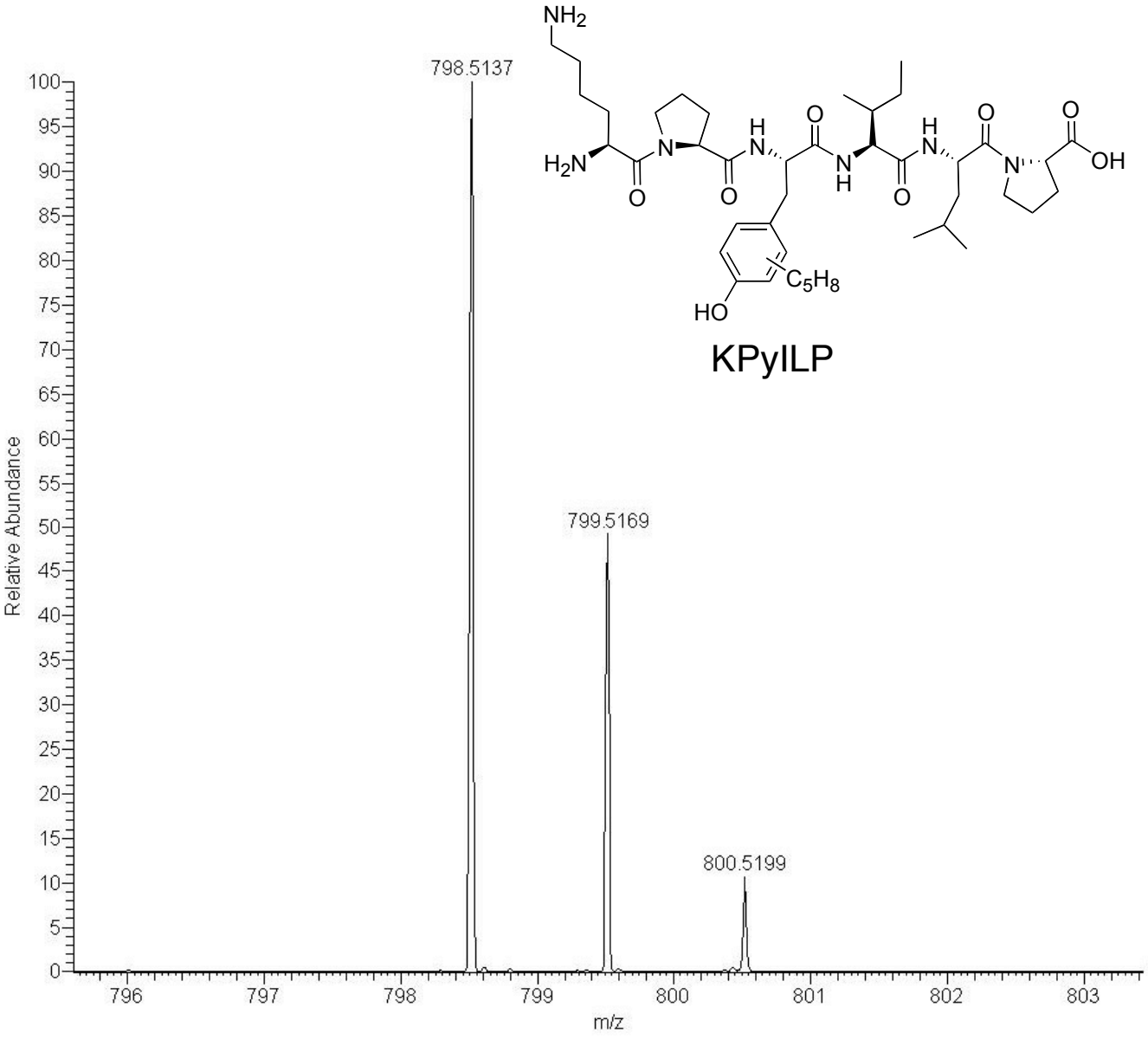


(bb) MS-MS assignments for prenylated cyclo[KPYILP] early (top) and late (bottom) eluting isomers

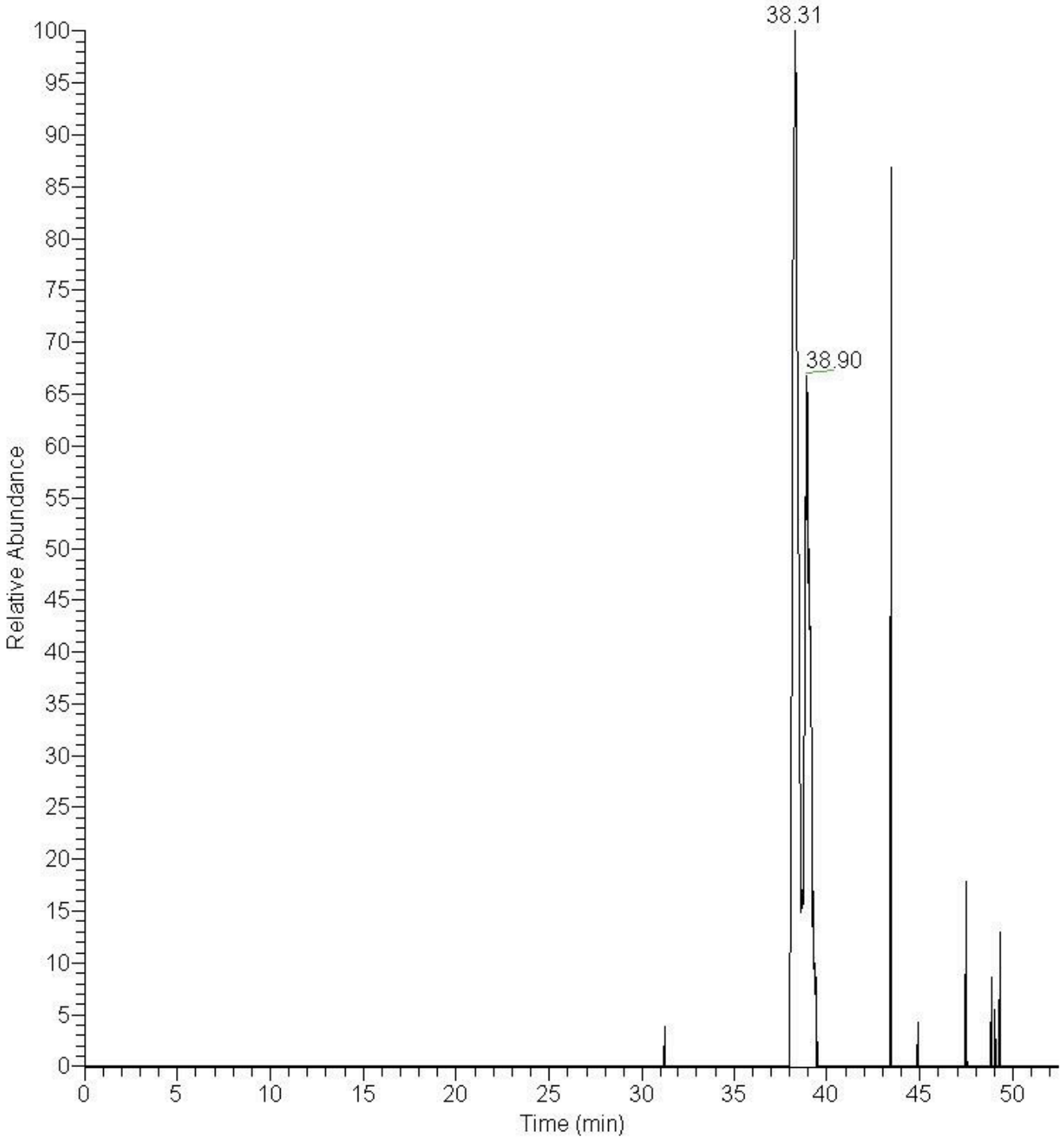
cyclo[KPYILP] 1st peak (C-prenyl)					
y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+2)**	**Expected**	**Observed**
KP	226.16	226.23	**KPyIL**	342.23	342.25
KPy	457.28	457.37	**PKPyl**	334.22	334.27
KPyl	570.36	570.39			
KPyIL	683.45	683.41	**Miscellaneous Ions**		
Py	329.18	329.17	**All -H2O**	762.49	762.44
Pyl	442.27	442.25	**All -acylium**	752.51	752.41
PyIL	555.35	555.32	**PKPy -H2O**	536.32	536.34
PyILP	652.40	652.46	**Py y- a- cleavage +1**	301.19	301.19
yl	345.22	345.19			
yIL	458.30	-			
yILP	555.35	555.32			
yILPK	683.45	683.41			
IL	227.18	-			
ILP	324.23	-			
ILPK	452.32	452.35			
ILPKP	549.38	549.36			
LP	211.14	-			
LPK	339.24	339.31			
LPKP	436.29	436.33			
LPKPy	667.42	667.43			
PK	226.16	226.23			
PKP	323.21	323.26			
PKPy	554.33	554.28			
PKPyl	667.42	667.43			

cyclo[KPYILP] 2nd peak (O-prenyl)					
y- b- cleavages (+1)	**Expected**	**Observed**	**y- b- cleavages (+2)**	**Expected**	**Observed**
KP	226.16	226.26	**KPyIL**	342.23	-
KPy	457.28	-	**PKPyl**	334.22	-
KPyl	570.36	-			
KPyIL	683.45	-	**Miscellaneous Ions**		
Py	329.18	329.11	**All -H2O**	762.49	762.47
Pyl	442.27	442.22	**All -acylium**	752.51	752.49
PyIL	555.35	555.25	**All -prenyl**	712.44	712.39
PyILP	652.40	652.38	**All -H2O, -prenyl**	694.43	694.42
yl	345.22	345.11	**PKPy -H2O**	536.32	536.30
yIL	458.30	-	**Py y- a- cleavage +1**	301.19	301.17
yILP	555.35	555.32			
yILPK	683.45	-			
IL	227.18	-			
ILP	324.23	-			
ILPK	452.32	-			
ILPKP	549.38	-			
LP	211.14	-			
LPK	339.24	-			
LPKP	436.29	436.25			
LPKPy	667.42	667.40			
PK	226.16	226.26			
PKP	323.21	323.13			
PKPy	554.33	554.38			
PKPyl	667.42	667.40			

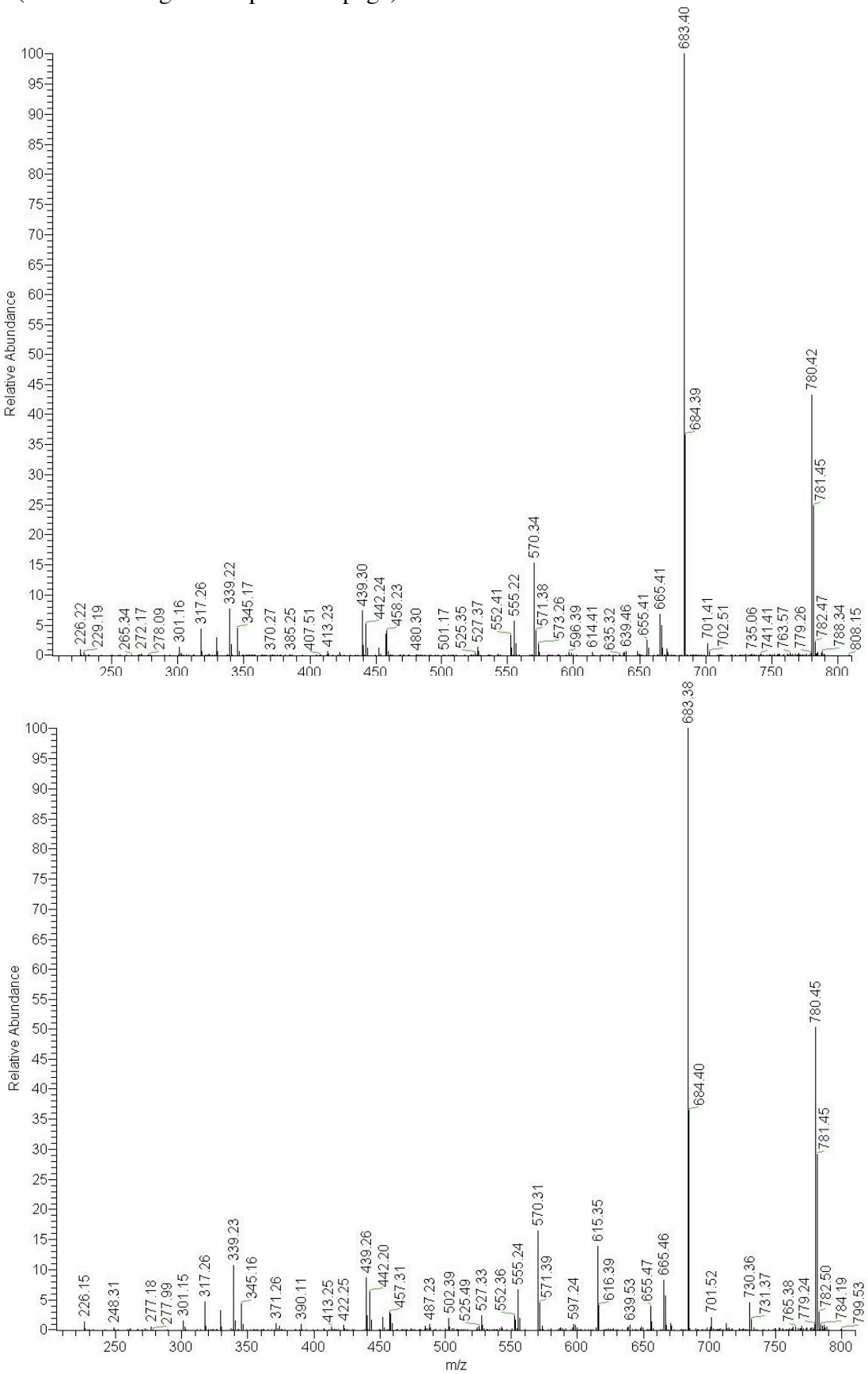
(cc) FT-ICR data showing ion whose mass (+/- 2 ppm) corresponds to prenylated KPYILP



(dd) LC-FT-ICR chromatogram selected for mass of prenylated KPYILP



(ee) MS-MS spectra for early (top) and late (bottom) eluting products of reaction of LynF with KPYILP (see chromatogram on previous page)

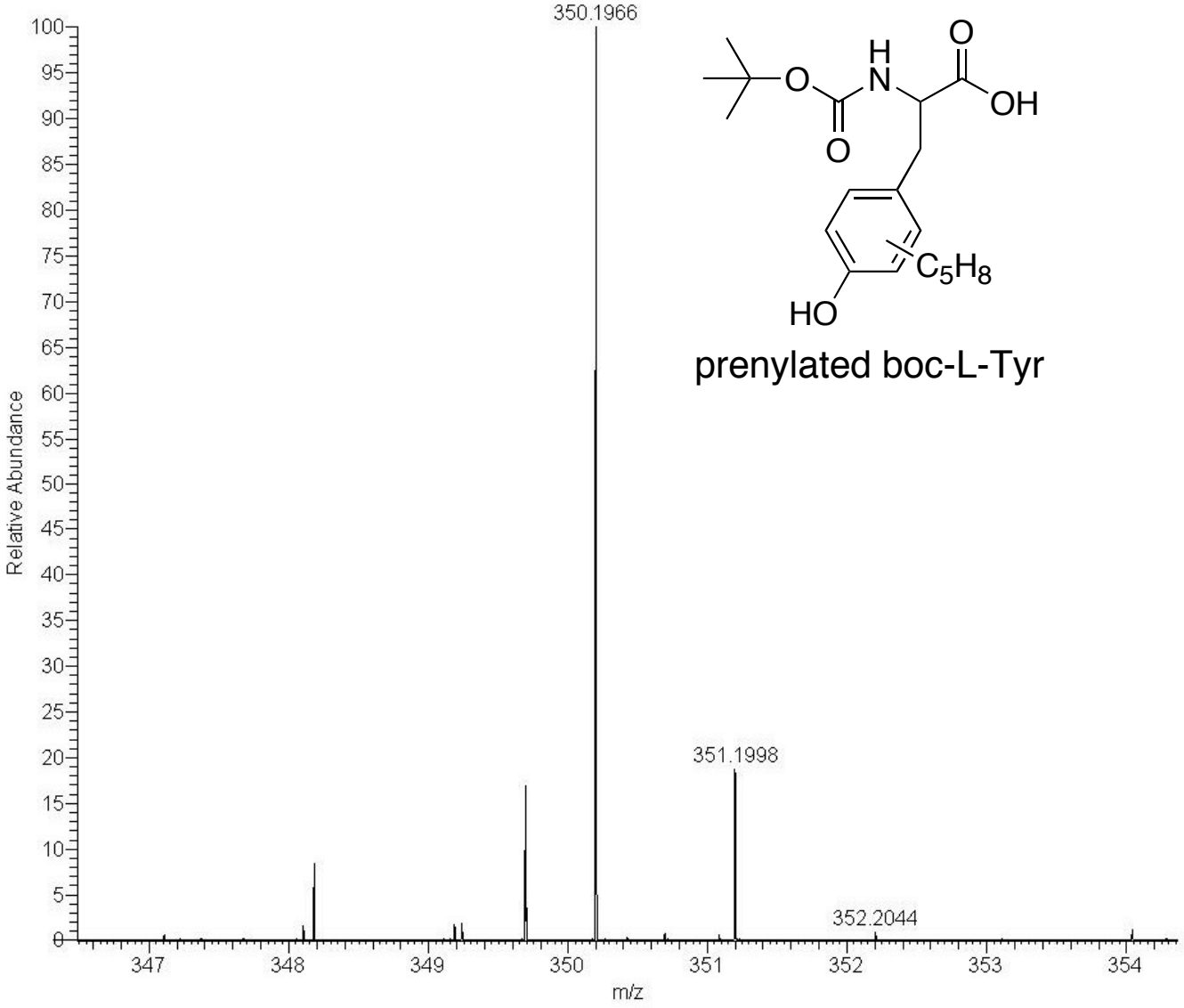


(ff) MS-MS assignments for prenylated KPYILP early (left) and late (right) eluting isomers

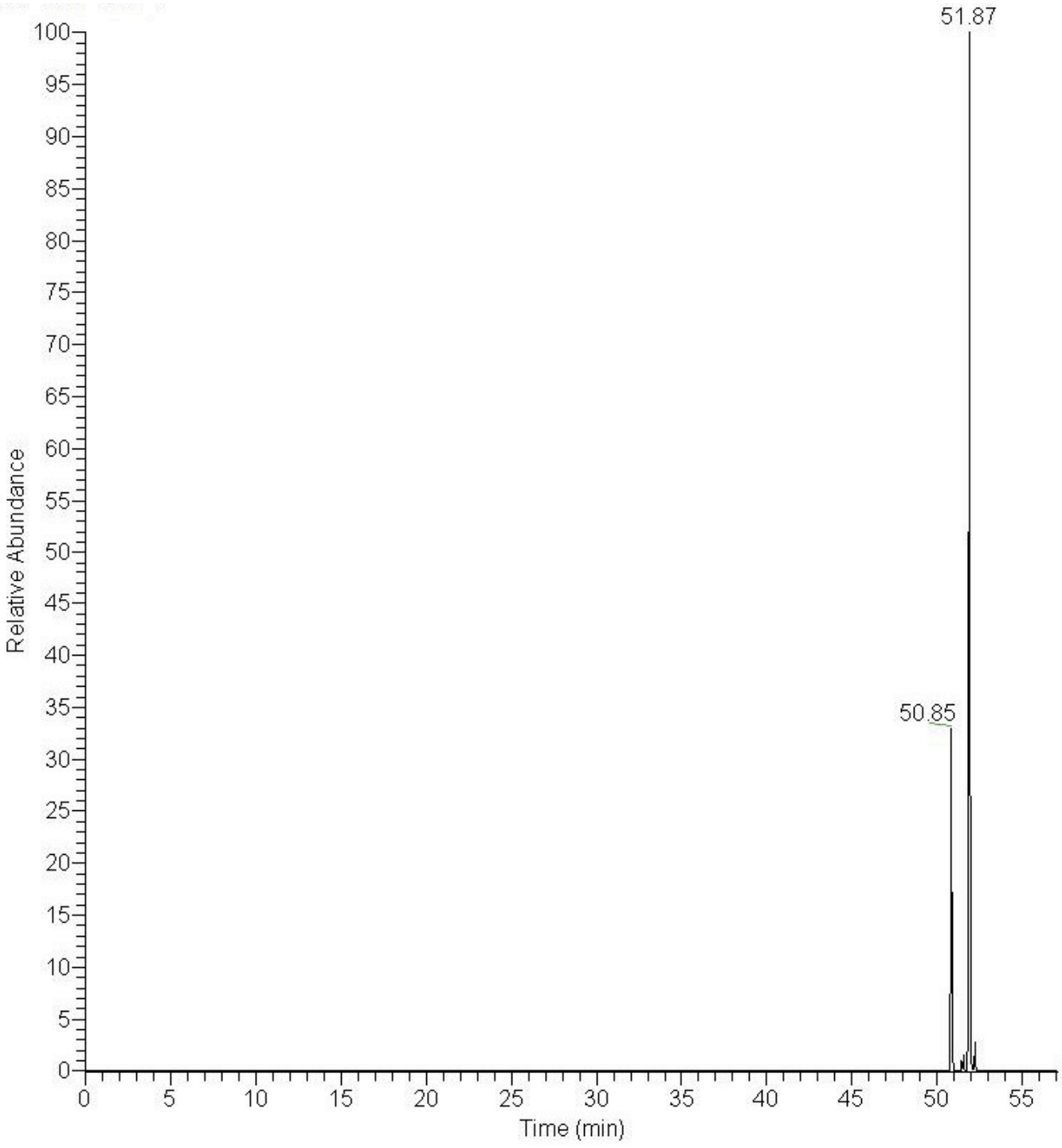
KPYILP 1st peak (C-prenyl)		
b-ions (+1)	**Expected**	**Observed**
KP	226.16	226.22
KPy	457.28	457.25
KPy°	439.27	439.30
KPyl	570.36	570.34
KPyl°	552.35	552.41
KPylL	683.45	683.41
KPYIL°	665.44	665.41
KPylLP°	780.50	780.42
y-ions (+1)		
PylLP	670.41	-
ylLP	573.36	573.26
lLP	342.24	-
LP	229.15	-
y- b- cleavages (+1)		
LPK	339.24	339.22
yl	345.22	345.17
ylL	458.30	458.23
Pyl	442.27	442.24
PylL	555.35	555.22
y- a- cleavages (+1)		
yl	317.22	317.26
ylLP	527.36	527.37
Miscellaneous Ions		
KPylL a-cleavage +1	655.45	655.47

KPYILP 2nd peak (O-prenyl)		
b-ions (+1)	**Expected**	**Observed**
KP	226.16	226.13
KPy	457.28	457.31
KPy°	439.27	439.26
KPyl	570.36	570.31
KPyl°	552.35	552.35
KPylL	683.45	683.38
KPYIL°	665.44	665.46
KPylLP°	780.50	780.44
y-ions (+1)		
PylLP	670.41	670.35
ylLP	573.36	-
lLP	342.24	-
LP	229.15	-
y- b- cleavages (+1)		
Py	329.19	329.17
LPK	339.24	339.24
Pyl	442.27	442.20
PylL	555.35	555.24
yl	345.22	345.15
ylL	458.30	458.31
y- a- cleavages (+1)		
yl	317.22	317.24
ylLP	527.36	527.31
Miscellaneous Ions		
KPYILP -prenyl +1	730.45	730.40
KPYILP a-cleavage +1	655.45	655.46
KPylL -prenyl +1	615.39	615.36
KPyl -prenyl +1	502.30	502.39

(gg) FT-ICR data showing ion whose mass (+/- 2 ppm) corresponds to prenylated boc-L-tyrosine



(hh) LC-FT-ICR chromatogram selected for the mass of prenylated boc-L-tyrosine



(jj) ESI-MS spectrum showing ion corresponding to the mass of prenylated N-acetyl-L-Tyr

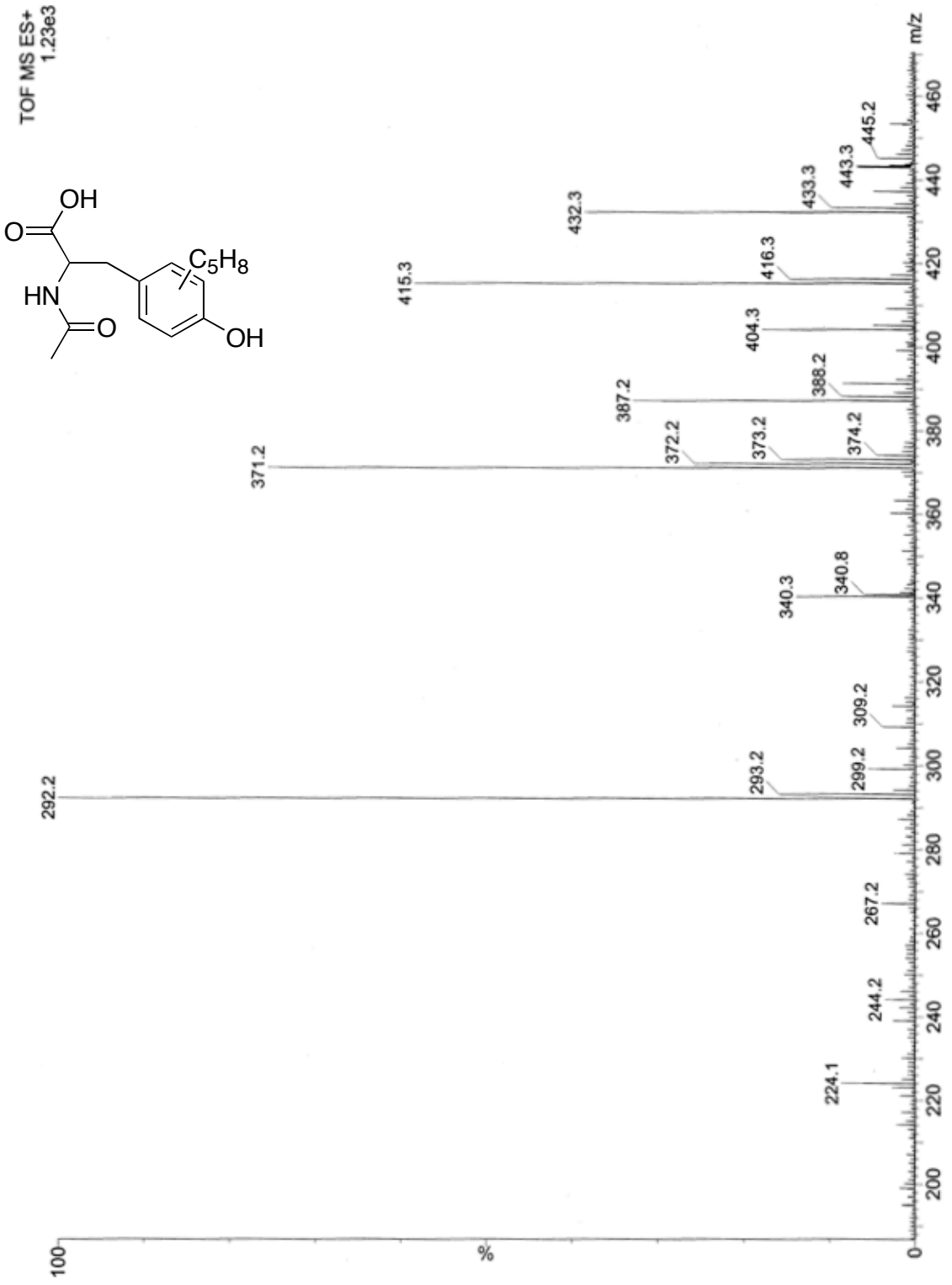
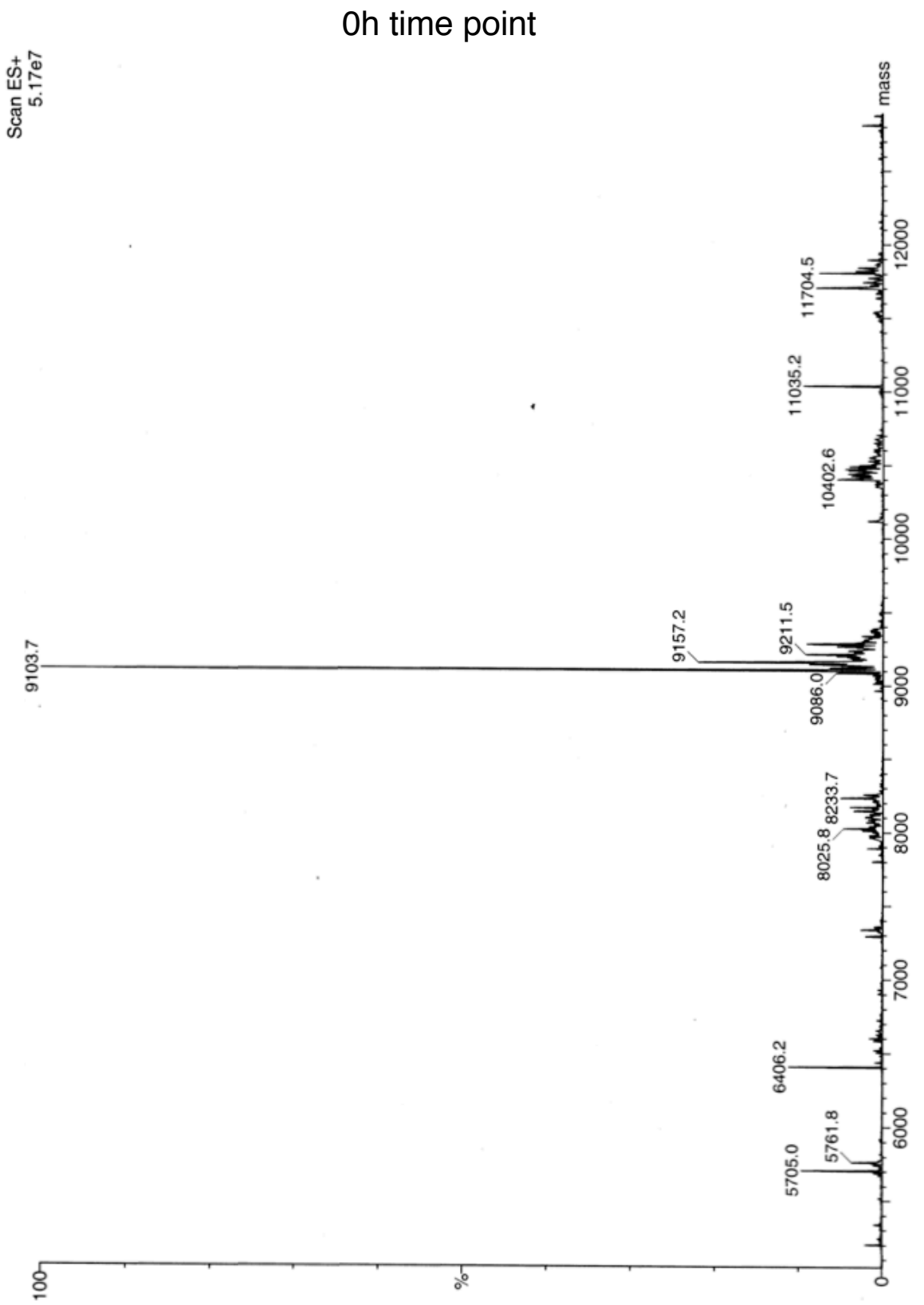
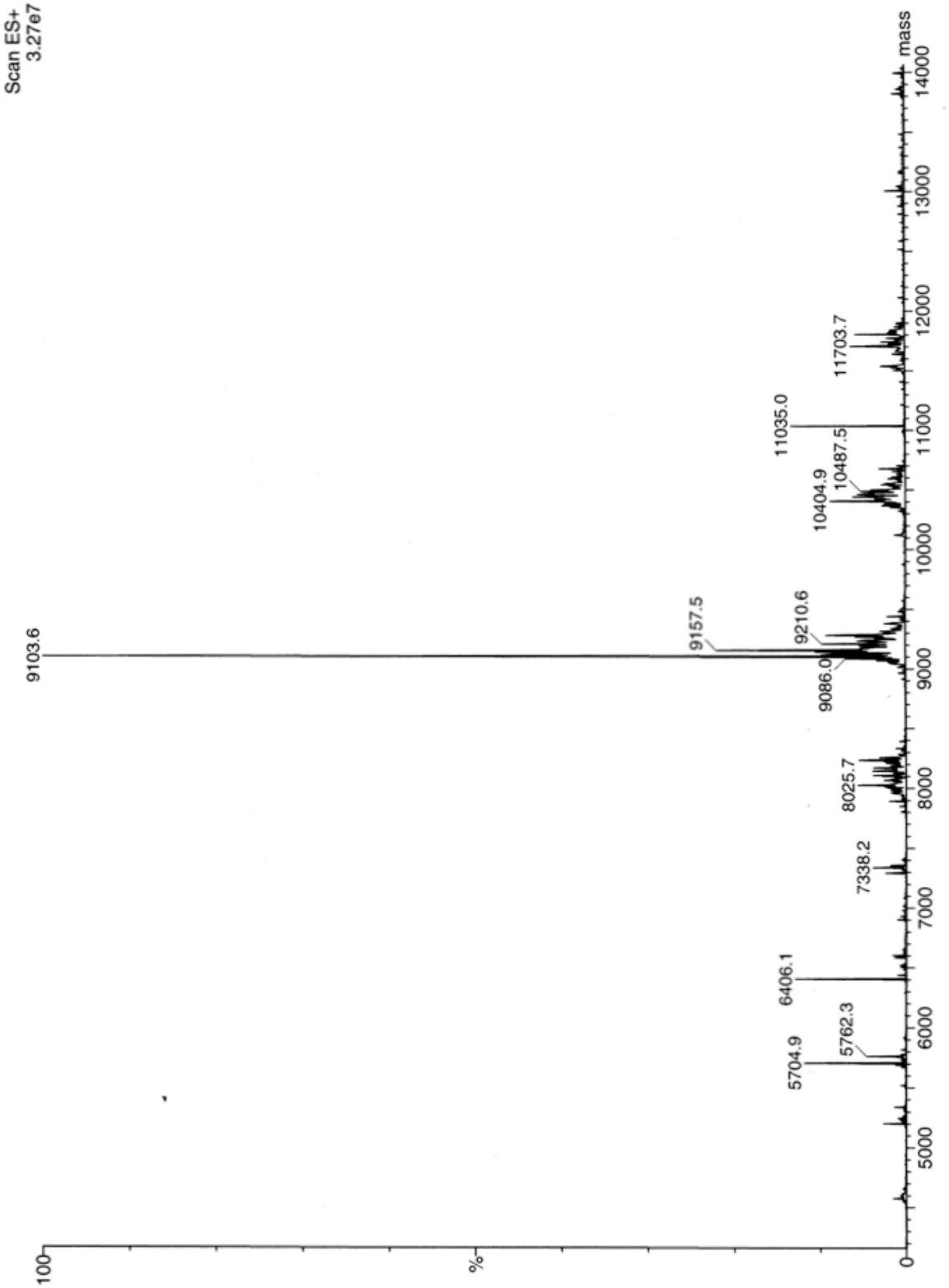


Figure S2. Precursor peptides are not substrates for prenyltransfer (a) ESI-MS intact analysis of time points taken from a reaction containing TruLy1, LynF and standard additives at 0, 6, 12, 24 h (deconvoluted spectra shown); expected mass of unmodified TruLy1 is 9104 Da. No reaction is observed even after 24 h incubation at 37 °C (b) Incorporation of tritiated DMAPP into various substrates; background subtracted counts-per-minute (CPM) shown

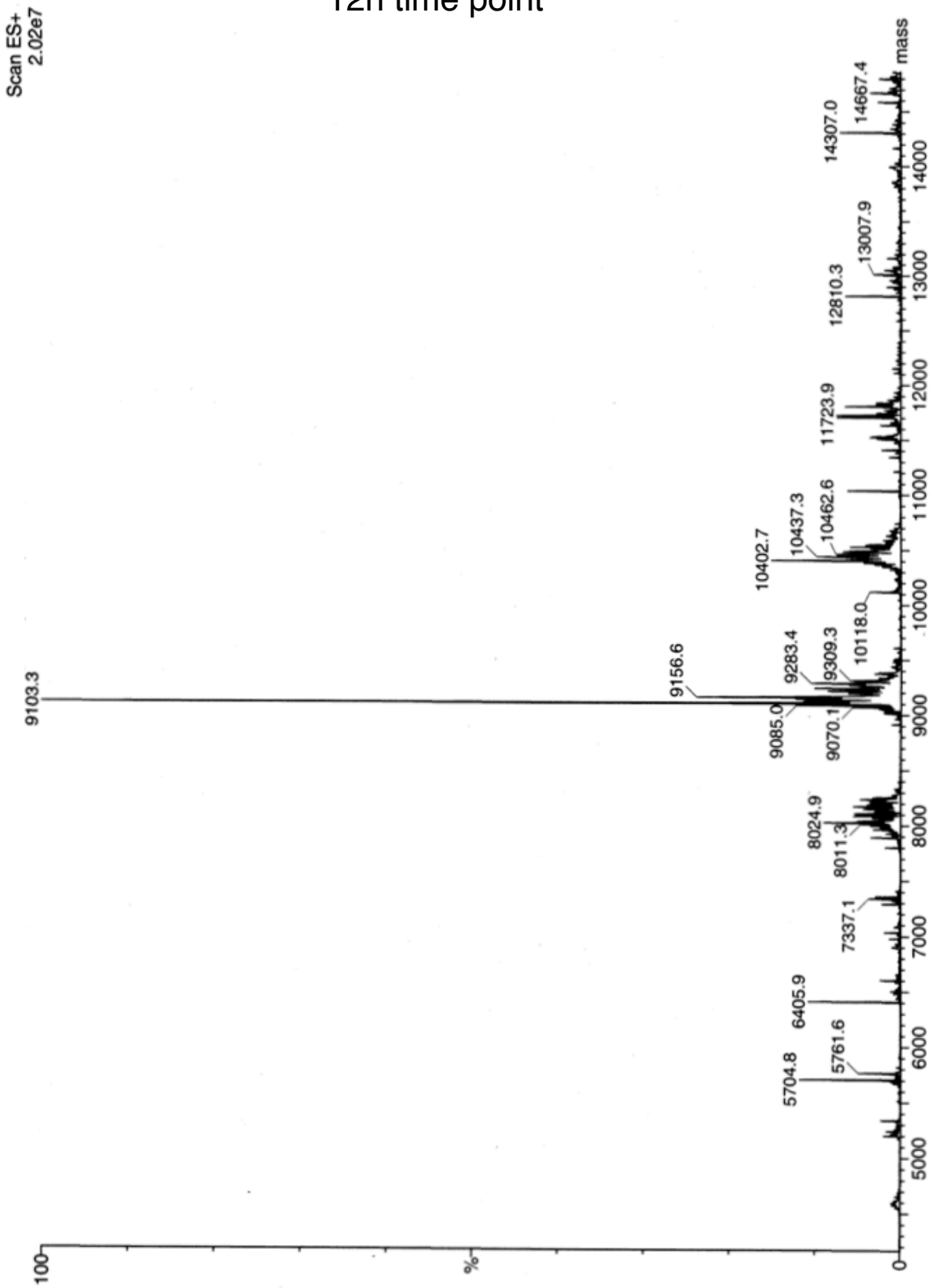
(a)


0h time point
Scan ES+
5.17e7
mass
12000
11000
10000
9000
8000
7000
6000
11704.5
11035.2
10402.6
9211.5
9157.2
9103.7
9086.0
8233.7
8025.8
6406.2
5761.8
5705.0
100
%
0

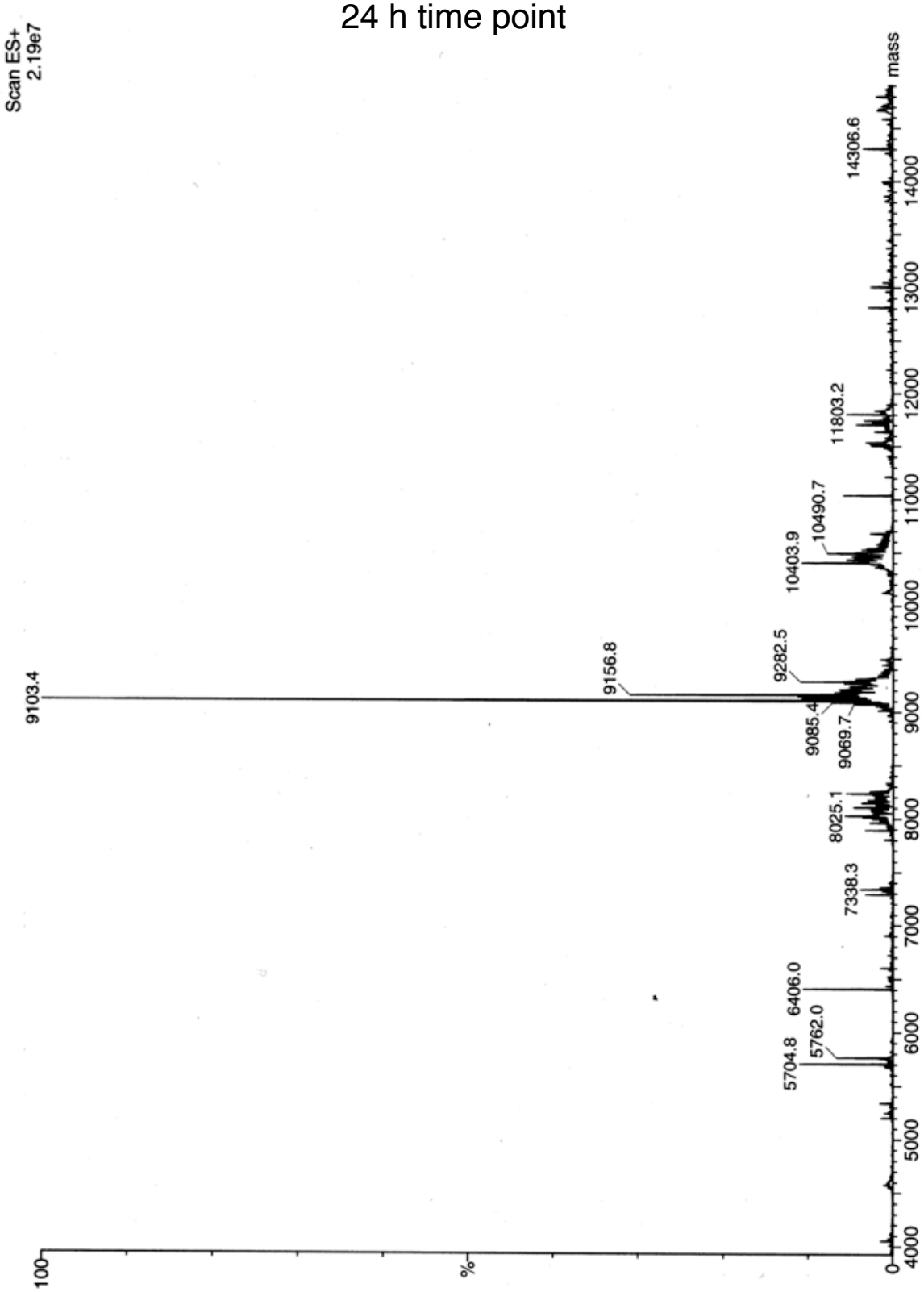
6h time point



12h time point



24 h time point



(b)

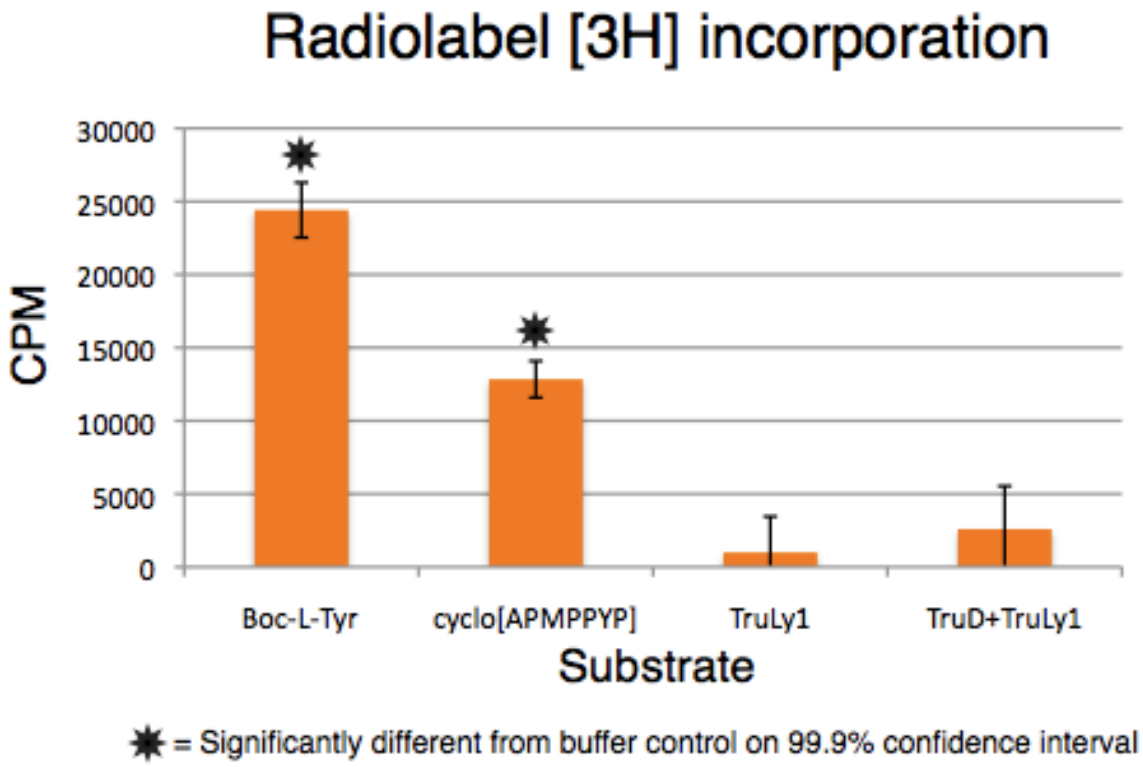
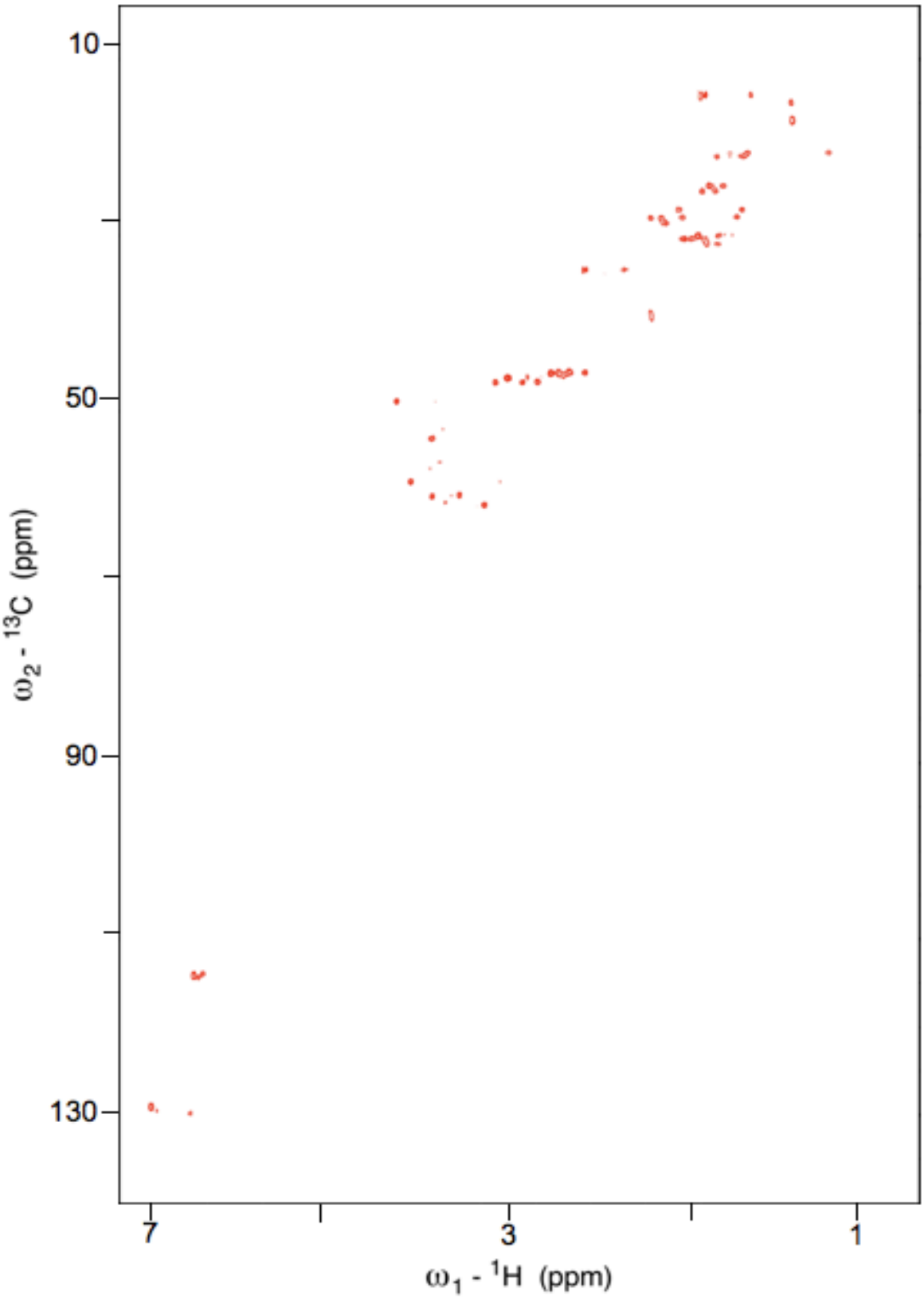
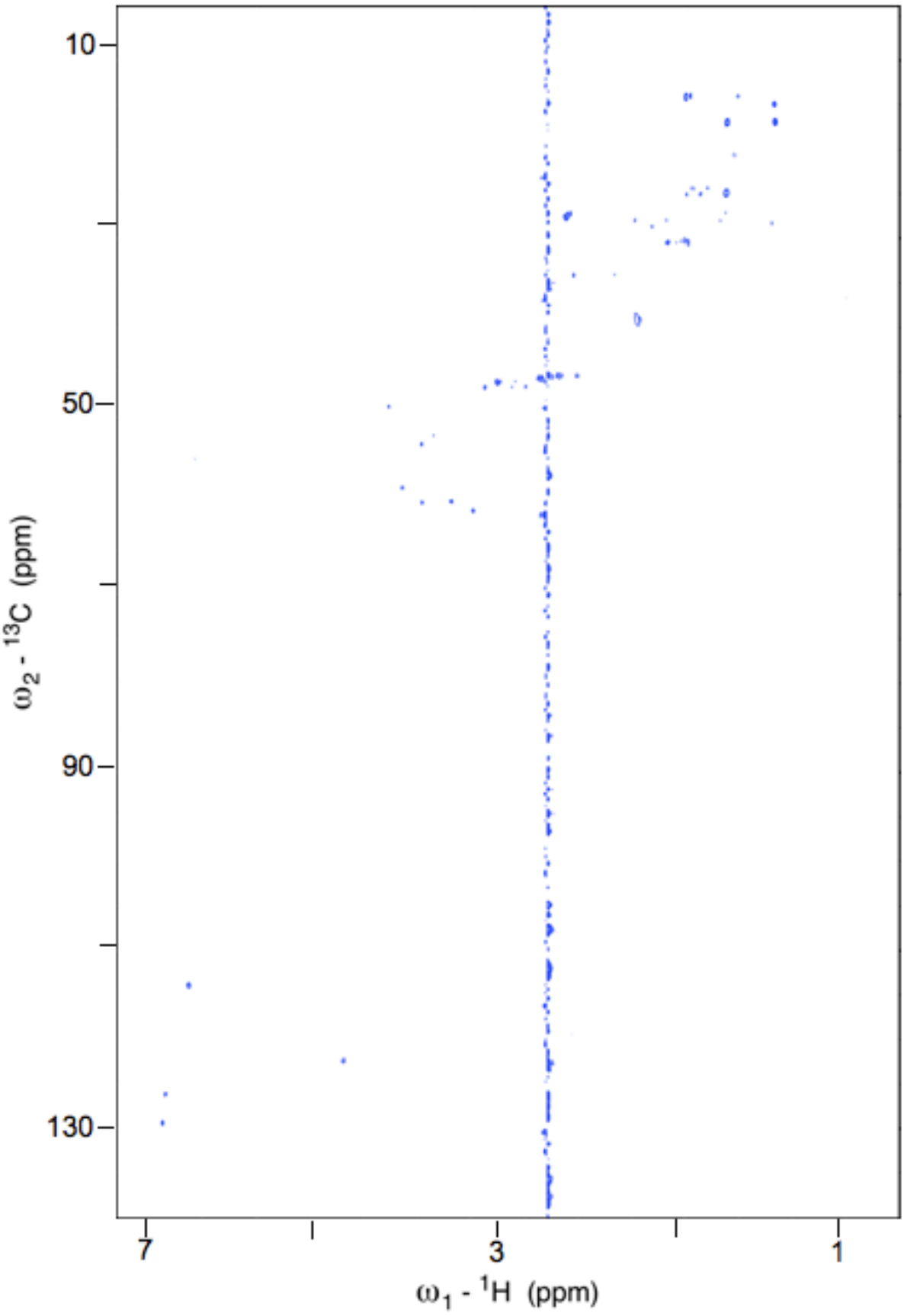

Radiolabel [3H] incorporation
30000
25000
20000
15000
10000
5000
0
CPM
Boc-L-Tyr
cyclo[APMPPYP]
TruLy1
TruD+TruLy1
Substrate
= Significantly different from buffer control on 99.9% confidence interval

Figure S3. NMR characterization of substrates cyclo[APMPPYP] and boc-L-tyrosine (a) HSQC of cyclo[APMPPYP] (b) HSQC of prenylated cyclo[APMPPYP] (c) Overlay of (a) and (b) data (d) table of assignments (e) HSQC of C-prenyl boc-L-Tyr (f) Table of assignments and numbered structure

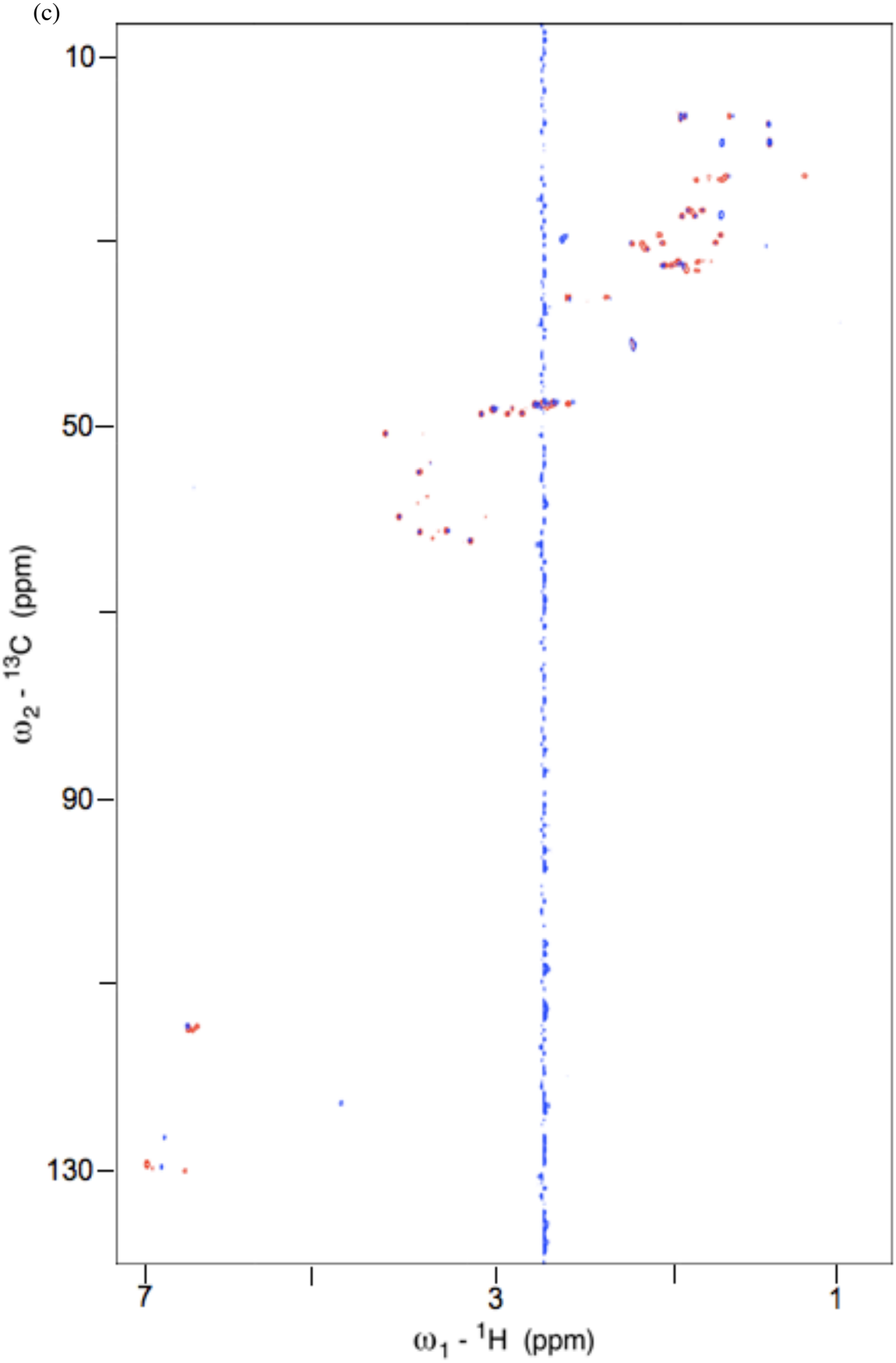
(a)



(b)



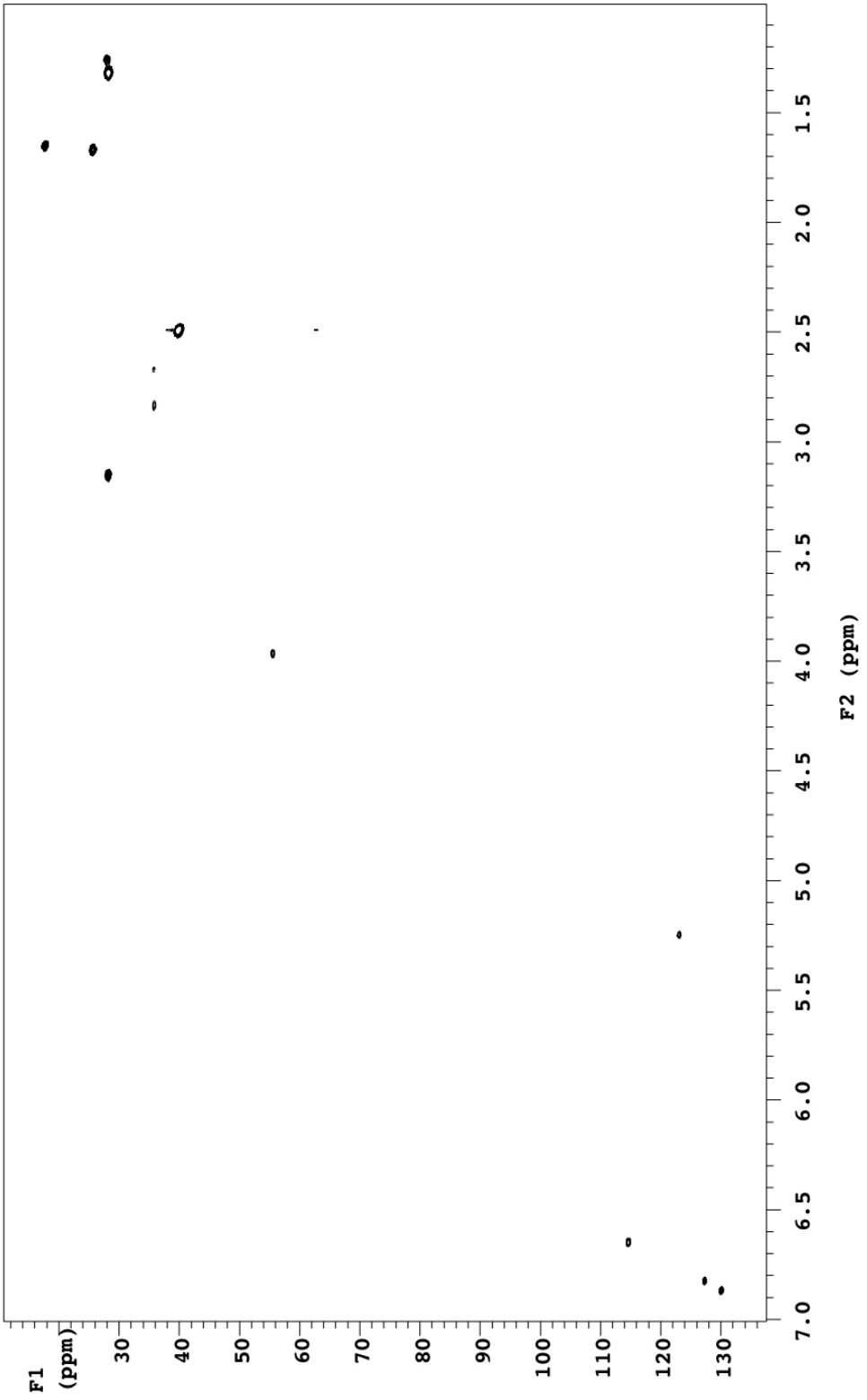
(c)



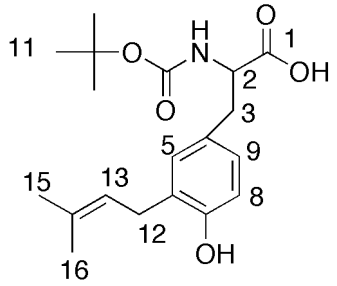
(d)

cyclo[APMPPYP] unmod	13C	1H	cyclo[APMPPY*P] product	13C	1H
Tyr Cδ	129.86	7.06	**Tyr Cδ1**	130.08	6.92
Tyr Cε	114.96	6.67	**Tyr Cδ2**	126.85	6.89
Pro Cα	61.12	4.01	**C2 of 3-methyl-2-buten-1-yl adduct**	122.9	5.23
Pro Cα	60.15	4.25	**Tyr Cε**	114.61	6.68
Pro Cα	60.25	4.50	**Pro Cα**	61.07	4.02
Pro Cα	58.64	4.69	**Pro Cα**	60.13	4.22
Tyr Cα	53.66	4.50	**Pro Cα**	60.13	4.50
Met Cα	49.52	4.82	**Pro Cα**	58.59	4.69
Ala Cα	47.22	3.91	**Tyr Cα**	53.57	4.50
Pro Cδ	47.22	3.67	**Met Cα**	49.48	4.81
		3.53	**Ala Cα**	47.24	3.91
Pro Cδ	46.74	3.81	**Pro Cδ**	47.06	3.66
Pro Cδ	46.12	3.41			3.53
		3.34	**Pro Cδ**	46.75	3.79
Pro Cδ	46.09	3.24			3.62
		3.09	**Pro Cδ**	46.02	3.40
DMSO	39.51	2.49	**Pro Cδ**	45.79	3.22
Tyr Cβ	34.53	3.11			3.05
		2.74	**DMSO**	39.51	3.49
Pro Cβ	31.4	1.98	**Tyr Cβ**	34.61	3.08
		1.88			2.70
Met Cγ	30.82	2.19	**C1 of 3-methyl-2-buten-1-yl adduct**	28.13	3.15
		2.12	**Pro Cβ**	31.34	1.88
Pro Cβ	30.48	2.07		30.87	2.02
		1.88	**Met Cγ**	30.83	2.20
Met Cβ	28.57	2.50			2.12
		2.40	**Pro Cβ**	30.52	2.05
Pro Cβ	28.36	2.21			1.89
		1.71	**Met Cβ**	29.18	2.39
Pro Cβ	27.68	2.24	**Pro Cβ**	28.45	2.21
		1.67			1.71
Pro Cγ	25.5	2.02	**Pro Cβ**	27.62	2.24
		1.91			1.67
Pro Cγ	24.89	1.96	**Pro Cγ**	25.39	2.02
		1.83			1.90
Pro Cγ	21.5	1.89	**trans methyl of 3-methyl-2-buten-1-yl adduct**	25.27	1.65
		1.65	**Pro Cγ**	24.82	1.97
Pro Cγ	21.14	1.77			1.83
		1.61	**Pro Cγ**	21.43	1.91
Ala Cβ (rotamers)	17.32	1.20			1.62
	15.42	1.21	**Pro Cγ**	21.03	1.77
Met Cε (rotamers)	14.54	2.03			1.58
		1.99	**cis methyl of 3-methyl-2-buten-1-yl adduct**	17.31	1.65
		1.58	**Ala Cβ (rotamers)**	17.31	1.20
				15.45	1.21
			Met Cε (rotamers)	14.06	2.03
					1.99
					1.55

(e)


F1 (ppm)
30
40
50
60
70
80
90
100
110
120
130
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
F2 (ppm)

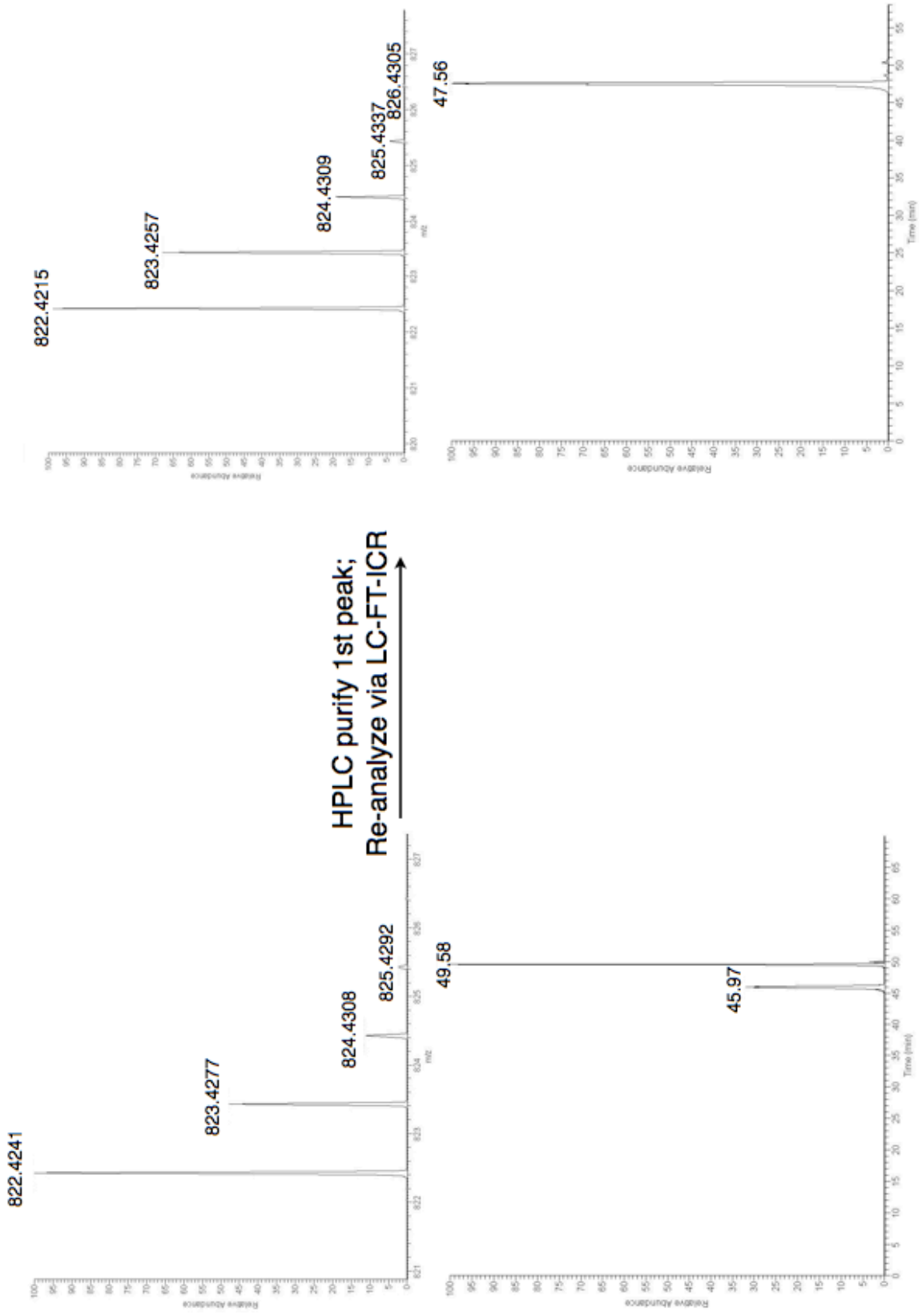
(f)



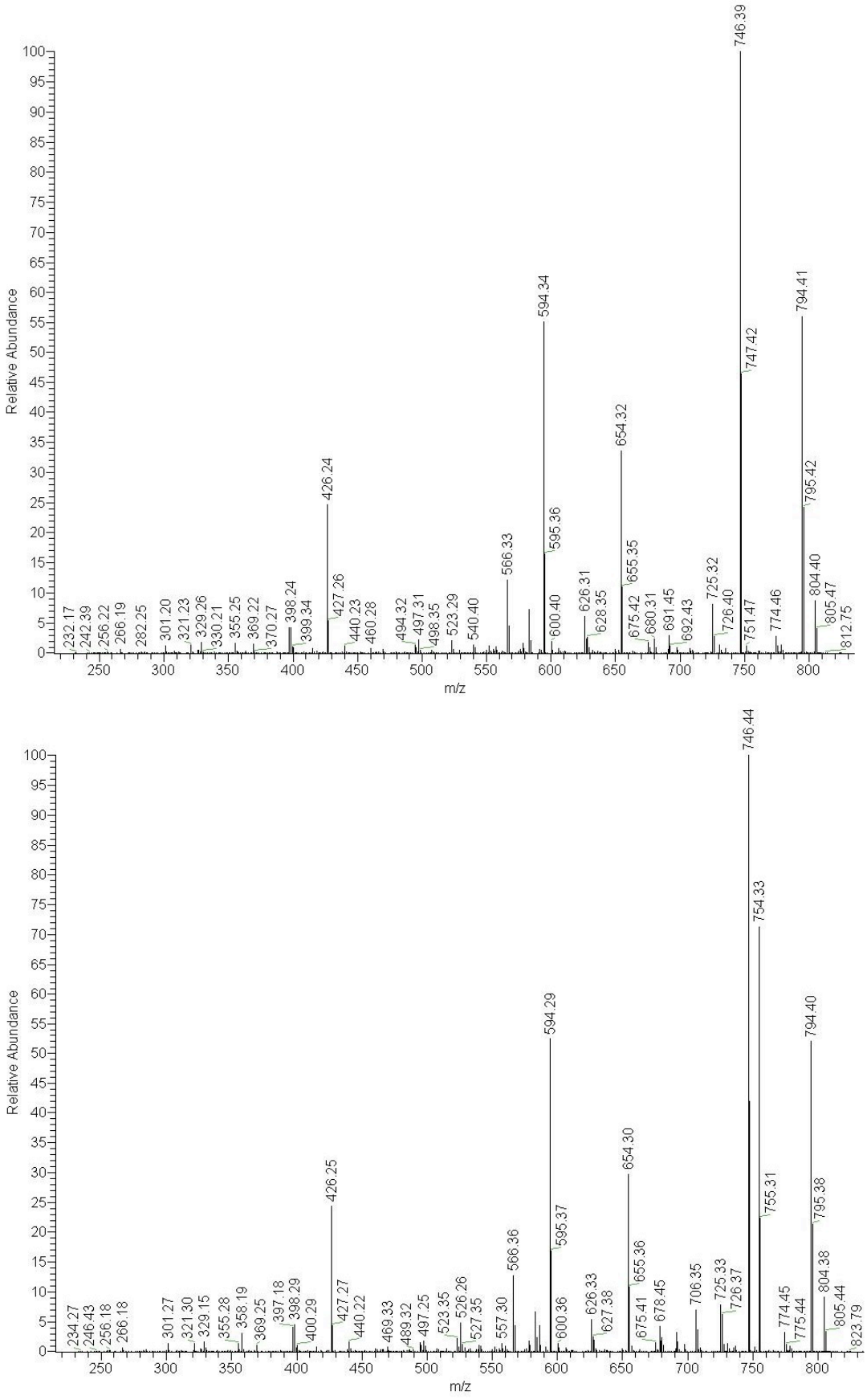
Position	δ 13C	δ 1H
5	129.5	6.87
9	126.8	6.82
13	122.6	5.25
8	114.2	6.65
2	55.0	3.96
DMSO	39.5	2.49
3a	35.5	2.66
3b	35.5	2.83
12	27.9	3.15
11a	27.7	1.32
11b	27.4	1.26
15	25.2	1.67
16	17.2	1.65

Figure S4. Spectral properties of C- and O-prenylated tyrosyl componds (a) Left: LC-FT-ICR peaks and chromatograms selected for the mass of prenylated cyclo[APMPPYP] from unpurified reaction mixtures; note that two separable compounds corresponding to the mass of cyclo[APMPPYP] are present. Right: Analysis by LC-FT-ICR of the NMR-characterized C-prenylated cyclo[APMPPYP]; for this compound only a single peak with a mass corresponding to prenylated cyclo[APMPPYP] is observed (b) MS-MS spectra for early (top) and late (bottom) eluting-species observed in initial cyclo[APMPPYP] reaction mixtures and tables of assignments; assignments for early-eluting species consistent with C-prenylation, while those for the late-eluting species consistent with O-prenylation (c) Table of assignments for O- and C-prenylated compounds (d) MS-MS spectrum for NMR-characterized C-prenylated cyclo[APMPPYP]; MS-MS shows no loss of isoprene (e) Table of assignments for C-prenylated reference compound.

(a)


822.4215
823.4257
824.4309
825.4337
826.4305
47.56
HPLC purify 1st peak;
Re-analyze via LC-FT-ICR
822.4241
823.4277
824.4308
825.4292
49.58
45.97

(b)

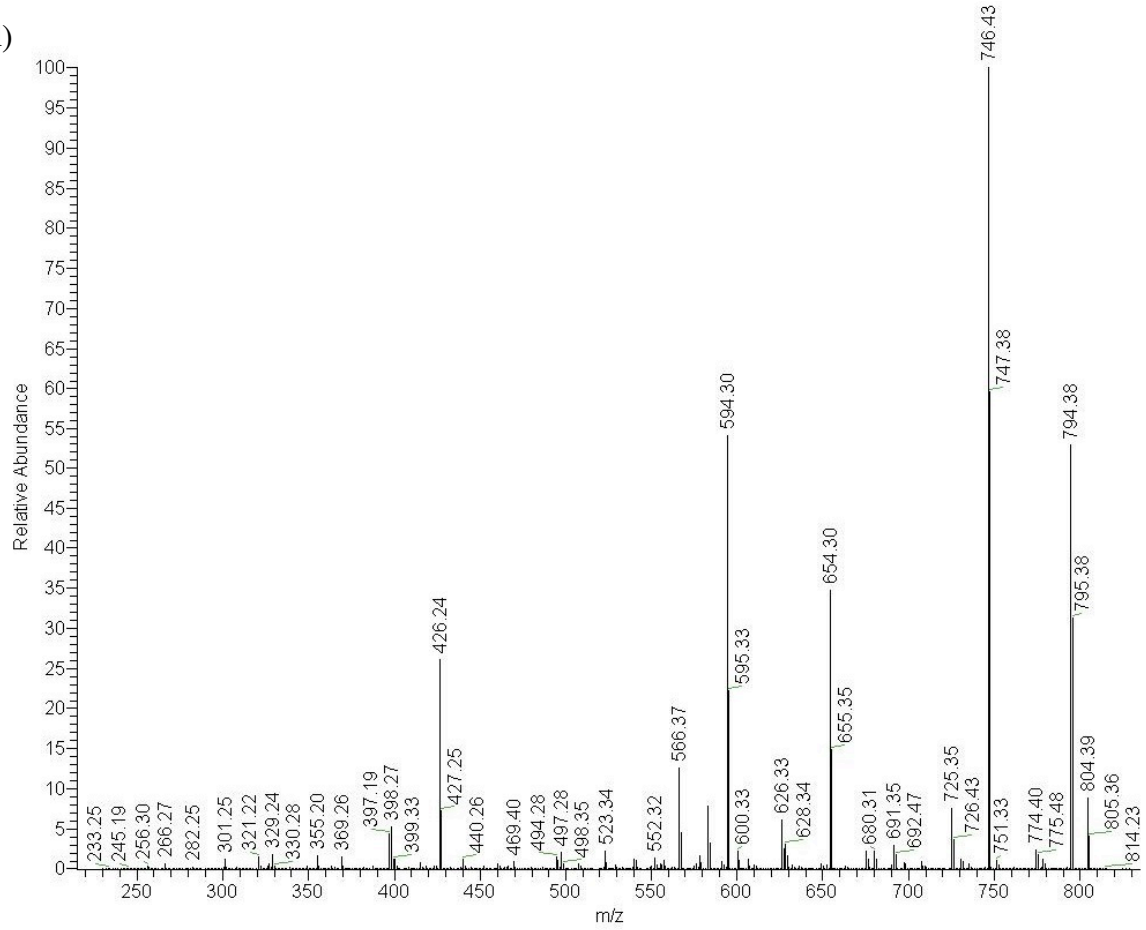

Relative Abundance
m/z
746.39
594.34
794.41
426.24
654.32
747.42
795.42
595.36
566.33
655.35
804.40
725.32
805.47
626.31
675.42
680.31
691.45
692.43
726.40
751.47
774.46
812.75
232.17
242.39
256.22
266.19
282.25
301.20
321.23
329.26
330.21
355.25
369.22
370.27
398.24
399.34
427.26
440.23
460.28
494.32
497.31
498.35
523.29
540.40
600.40
628.35
Relative Abundance
m/z
746.44
754.33
594.29
794.40
426.25
654.30
595.37
755.31
795.38
566.36
655.36
804.38
626.33
725.33
627.38
706.35
726.37
774.45
775.44
805.44
823.79
234.27
246.43
256.18
266.18
301.27
321.30
329.15
355.28
358.19
369.25
397.18
398.29
400.29
427.27
440.22
469.33
489.32
497.25
523.35
526.26
527.35
557.30
600.36
675.41
678.45

(c)

APMPPYP 1st peak (C-prenyl)		
b-ions (+1)	**Expected**	**Observed**
AP	169.10	-
APM	300.14	-
APMP	397.19	397.22
APMPP	494.24	494.33
APMPPy	725.37	725.31
y-ions (+1)		
PMPPyP	769.39	769.36
MPPyP	672.34	672.27
PPyP	541.30	541.25
PyP	444.25	444.26
Py	347.19	347.23
y- b- cleavages (+1)		
Py	329.19	329.17
PPy	426.24	426.24
MPPy	557.28	557.25
y- a- cleavages (+1)		
Py	301.19	301.22
Miscellaneous Ions		
APMPPy a-cleavage +1	697.37	697.31

APMPPYP 2nd peak (O-prenyl)		
b-ions (+1)	**Expected**	**Observed**
AP	169.10	-
APM	300.14	-
APMP	397.19	397.22
APMPP	494.24	-
APMPPy	725.37	725.31
y-ions (+1)		
PMPPyP	769.39	769.36
MPPyP	672.34	672.27
PPyP	541.30	541.25
PyP	444.25	444.26
Py	347.19	347.23
y- b- cleavages (+1)		
Py	329.19	329.17
PPy	426.24	426.24
MPPy	557.28	557.23
y- a- cleavages (+1)		
Py	301.19	301.22
Miscellaneous Ions		
All -prenyl	772.37	772.28
APMPPy a-cleavage +1	697.37	697.29
APMPPy -prenyl	657.31	657.26
PPyP -prenyl	473.24	473.23

(d)

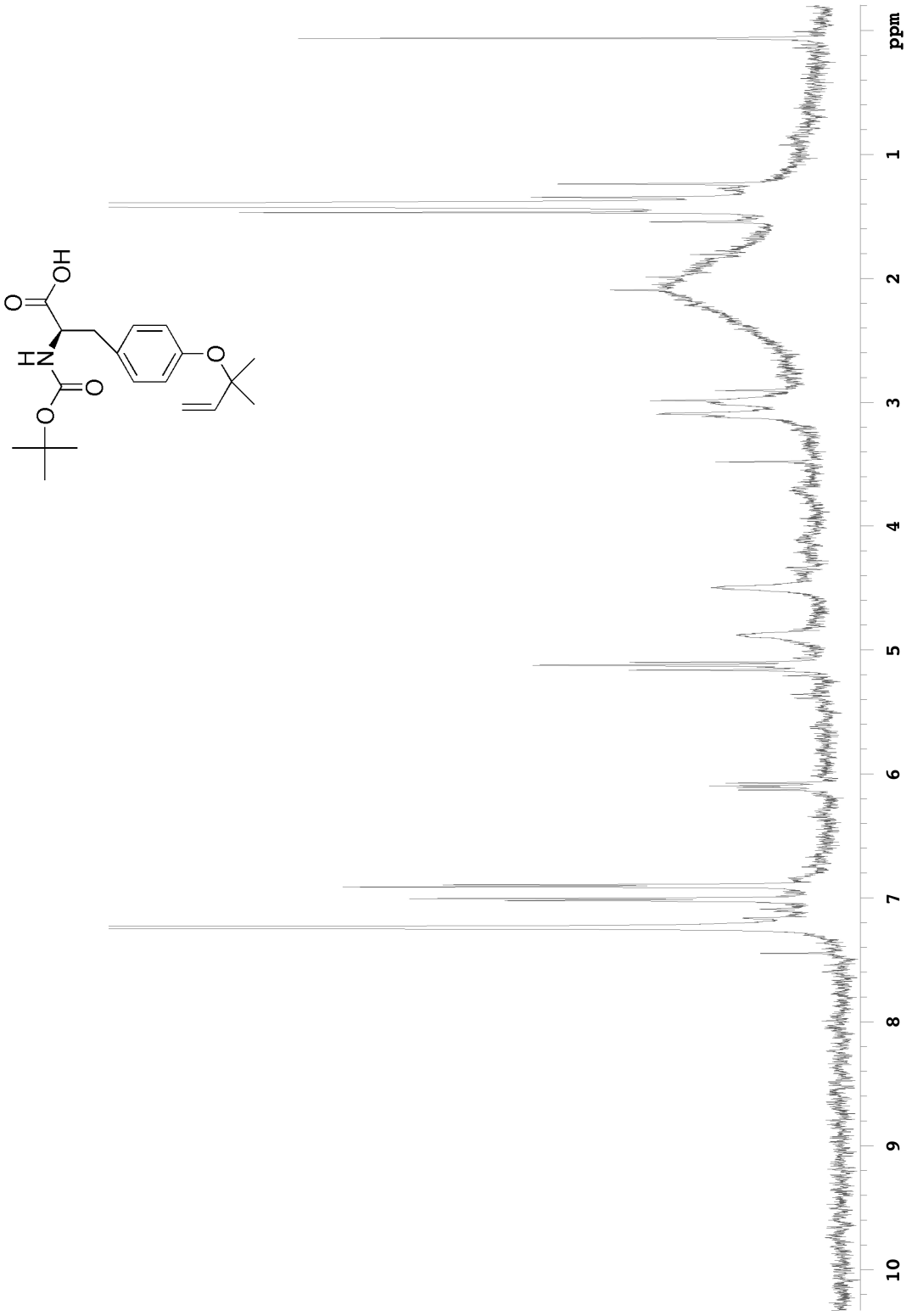

Relative Abundance
m/z
746.43
747.38
794.38
594.30
795.38
654.30
426.24
595.33
655.35
566.37
804.39
626.33
628.34
725.35
680.31
691.35
692.47
726.43
751.33
774.40
775.48
805.36
814.23
397.19
398.27
399.33
427.25
440.26
469.40
494.28
497.28
498.35
523.34
552.32
600.33
233.25
245.19
256.30
266.27
282.25
301.25
321.22
329.24
330.28
355.20
369.26

(e)

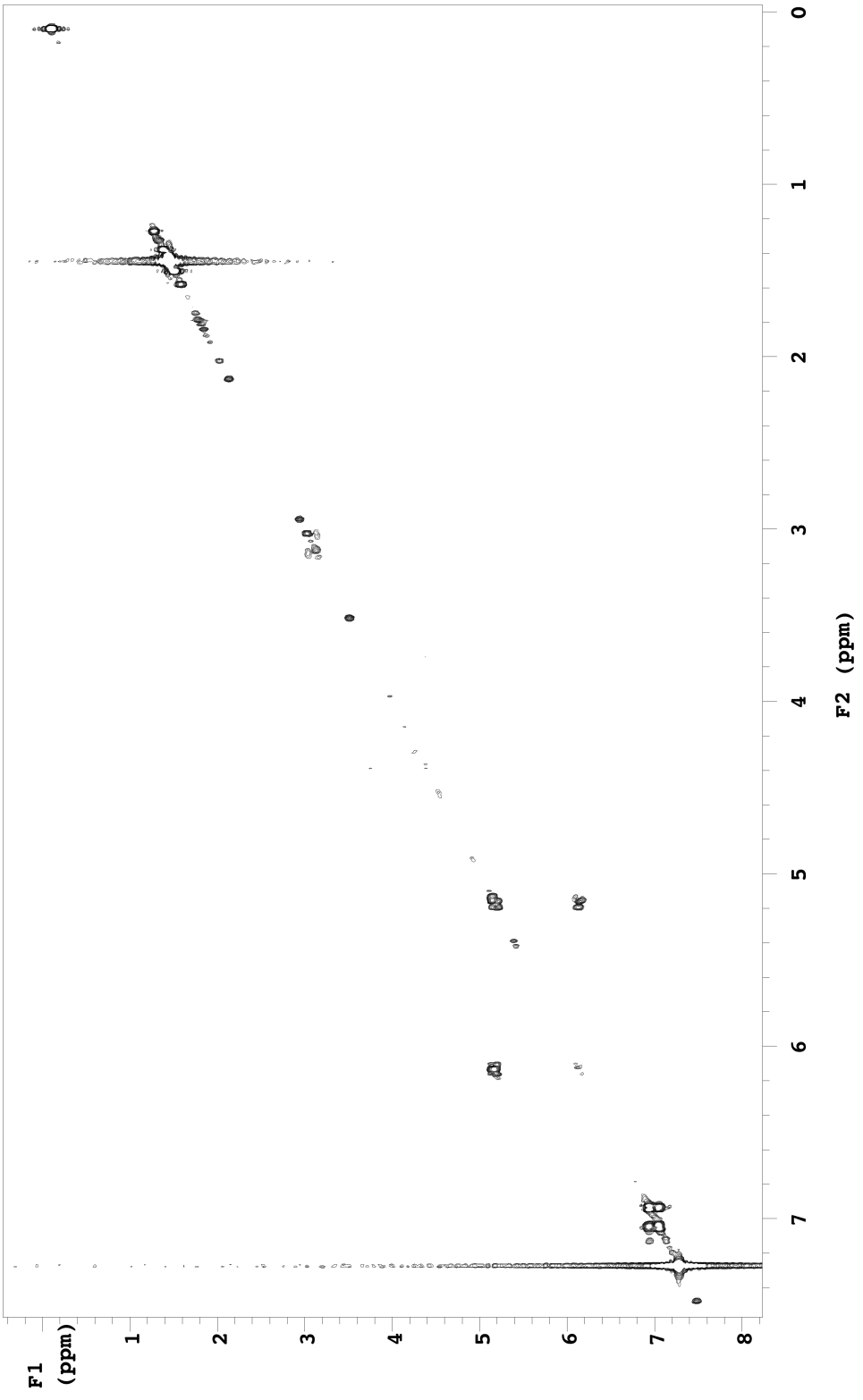
cyclo[APMPPYP] NMR-characterized C-prenyl reference compound					
y- and b- cleavages (+1)	**Expected**	**Observed**	**y- and b- cleavages (+1)**	**Expected**	**Observed**
AP	169.10	-	**yP**	329.19	329.24
APM	300.14	-	**yPA**	400.22	-
APMP	397.19	397.19	**yPAP**	497.28	497.24
APMPP	494.24	494.28	**yPAPM**	628.32	628.34
APMPPy	725.37	725.35	**yPAPMP**	725.37	725.35
PM	229.10	-	**PA**	169.10	-
PMP	326.15	-	**PAP**	266.15	266.28
PMPP	423.21	423.35	**PAPM**	397.19	397.19
PMPPy	654.33	654.30	**PAPMP**	494.24	494.28
PMPPyP	751.39	751.32	**PAPMPP**	591.30	-
MP	229.10	-			
MPP	326.15	-	**Miscellaneous Ions**		
MPPy	557.28	557.29	**-Acylium (C=O)**	794.42	794.38
MPPyP	654.33	654.30	**-Methyl sulfide**	774.42	774.40
MPPyPA	725.37	725.35	**-Met side-chain**	746.39	746.43
PP	195.11	-	**-H2O**	804.41	804.39
PPy	426.24	426.24	**PyPAP y- a- cleavage**	566.33	566.38
PPyP	523.29	523.34	**PAPM y- a- cleavage**	369.20	369.21
PPyPA	594.33	594.30	**PAPM -Met sidechain**	321.16	321.22
PPyPAP	691.38	691.35	**PAPM y- a- cleavage**	301.19	301.25
Py	329.19	329.24			
PyP	426.24	426.24			
PyPA	497.28	497.28			
PyPAP	594.33	594.30			
PyPAPM	725.37	725.35			

Figure S5. Analysis of reverse O-prenylated intermediate and C-prenylated product (a) 1H NMR of boc-D-Tyr reverse O-prenylated intermediate (b) 2D COSY spectrum of boc-D-Tyr reverse O-prenylated intermediate (c) 1H NMR of boc-D-Tyr forward C-prenylated final product (d) circular dichroism spectral overlays of (top) boc-L-Tyr (blue) and boc-D-Tyr (red) and (bottom) C-prenyl boc-L-Tyr (blue) and C-prenyl boc-D-Tyr (red)

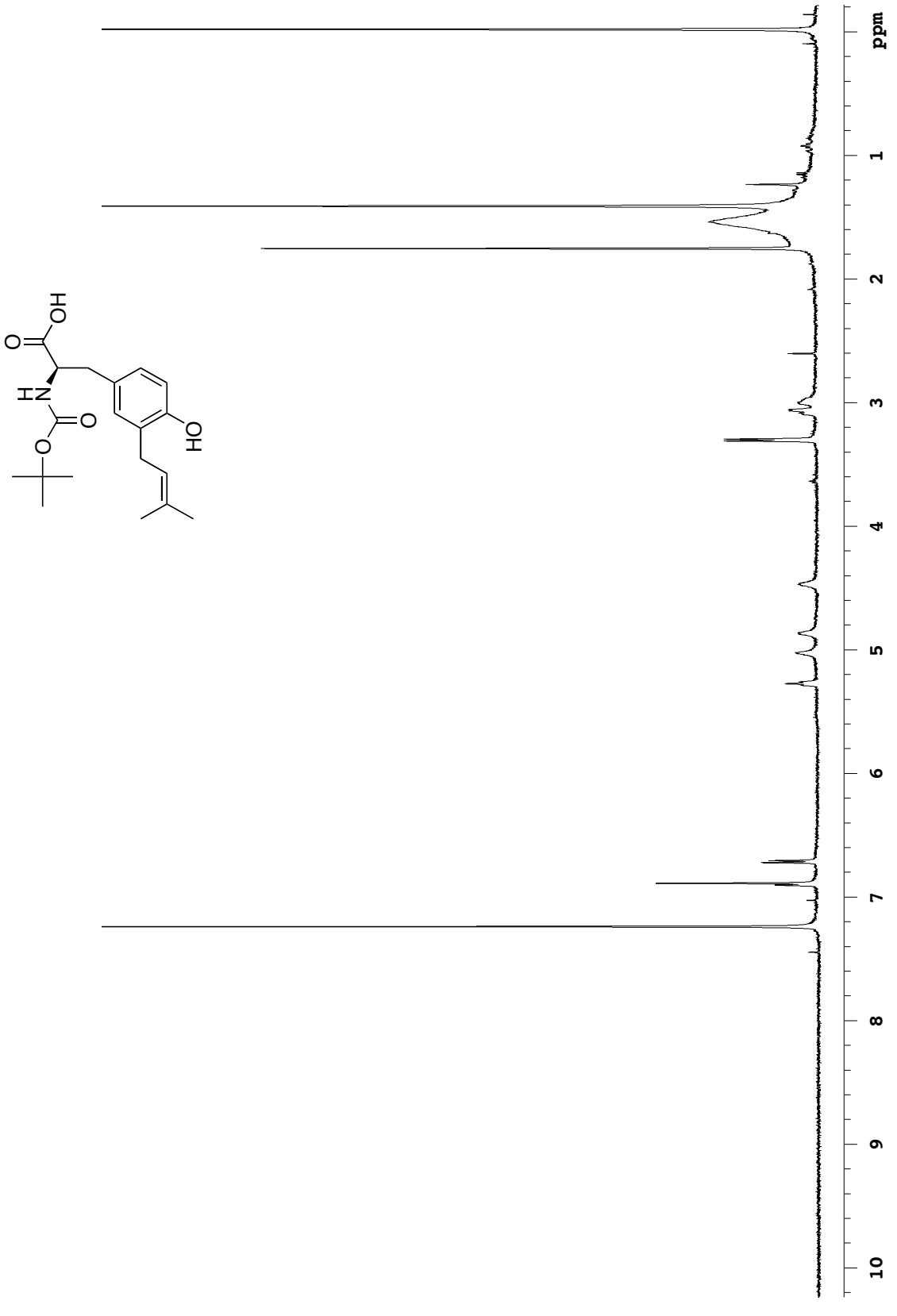
(a)



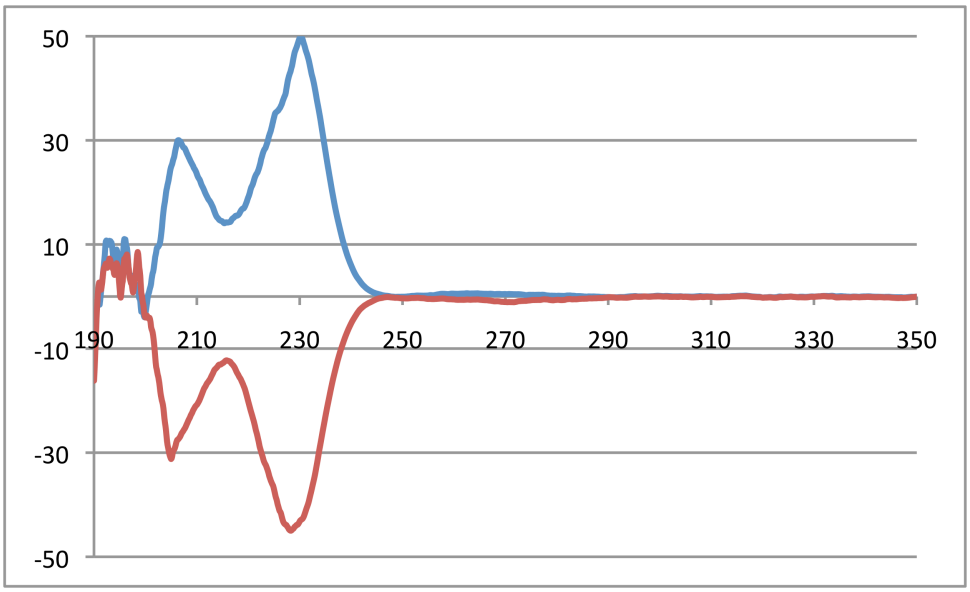
(b)



(c)



(d)


50
30
10
-10
-30
-50
190
210
230
250
270
290
310
330
350

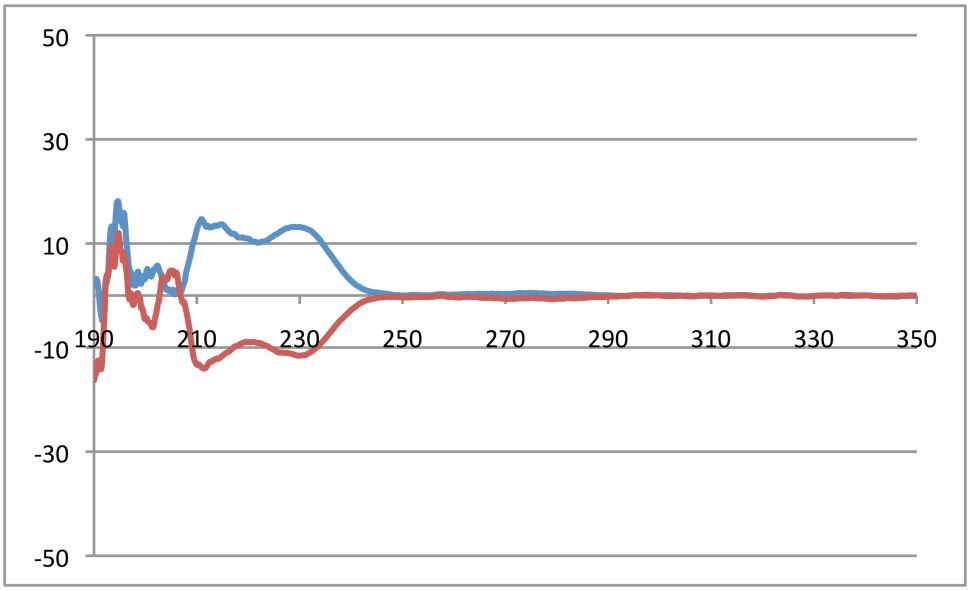

50
30
10
-10
-30
-50
190
210
230
250
270
290
310
330
350

Figure S6. Non-enzymatic rearrangement of reverse O-prenylated tyrosine. Bar graph showing proportions of reverse O-prenylated intermediate and C-prenylated final product after incubation at 37 °C for 0 and 8 h under different conditions. The O-prenylated intermediate is sufficiently unstable that after islolation it was partially rearranged. Proportions of products determined by integration of A220 diode array trace of the relevant peaks, which are well-separated by HPLC.

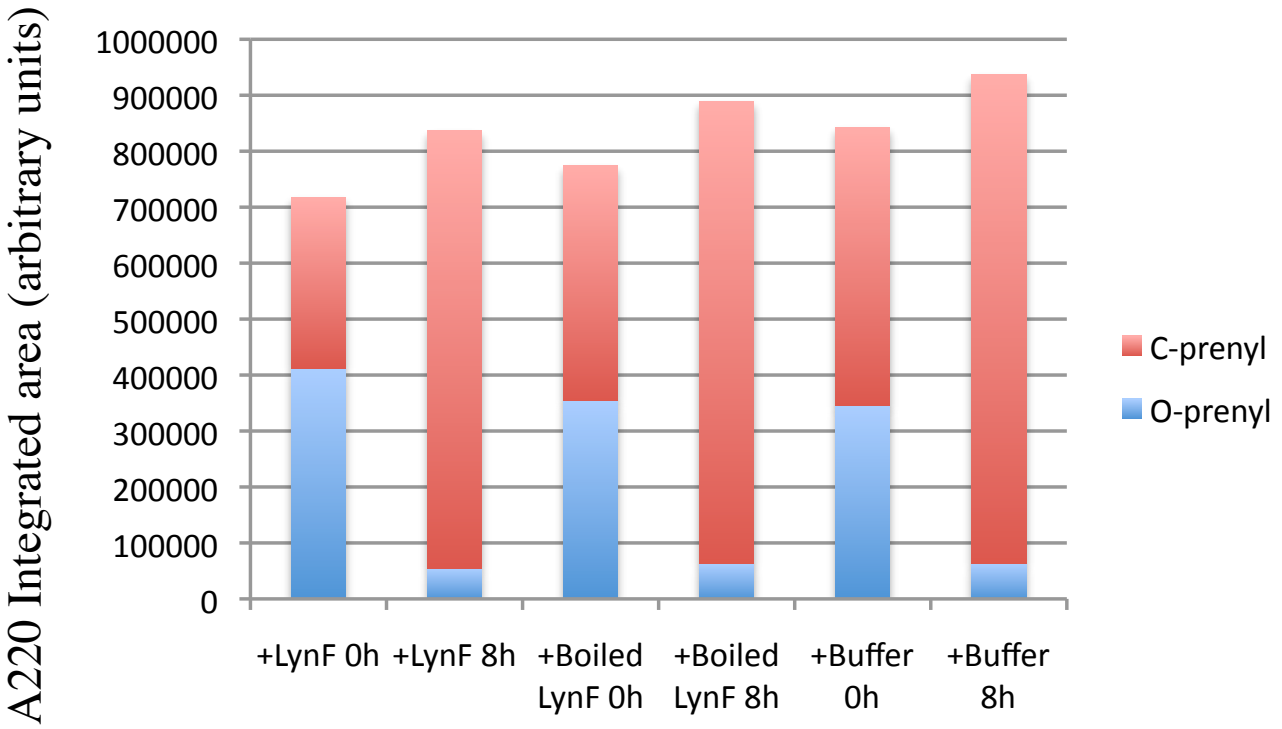
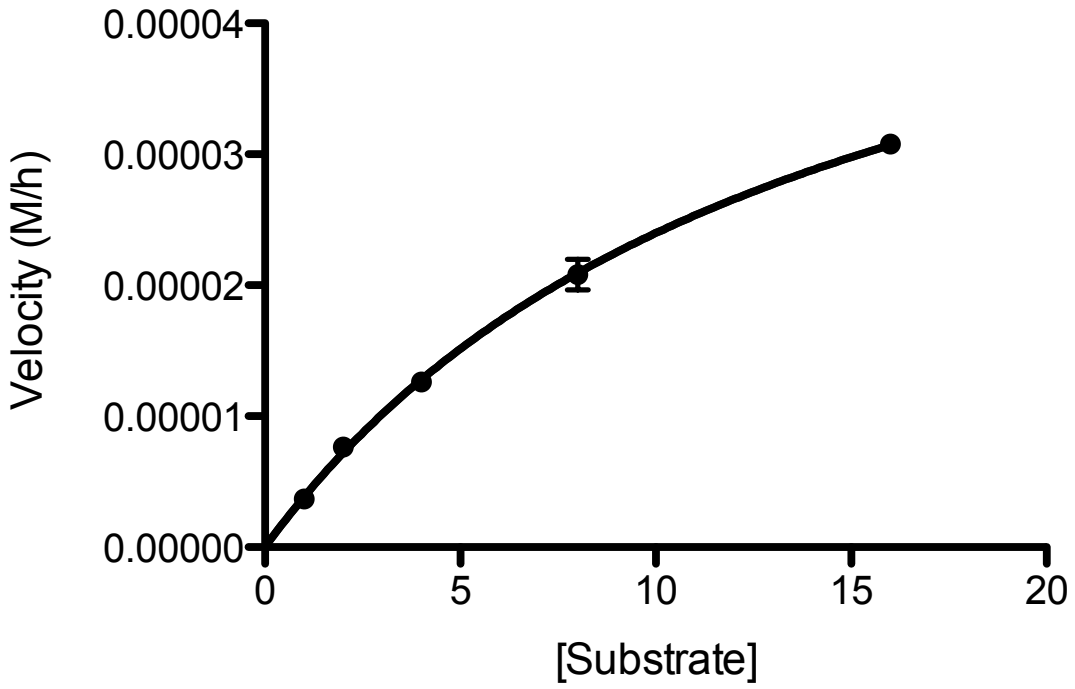


Figure S7. Kinetics of prenylation of cyclo[APMPPYP] and boc-L-tyrosine. Rates were measured by integration of product peaks observed in the monitoring of the HPLC elution 220 nm. Both the C- and O-prenylated products were summed to obtain the total amount of prenylated products at each point. Areas were then plotted on a calibration curve created via injections of known quantities of boc-L-Tyr to derive concentrations (a) Kinetics for reaction of Boc-L-Tyr with LynF (b) Kinetics for reaction of cyclo[APMPPYP] with LynF (c) Controls showing linearity of reaction rate through 6 h.

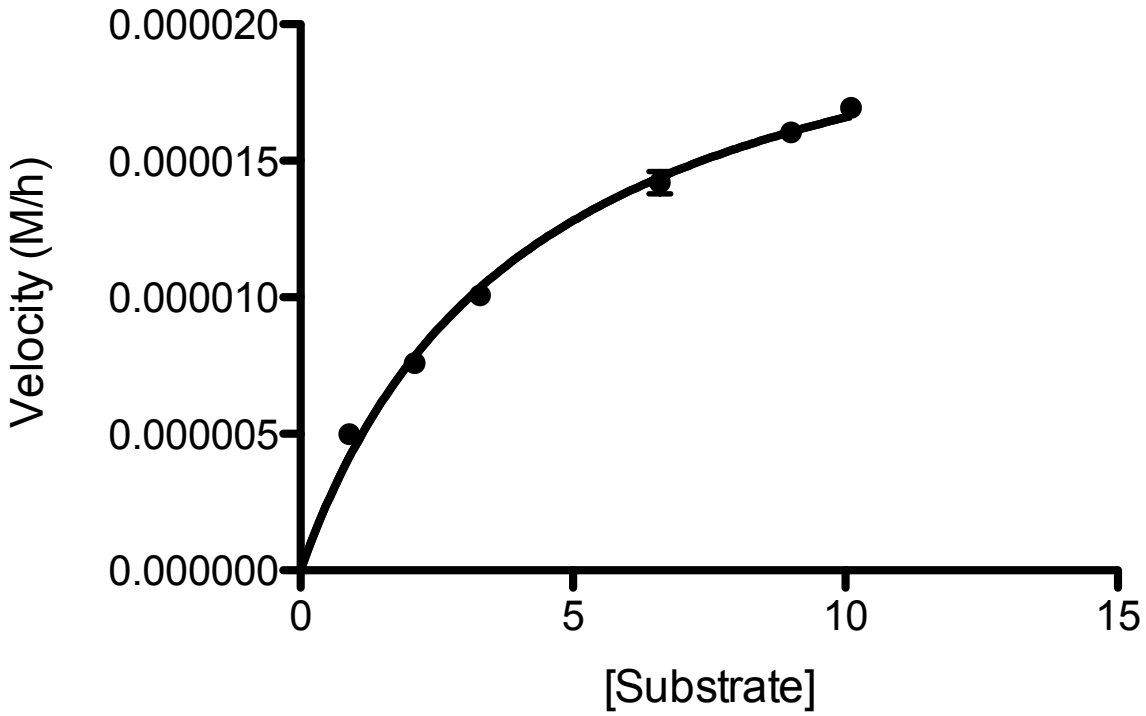
(a)

Best-fit values for Boc-L-Tyr kinetics	
Vmax	5.7E-05 M/h
Km	14.0 mM
kcat	63.3 h-1
Std. Error	
Vmax	3.9E-06 M/h
Km	1.6 mM
95% Confidence Intervals	
Vmax	4.9e-005 to 6.6e-005 M/h
Km	10.49 to 17.59 mM

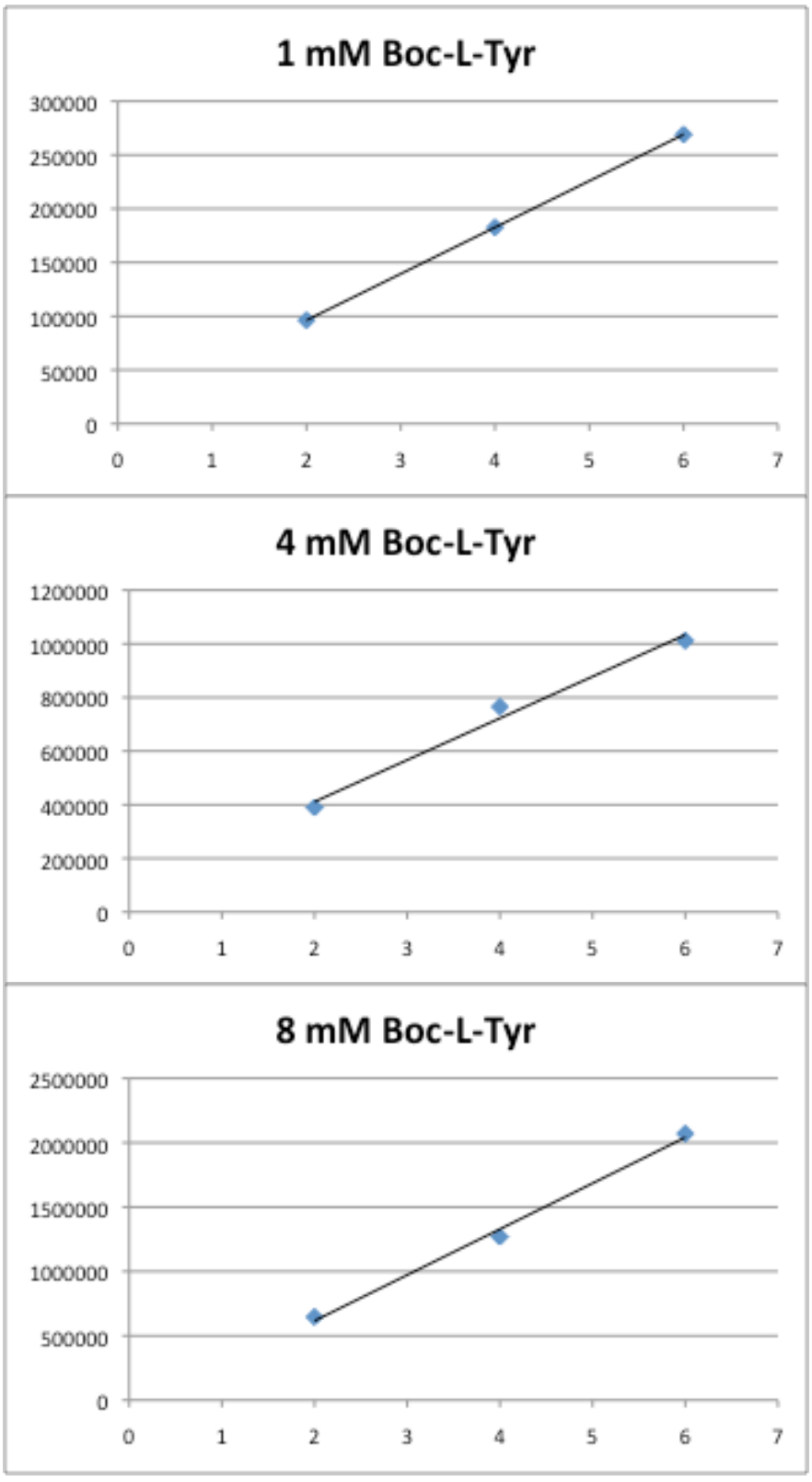


(b)

Best-fit values for cyclo[APMPPYP] kinetics	
Vmax	2.4E-05 M/h
Km	4.2 mM
kcat	13 h-1
Std. Error	
Vmax	8.3E-07 M/h
Km	0.4 mM
95% Confidence Intervals	
Vmax	2.2e-005 to 2.5e-005 M
Km	3.4 to 5.0 mM



(c)



Integrated Area (arbitrary units)

Time (h)

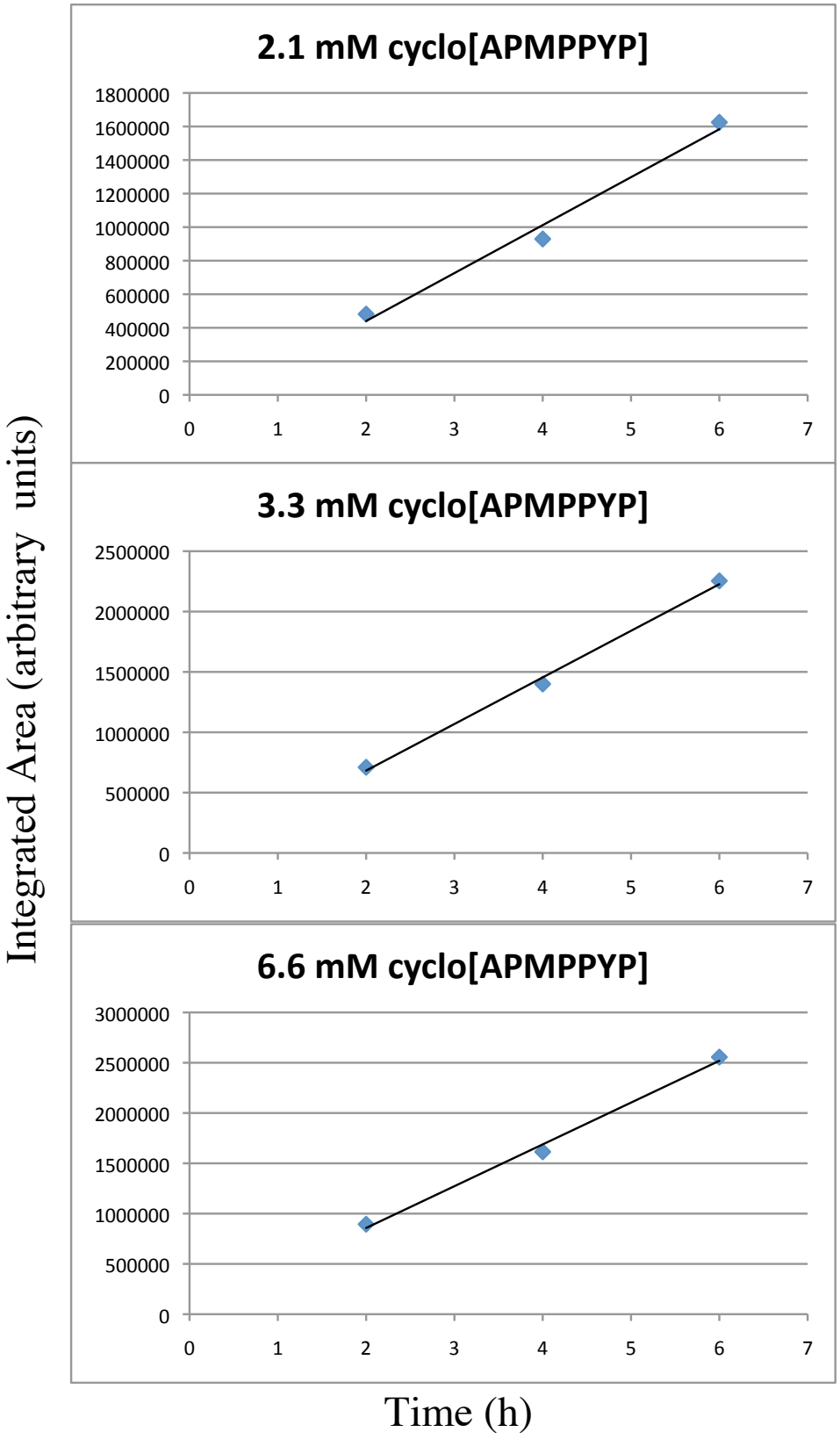

2.1 mM cyclo[APMPPYP]
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
0 1 2 3 4 5 6 7
3.3 mM cyclo[APMPPYP]
2500000
2000000
1500000
1000000
500000
0
0 1 2 3 4 5 6 7
6.6 mM cyclo[APMPPYP]
3000000
2500000
2000000
1500000
1000000
500000
0
0 1 2 3 4 5 6 7
Integrated Area (arbitrary units)
Time (h)

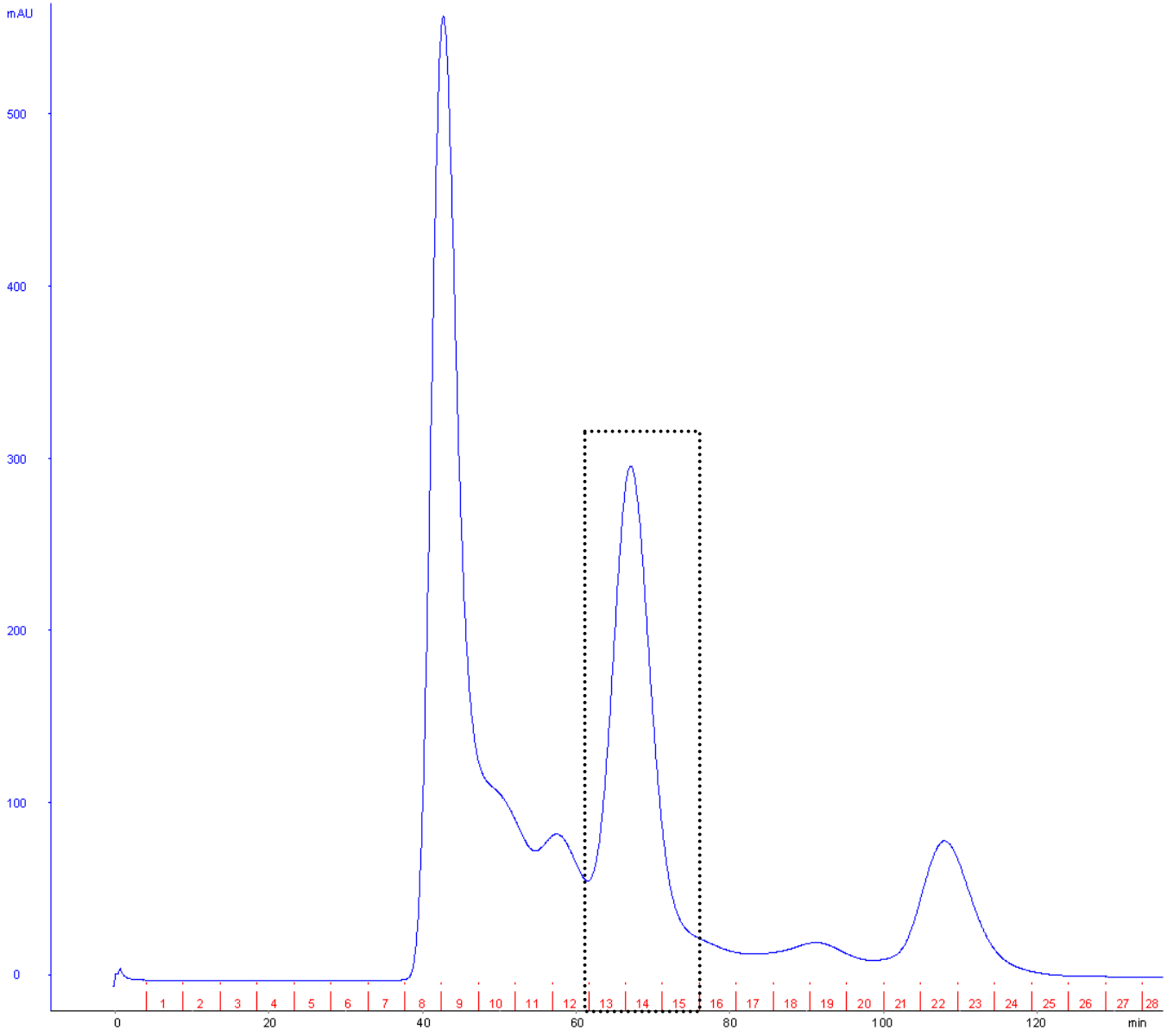
Figure S8. Multiple sequence alignments of LynF relatives. Highlighted in yellow are residues conserved across all known LynF homologues, in green are residues conserved across enzymes predicted to catalyze prenylation, in cyan are residues conserved across predicted non-prenylating enzymes

```
TruF1          ---------MIMTTTWPDSYAKERRIQRLRHHFESFDVERAFPLPLFEQAVLSLDSCPLL
LynF           ------MTIMAIANRVPYNYLREQRIQFMHAHQDAFDVSTVFPLPLFEKLVTELEGSNVI
AcyF           ----------MIANVTQKDRFQEQKLQFIRNHQQAFDVEPIYPLPLFEDFVMNVEGDCSI
PagF           ----------MIVNVIQKDRLKEQKLQFIRNHQQAFDVEPIYPLPLFEDFVTSIEGDCSL
Mic843F        ----------MIVADIQKSSLKEQRLQFIRNHQQAFDVEPIYPLRLFEDFVMGVEGDCTI
ArtF           ---------MNCTSVLQQNHLREKRLQFIRAHQTAFDVEPVFPLQVFEDFVFGVEGDCTI
TheF           --------------------MPREQRLQFIKAHQAAFEVEPLYPLALFEALVETFDEDCAL
OscF           MIILSASDSTRPIFTLPKPLTQEQKLHCINAHRQAFDVQPLYPLDIFQDFITKTDGIDTI

MicF1          ---------MTLTSMLKNNHLKARRLQFLRGHQEAFDVEPTFMLSLFEEAVLGIEETCGV
MicF2          ---------MTLTSMLTNNHLKARRLQFLRGYQEAFDVEPNFMLSLFEEAVLGIEETCGV
TenF           ---------MTLTSMLQNNRLKDRRLQFIRTHQEAFDVEPTFILSLFEEAVLGIEGTCGV
PatF           ---------MDLIDRLQNNQRKDRRLQFVRTHQEAFDVKPTFPLPLFEEAILEIEGSCSV
TruF2          ---------MVLSQLSKQTNLRENRLRCIRTHLEAFDIEPVLQISLFEEVIMEVEGSCNV

TruF1          EPSFKVQEGILFAGRVTTST-GT-EDWQHLISTALNFFDAVESRVEVTIDRGLLEKFLTL
LynF           ELSCKIEADKLLAGRFLIFS-DQENNWHQSLAQALQFLDSIESRVGVEINRESLDKFLAA
AcyF           EASCKIELDKLIASRFMFFFKDKAQQWQKYLHQSLTFFNRVENLVGVQVDYSLLRQFLGS
PagF           EASCKIESDKLIASRFLLFFEDKTQEWQKYLHQSLTFFGLVENRVGVKINYSLLQQFLGS
Mic843F        QASCKIELDQLIASRFMLFFKDKAQEWQNYLAQSLAFFRQVENRVGVKLDYSLLQQFLGL
ArtF           EASCKVESDHLIASRFLLFFQEMTQSWPQKLDQAFRFFHQTENQVGVRLDYGLLQHFLGD
TheF           EASCKIEFDQLIASRFLIFF---SQNFEQNLARVLNFMTQVNQRVDVQINTDLLYHFLGQ
OscF           EASCKIEADKLQAARFVALS---SQEIERKLTEFLTFFRQVESRVDVQLNYDLLHKFLGK

MicF1          ESKCNVEKDQLFAIDFQVCN-DQGRTWPMSLTHAVKFMDKIESTVGVRLNRNLLEQFATL
MicF2          ESKCNVEKDQLFAIDFQVCN-DQGRTWPMSLTHAVKFMDKIESTVGVRLNRNLLEQFATL
TenF           ELMCHVEGDQLFAVDFQVSN-ER-HTWPRSLTDAVKFLDKVESQVGVRLNRDLLQQFVAV
PatF           ESSCQVEGDRLQGGRYEVCN-NQGTTWPESLTHAFKLLDKIDSQLGVRINRDSFDRFAAA
TruF2          KCSCKVERDRLFACQFTLAY-SQ-QKWPKTLKYNAILFDKIKSQVGICIDSSKFEQFSRL

TruF1          HQNSDKIEASLMGIDLRPNVKESSLKVHLRLDPQQDADELVMTAIDLDGGDYSPELTQVL
LynF           HINSGKIMGISTGLDLRPELENSSVKIHIMLG--ENSEELVRTAIAIDGSHYPVELAQVL
AcyF           DFDFRKVTVLSAGIDLRSNIAESSLKMHIRIKDYPE---KLDQALSLAS-NAED--LISV
PagF           SFDFSKVTVLSAGIDLRNNLAESSLKMHIRIKDYPE---KLDKAFALSD-GAAD--GNYL
Mic843F        NFNFSKITVFSTGIDLRTNLADSSLKMHIRLKDYPE---KINQALLLTS-DSDD--LIAV
ArtF           DFDFSKISVLSTGIDLRQNLADSSLKMHITIEDYPE---KIATAFSLAK-LPRDKFHQIL
TheF           KFDFRKMIRLATGVDLRSNLADSSLKIHIRLEDYPE---KIESALALHG-NPDDASYWAD
OscF           SFDFSKVTRITTGVDLRPNISDSSLKIHIRLNDHPENLKKIEAALTLDG-NDSTAQRWIA

MicF1          HMDSHKIENNTVGIDLRPKNEDSCIKVCLHLGSEEEPEELVRTALELDGGSYSPELLQVL
MicF2          HLDSHKIQDNTVGIDLRPRNEDSCIKVYLHLGSEEEPEELVRTALELDGGSYSPELLQVL
TenF           HIGSSKILNNTIGIDLRPRHENSCIKVYMHIEHEEDPEELVRTALKLDGDSYSSEMLQVL
PatF           HVNSRKIINNTIGVHLGSKLEDSSVMLYIHIKPEEDTEELARTALVLDGGRYSDELTRVL
TruF2          HVNSDKILDSTVGIDLRPKSQDSCIRISVHLEPKESPEELVRTALALDNATYTSELTQVF
```

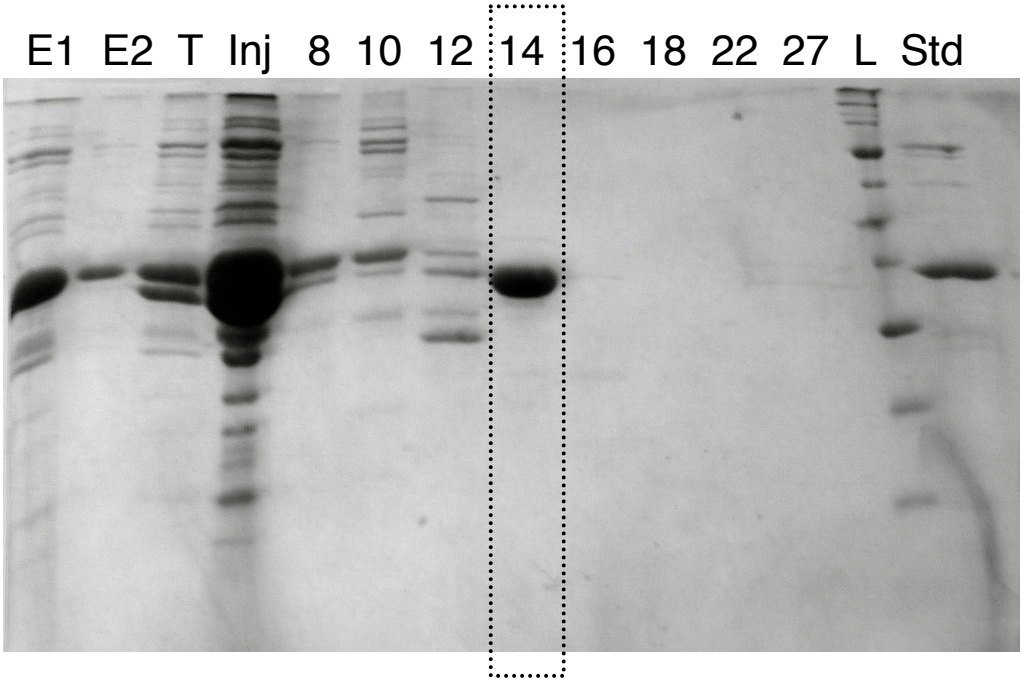
```
TruF1           LKDTFLIGFDFFLDGGSAVEMYTICPGKKPLAMLGKKGAYLKPYVLSNFSHKVTSLLQEV
LynF            LKDTMMIGFDFFLNGHSEVELYISCSRKKDSLPN-NRGESTRYYIRQKFSPKVSSLLDAS
AcyF            RPFLSLVGFDFYFNGRSEIELYPEIQAEDFAKSE------TQNLVWRHFPKFVLDPLEVT
PagF            KDFVNLIGFDFYFNGKSEIEIYAEVQEDDFFKPE------INNLVWQHFPKTALQPLKAS
Mic843F         RDFLSIVGFDFYFDGRSAIKIYPEVAETDFFKPE------TQDKVWRHLPKFVLEPLKAT
ArtF            LSSVSLIGFDFYLDGRSEIELYASLKEEEFNSPH------VQSFLTSNFCASALKPLAAS
TheF            LNAIANIGLDFYLDGRSEIEFYPELSEERFQQPE------MQVLLQQMFPPFVLAPLKAS
OscF            LQTVHLIGFDFYLNGRSEIELYCELTEKQFQQPD------IQSFLQQTFPPFVLEPLKVS

MicF1           LKSTIVIGFNLFLNGYSDIELWALAPGEQYEITNSDRGKYLKHYIQRNFSPKVNSLLKEC
MicF2           LKSTIVIGFNLFLNGYSDLELWATCPGEQYEVPNSDRGKYLKQYVQNNFSQKINDLLRES
TenF            LKSTIIIGFNIFFNGYSDVELLVATVGDKYESHKFNRGKYLKHYIQKNFSLKANYMLRES
PatF            LRDTMVIGFELFFDGRSRVDLGPCAPGK--SGTLKMKGKHLEQYTQKNLSRKVNSIFREG
TruF2           LQDCTAIIFECFFDGRSRIELGAVAPGKKHGFSG-NHGRALTAYAQKYFSPKAVSLSEVS


TruF1           AALTVGFSKEN-PRPVLYFEFETLREVKYNLLFNSLGDKIYDFCLHNQIENFVSIGVTEP
LynF            DFFVGGFSKAN-VEPVLYYAFENIKDIPKYFVFNDLGNRVYDFCRSQDSITMTWIGINER
AcyF            GSFLVGFSKAN-PNPVLYYNLKNKQDLANYFKLNDAAQRVHSFYQNQDILPHMWVGTVQK
PagF            SLFFTGLSKAN-NNPVLYYHLKNRQDLTNYFKLNDTAQRVHSFYQHQDILPYMWVGTAQK
Mic843F         SLFGFGFSKTN-NNPVLYYRLKNRQDLTNYFKLNDTAQRVHSFYQHQDILSSVWLGTAQQ
ArtF            SAFYMGLSIAN-ENPVLYYLLKNKQELQNYFRLNDTGNRVHSLL----------------
TheF            EIFGFGLSKAN-PSAVLYYQLKNKQDLPSYFAINDKAHQVHGYYLHQDTRPYMCVGVAQS
OscF            SVFFTGLSKDN-TEPVLYYCLKDKKDLLSYFPINDTAQRVHAFYQNQPVYSSMWVGVAQG

MicF1           TFFCVSFFHKK--EPVIIFHYEDTKEIPKNFFFNSLGDRIYSFCQGQDCMTYAGVAVTER
MicF2           TFLVVSFSNQK--EPALIFHYEDIKEIPKNFLLNSLGDRIYSFCQGQDCMTYAGVAVTER
TenF            NILLVSFSQAKKVNPLLIFHYEDIKDIRKYFSFNSLGDRSYSFFQSQDCITYAGVSVREL
PatF            YLFGAFFSKTR-VEPILFFYHSIIKDLPKYFTFNSLGDKIYNFCQSQGCITDVAIAVTET
TruF2           DLFGMTISKYK-AEPVLHFGFNNIKDISNYFLFNTLGNRIYSFCQNQDCILLAIIGVNEK


TruF1           DLEKRRLENFRFYYRKAV---------------
LynF            DLDRERLNNFRLYYRRSFG--------------
AcyF            ELEKTRIENVRLYYYKFFN--------------
PagF            ELEKTRIENIRLYYYKSFKMESN----------
Mic843F         ELEKTRIENVRLYYYKLFGKL------------
ArtF            -----------------------------
TheF            ELAKTRIDQIRLYYHQFFKVQNP----------
OscF            ELQKTRIDNIRLYYSKKNYSK------------

MicF1           ELEKDRLENFSILYNQRDECKPLLHIKKREDFS
MicF2           ELEKDRLENFSILYNQRDECKPLLHIKKREDFS
TenF            ELQKDRLEKFSLFYNKRDKCQHLPLFSTLNE--
PatF            ELEKSRLENFCFYYDQWDECKPSSDYDTERHLH
TruF2           ELYSNRLENFLFDYAKNDESRMMRV--------
```

Figure S9. Sizing column chromatogram for LynF and resulting SDS-PAGE gel (a) Size-exclusion chromatogram showing elution of LynF. Protein elution was detected at 280 nm, and is shown in mAu; along the x-axis is time in minutes and fraction number (the fraction size was 4.5 mL). Purest fractions ultimately used for experiments are highlighted with box (b) SDS-PAGE gel showing analysis of size-exclusion chromatography fractions; fractions 13-15 of size purification used for most experiments described in paper. Key to abbreviations: E1=Ni-NTA elution 1, E2=Ni-NTA elution 2, T=after treatment with thrombin to cleave his-tag, Inj=sample of material injected on sizing column, 8-27=sizing column fraction number, L=ladder, Std=previously prepared LynF sample purified by Ni-NTA chromatography only.

(a)



(b)



Materials and Methods

Synthesis of substrates.

Dimethylallyl pyrophosphate (DMAPP) was synthesized following previously established procedures.[1-3] Briefly, disodium dihydrogen pyrophosphate (15 mmol, 3.3g) was dissolved in 15 mL of a 10% (v/v) solution of ammonium hydroxide. That solution was put over a column containing Dowex AG 50W-X8 column (58 mequiv, acidic form), and eluted with 110 mL of deionized water to give the free acid. The eluent was titrated to pH 7.3 (as measured by a pH meter) with a 40% solution of tetra-*n*-butylammonium hydroxide. The solution was then dried by lyophilization to give tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (**32**). Next, **32** (2.5 mmol, 2.3g) was dissolved in 2.5 mL of acetonitrile. The pyrophosphate solution was placed on ice, with stirring under a blanket of argon. Dimethylallyl bromide (1.2 mmol, 1 M in acetonitrile) was then added dropwise to the pyrophsophate solution. The reaction proceeded for 2 h, during which time it was allowed to come to room temperature. After solvent removal, the residue was dissolved in ion-exchange buffer (1:49 v/v Isopropanol : 25 mM aqueous ammonium bicarbonate) and run on a column containing Dowex AG 50W-X8 (18.8 mequiv, ammonium form). Finally, chromatography on cellulose (2, 3) afforded dimethylallyl pyrophosphate, ammonium salt. ^{1}H NMR (400 MHz, D_2O) δ 5.39 (1H, t, J=7.03 Hz), 4.39 (2H, dd, $J_{H,H}$=6.64 Hz, $J_{H,P}$=6.64 Hz), 1.70 (3H, s), 1.66 (3H, s); ^{31}P NMR (162 MHz, D_2O) δ -5.96 (1P, d, $J_{P,P}$=21.7 Hz), -9.23 (1P, d, $J_{P,P}$=20.6).

Unless otherwise noted, peptide substrates were synthesized at the University of Utah DNA/peptide synthesis core facility using standard Fmoc chemistry. Cyclic peptide **6** was cyclized in solution, leading to small amounts of **9** and **10**. HPLC purification effected purification of **6** from **9** and **10**, which co-eluted and were used as a mixture in prenyltransferase experiments. Substrates **11** and **12** were was synthesized as a linear precursor (KKPYILPAYDGE) and then enzymatically cyclized as reported elsewhere.[4] Synthesis and characterization of **26** and **27** has been previously reported.[5,6]

Boc-protection of 4-iodo-L-phenylalnine and 4-methoxy-L-phenylalanine was performed according to previously established procedures.[7] Briefly, to a stirred solution of amino acid (0.3 M in 50/50 THF/H_2O) was added di-tert-butyl dicarbonate (1.1 equiv), and triethylamine (1 equiv). The reactions were stirred at room temperature until the starting material was consumed, as determined by TLC. Reaction mixtures were then dried by rotary evaporation to remove THF, and then lyophilized. The residue was then dissolved in aqueous sodium hydroxide (1 M, 2 equiv) and extracted twice with CH_2Cl_2; the solutions were acidified and extracted three times with CH_2Cl_2. The acid extracts were then combined and dried by rotary evaporation. Prior to use in enzyme reactions, small amounts of each boc-protected amino acid were subjected to additional purification by reversed-phase HPLC. To wit, the amino acid derivatives were injected on a C18 onyx monolithic semiprep column (Phenomenex) on a linear gradient from 50% buffer A (H_2O, 0.05% TFA) to 100% buffer B (acetonitrile) over 60 minutes.

Boc-4-iodo-L-Phe: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (2H, br), 6.93 (2H, br), 4.90 (1H, s), 4.56 (1H, br), 3.13 (1H, br), 3.03 (1H, br), 1.42 (9H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ 173.4, 155.4, 137.9, 136.8, 131.6, 92.2, 78.1, 54.9, 35.9, 28.1. **Boc-4-methoxy-L-Phe**: ^{1}H NMR (400 MHz, DMSO d_6) δ 7.14 (2H, br), 6.83 (2H, br), 4.00 (1H, br), 3.70 (3H, br), 2.91 (1H, br), 2.74 (1H, ap t, J=10.8 Hz), 1.31 (9H, s). ^{13}C NMR (100 MHz, DMSO d_6) δ 173.7, 157.9, 155.5, 130.1, 129.9, 113.6, 78.0, 55.5, 55.0, 35.6, 28.2.

Boc-L-tyrosine, sodium hydrogen pyrophosphate, dimethylallyl bromide, tetrabutylammonium hydroxide, and dopamine HCl were purchased from Sigma. N-acetyl-L-tyrosine and phenol were purchased from Fisher Scientific. All other Tyr and Phe derivatives were purchased from ChemImpex.

Genes and Cloning

A codon-optimized version of *lynF* was synthesized and cloned into pET28 in frame with the N-terminal his-tag sequence using NdeI and EcoRI (Genscript). The vector pRSF-truE1-DlacI was described previously.[8] Oligonucleotides were designed to swap the region of truE1 encoding patellin 2 (TVPTLC) for a region encoding a cassette found in the *lyn* pathway (VCMPCYP). The resulting construct (pRSF-LynE-Dlac) was confirmed using DNA sequencing. This construct was then cloned into pET28b using NdeI and BamHI.

Protein Expression and Purification

The plasmid encoding *lynF* was transformed into BL21(DE3) cells via electroporation and grown at 30 °C. Colonies were picked the following day and grown overnight in 2 x 45 mL starter cultures (LB media, 50 μg/mL kanamycin) at room temperature. The starter cultures were then used to inoculate 8 x 1 L cultures (LB, 50 μg/mL kanamycin) in 2.8 L Fernbach flasks and grown at 30 °C until an A600 of 0.4 was reached. Upon reaching the desired optical density, the temperature was lowered to 15 °C and IPTG (0.1 mM final concentration) was added, and expression was allowed to proceed overnight. Cells were then pelleted by centrifugation, and frozen at -80 °C until purification. Cell lysis was performed according to previously established procedures. Briefly, cells were resuspended in cold lysis buffer (200 mM NaCl, 50 mM Tris pH 7.5, 100 μM EDTA, 5 mL per gram of cell paste) to which lysozyme (600 μg/mL), PMSF (1 mM), and imidazole (10 mM) were added. The suspension was then incubated on ice with stirring for 1 h, after which time $MgCl_2$ (10 mM) and DNase I (20 μg/mL) were added and incubation on ice was continued for a further 0.5 h. After lysis was complete, the suspension was centrifuged at 14,000 RPM in a JA-20 rotor for 1 h at 4 °C. Cleared lysates were then passed through a 0.45 μm syringe filter, and then ATP (2 mM) was added. Lysate was then applied to a gravity column containing a 5 mL bed of nickel-NTA resin (Qiagen). The lysate was allowed to equilibrate on resin for 15 minutes and then allowed to flow through. The column was then washed with 20 column volumes of wash buffer (500 mM NaCl, 25 mM imidazole, 2 mM ATP pH 8.0), and then eluted with 2 x 15 mL of elution buffer (750 mM NaCl, 250 mM imidazole pH 8.0). In order to cleave the his-tag, thrombin was added to the eluents (33 nM), which were then placed in 8,000 MWCO dialysis tubing and stirred in dialysis buffer (0.5 M NaCl, 25 mM HEPES pH 8.0) overnight at 4 °C. Following dialysis, LynF-containing fractions were concentrated and run on an S-75 sizing column, for which an isocratic buffer (0.5 M NaCl, 25 mM HEPES pH 8.0) was employed. Fractions were tested for activity, the portion eluting between 65 and 77 mL was significantly more active as determined by MALDI-MS analysis of reactions containing **6**. The active fractions were then dialyzed into storage buffer (0.5 M NaCl, 25 mM HEPES pH 8.0, 10% glycerol), flash frozen in liquid nitrogen, and frozen at -80 °C until use.

Expression of *truLy1* was performed in a similar fashion, with the main difference being that rather than attempting to isolate soluble protein, *truLy1* was strongly overexpressed with the intent of driving the protein into inclusion bodies, after which time purification under denaturing conditions was performed. To wit, 8 x 1L cultures (sLB: 10 g/L soytone, 10 g/L NaCl, 5 g/L yeast extract, 50 μg/mL kanamycin) containing the *truLy1* plasmid were grown at 37 °C and upon reaching an A600 of 0.4, induced with 1 mM IPTG and allowed to grow overnight. Cell lysis and denaturing Ni-NTA purification was effected according to the manufacturer's protocol (Qiagen). The concentration of TruLy1 was assessed by amino acid analysis.

Enzyme Assays

Enzyme reactions typically contained enzyme (3.8 μM), variable substrate concentration (100 μM for most substrates; higher concentrations, i.e. 1 mM, were occasionally employed with boc-protected amino acid derivatives. Exceptions include substrates **11** and **12**, which were used at

20 μM final concentration as well as substrates **9**, and **10**, which were used at 70 and 30 μM, respectively), and several additives (1 M NaCl, 40 mM glycylglycine pH 9.0, 12 mM $MgCl_2$, 3 mM tris(2-carboxyethyl) phosphine (TCEP), and 1 mM DMAPP). Reactions were incubated at 37 °C for 24 h in a DNA Engine Peltier thermocycler (Bio-Rad). Enzyme reactions with full-length precursor peptide contained TruLy1 (28 μM), ATP (0.8 mM), with or without heterocyclase enzyme TruD (90 nM), and additives as above. Controls were run to ensure that LynF was active in the presence of TruD and TruLy1, and vice versa.

Reactions prepared for kinetic analysis of boc-L-Tyr were prepared in triplicate, and contained variable substrate concentrations (1, 2, 4, 8, and 16 mM), constant enzyme (0.9 μM), DMSO (4% v/v) for solubility, and additives as described above. The reactions were incubated for 3 h at 37 °C as above, halted by addition of guanidinium HCl (1 M), and then frozen at -80 °C until HPLC analysis. A sample of each reaction (15 μL) was injected onto a 214MS C4 5μ column (Grace-Vydac) and run on a linear gradient from 99% buffer A (H_2O, 0.5% TFA) / 1% buffer B (AcN) to 0% A / 100% B over 45 minutes. Peaks corresponding to O-prenyl and C-prenyl products were integrated, and summed to arrive at the total integrated area corresponding to prenylated product. The integrated areas were plotted on a calibration curve to determine what molar amounts of product they represented. The data were then analyzed using GraphPad Prism to derive kinetic curves and parameters.

Kinetic analysis of cyclo[APMPPYP] was performed in a similar fashion, with variable substrate concentrations (0.9, 2.1, 3.3, 6.6, 9, and 10.1 mM) and constant enzyme (1.8 μM). Reactions were incubated as above and quenched in an identical manner. HPLC conditions differed only in that the run began by holding the solvent mixture at 99% buffer A / 1% buffer B for the first 5 minutes, then stepped to 80% buffer A / 20% buffer B at which time a linear gradient to 60% buffer A / 40% buffer B over 34 minutes was begun. Additionally, controls were run with both boc-L-Tyr and cyclo[APMPPYP] to ensure that the rate of product formation was linear over the first several hours. To wit, reactions containing boc-L-Tyr (1, 4, and 8 mM), constant enzyme (0.9 μM), DMSO (4% v/v) for solubility, and additives as described above were incubated at 37 °C. Aliquots were removed at three time points (2, 4, and 6 h), and analyzed as above. A similar set of controls was performed containing cyclo[APMPPYP] (2.1, 3.3, and 6.6 mM), constant enzyme (1.8 μM), DMSO (4% v/v), and additives as described above. Data presented in figure 5 were generated via analysis of a time-course of the LynF (5.4 μM) reaction with boc-L-Tyr (1 mM) containing standard additives. Reaction aliquots (17.5 μL) were removed at 1, 2, 4, 6, and 24 h and immediately mixed with guanidinium HCl (3.5 μL, 6 M) and then placed at -80 °C until HPLC analysis.

Experiments assaying prenylation of TruLy1 were performed as follows: LynF (1.5 μM) or buffer control was incubated with either TruLy1 (14 μM), boc-L-Tyr (14 μM), cyclo[APMPPYP] (14 μM), without substrate, additives as above with the exception of DMAPP, and ^{3}H-labeled DMAPP (0.1 μM) (American Radiolabeled Chemicals), which had previously been dried under a stream of argon, and resuspended in ultrapure water. Reactions were covered with a mineral oil overlay (30 μL) and placed in a heat-block at 37 °C for 24h. Once complete, reaction aliquots (20 μL) were then quenched by addition of acid (2 M HCl in 80% EtOH, 4 μL), incubated a further 30 minutes to hydrolyze any remaining DMAPP. The resulting mixture was then added to a 7 mL scintillation vial along with water (150 μL), and placed in a sand-bath at 110 °C for 1.5 h. Resolubilization was then effected via addition of Soluene 350 (1 mL) (Perkin-Elmer), and heating in a sand bath at 50 °C for 1 h. Scintillation fluid (up to 7 mL) (Hionic-Fluor, Perkin-Elmer) was then added. Samples were placed in the dark for 30 minutes and then read by scintillation counting (5 minutes per sample). Radioassay samples were also analyzed by autoradiography, in which case a reaction aliquot (15 μL) was mixed with 6X SDS-PAGE buffer (0.6 M DTT, 0.35 M tris pH 6.8, 30% (v/v) glycerol, 10% (w/v) SDS, 0.012% (w/v) bromophenol blue, 3 μL), boiled for 5 minutes, and run on an 18%

polyacrylamide gel. After staining with Coomassie R250, gels were destained, and enhanced for autoradiography using En3hance (Perkin-Elmer) according to the manufacturer's instructions. Film (BioMax XAR, Kodak) was then exposed to the enhanced gel at -80 °C for at least 24 h and then developed. Lastly, possible prenylation of TruLy1 was assessed via ESI-MS intact analysis, as well as by proteolytic digest followed by LC-FT-ICR. In those cases, LynF (1.5 μM) was incubated with TruLy1 (14 μM) or boc-L-Tyr (640 μM), additives as above, and ATP (800 μM). Reactions were run at 37 °C for 24h; samples were taken at 0, 6, 12, and 24h time points, and were then analyzed by ESI-MS. Control reactions were run containing TruD (90 nM), or lacking ATP and TruD, and also analyzed by ESI-MS after 24h incubation. Boc-L-Tyr containing reactions were analyzed by HPLC as positive controls. Samples analyzed by LC-FT-ICR were digested either with previously characterized cyanobactin protease PatA (2 μM) including added calcium (10 mM),[6] or with trypsin and chymotrypsin.

Isolation of O-prenylated intermediate was performed as follows: a crude fraction of LynF (6.3 μM) was incubated with boc-D-Tyr (3.5 mM, 8.8 μmol), DMSO (1% v/v) for solubility, and additives as above. The reaction was allowed to proceed for 48 h at 37 °C. The reaction was then acidified (pH 4) with 1 M HCl and then extracted 5X with methylene chloride. The combined organic phases were then dried, and purified by HPLC. Purification was performed using a Onyx monolithic C18 semi-preparative column on a linear gradient from 99% buffer A (H_2O, 0.05% TFA) / 1% buffer B (AcN) to 100% buffer B over 60 minutes. The dominant C-prenylated product was purified along with the O-prenyl product.

To assess whether the conversion of reverse O-prenylated Tyr to forward C-prenylated Tyr was accelerated by LynF, the O-prenylated intermediate was purified as above, and then incubated at 37 °C with additives as above, excepting DMAPP, and either LynF (2.3 μM), boiled LynF (2.3 μM), or buffer only. Time points were taken at 0 and 8 h, and then frozen at -80 °C until analysis.

Phylogenetic Tree Construction

The amino acid sequences of LynF homologues from the functionally characterized cyanobactin pathways were aligned using CLUSTALX. Maximum likelihood analysis with molecular clock PROMLK (PHYLIP) using the bootstrap test method (1000 replicates) was performed to assess the phylogenetic relationship between the different homologues. The same tree branches were also supported using other phylogenetic experiments such as Maximum Parsimony (MEGA 4.0) using 1000 bootstrap replicates.

Preparative Enzymatic Synthesis

Preparative synthesis of prenylated boc-L-Tyr was performed as follows: a crude fraction of LynF (6.3 μM) was incubated with boc-L-Tyr (3.5 mM, 17.8 μmol) and additives as above. The reaction was incubated at 37 °C for 48 h and extracted as described above for boc-D-Tyr. Purification was accomplished using a Vydac C4 214TP1010 semi preparative column on a linear gradient from 99% buffer A (H_2O, 0.05% TFA) / 1% buffer B (AcN) to 100% buffer B over 45 minutes.

Preparative synthesis of prenylated boc-D-Tyr was performed as described in the previous section. The purified O- and C-prenylated products were subjected to NMR analysis: **Boc-4-O-(2-methylbut-3-en-2-yl)-D-tyrosine**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01 (2H, d, *J*=8.5 Hz), 6.90 (2H, d, *J*=8.5 Hz), 6.10 (1H, dd, *J*=17.3, 10.5), 5.14 (1H, ap d, *J*=17.8), 5.11 (1H, ap d, *J*=11.5), 4.88 (1H, s), 4.49 (1H, br), 3.10 (1H, br), 2.98 (1H, br), 1.42 (6H, s), 1.40 (9H, s). **Boc-3-(3-methylbut-2-en-1-yl)-D-tyrosine**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.90 (1H, d, *J*=8.0 Hz), 6.89 (1H, s), 6.71 (1H, d, *J*=8.0 Hz), 5.27 (1H, t, *J*=6.6 Hz), 4.86 (1H, s), 4.47 (1H, br), 3.30 (2H, d, *J*=7.0 Hz), 3.07 (1H, m), 3.00 (1H, br), 1.75 (6H, ap s), 1.41 (9H, s)

Preparative synthesis of prenylated cyclo[APMPPYP] was performed by incubation of sizing-column purified LynF (5 μM) with cyclo[APMPPYP] (0.4 mM) and additives as above. The reaction was incubated at 37 °C for 88 h, and then frozen at -20 °C. Purification was accomplished using a Vydac C4 214TP1010 semi preparative column on a linear gradient from 99% buffer A (H_2O, 0.05% TFA) / 1% buffer B (AcN) to 100% buffer B over 45 minutes.

Preparative synthesis of prenylated boc-D-Tyr was performed as described in the previous section. The purified O- and C-prenylated products were subjected to NMR analysis: **Boc-4-O-(2-methylbut-3-en-2-yl)-D-tyrosine**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01 (2H, d, *J*=8.5 Hz), 6.90 (2H, d, *J*=8.5 Hz), 6.10 (1H, dd, *J*=17.3, 10.5), 5.14 (1H, ap d, *J*=17.8), 5.11 (1H, ap d, *J*=11.5), 4.88 (1H, s), 4.49 (1H, br), 3.10 (1H, br), 2.98 (1H, br), 1.42 (6H, s), 1.40 (9H, s). **Boc-3-(3-methylbut-2-en-1-yl)-D-tyrosine**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.90 (1H, d, *J*=8.0 Hz), 6.89 (1H, s), 6.71 (1H, d, *J*=8.0 Hz), 5.27 (1H, t, *J*=6.6 Hz), 4.86 (1H, s), 4.47 (1H, br), 3.30 (2H, d, *J*=7.0 Hz), 3.07 (1H, m), 3.00 (1H, br), 1.75 (6H, ap s), 1.41 (9H, s)

Preparative synthesis of prenylated cyclo[APMPPYP] was performed by incubation of sizing-column purified LynF (5 μM) with cyclo[APMPPYP] (0.4 mM) and additives as above. The reaction was incubated at 37 °C for 88 h, and then frozen at -20 °C. Purification was accomplished using a Vydac C4 214TP1010 semi preparative column; the run was begun by holding the solvent mixture at 99% buffer A / 1% buffer B for the first 5 minutes, then stepped to 80% buffer A / 20% buffer B at which time a linear gradient to 60% buffer A / 40% buffer B over 55 minutes was begun.

General Methods

ESI-MS, and FT-ICR analyses were performed at the University of Utah Mass Spectrometry and Proteomics core facility. MALDI-MS analyses were performed on a Micromass MALDI micro MX instrument (Waters). HPLC separations were performed on a LaChrom Elite system (Hitachi). NMR spectra were collected either on 400 or 500 MHz spectrometers (Varian). CD spectra were collected on a Jasco J-815 spectrometer, and data were plotted in Excel.

Table S1. Substrates used in this study.

#	Substrate	Result	Analytical Method(s)
3	TruLy1 precursor peptide	NR	ESI-MS, LC-FT-ICR, radioassay, autoradiography
4	TruD-treated TruLy1	NR	ESI-MS, LC-FT-ICR, radioassay, autoradiography
5	APMPPYPSYDDAE	NR	MALDI-MS, HPLC
6	cyclo[APMPPYP]	48%	MALDI-MS, HPLC, LC-FT-ICR, NMR, radioassay
7	APMPPYP	12%	MALDI-MS, HPLC, LC-FT-ICR
8	N-acetyl APMPPYP	10%	HPLC
9	cyclo[APMPPPAPMPPYP]	47%	MALDI-MS, LC-FT-ICR
10	cyclo[APMPPYPAPMPPYP]	43%	MALDI-MS, LC-FT-ICR
12	cyclo[KKPYILP]	37%	MALDI-MS, LC-FT-ICR
12	cyclo[KPYILP]	94%	MALDI-MS, LC-FT-ICR
13	KPYILP	1%	MALDI-MS, LC-FT-ICR
14	Boc-L-Tyr	71%	HPLC, FT-ICR, NMR, CD, radioassay
15	Boc-D-Tyr	66%	HPLC, NMR, CD
16	Boc-4-cyano-L-Phe	NR	HPLC
17	Boc-O-allyl-L-Tyr	NR	HPLC
18	Boc-4-iodo-L-Phe	NR	HPLC
19	Boc-4-methoxy-L-Phe	NR	HPLC
20	L-Phe	NR	HPLC
21	N-acetyl-L-Tyr	3%	HPLC, ESI-MS
22	L-Tyr	NR	HPLC
23	Dopamine	NR	HPLC
24	Phenol	NR	HPLC
25	L-Trp	NR	HPLC
26	cyclo[QGGRGDWP]	NR	MALDI-MS
27	QGGRGDWPAYDGE	NR	MALDI-MS

```
TruLy1:
MGSSHHHHHHSSGLVPRGSHMNKKNILPQLGQPVIRLTAGQLSSQLAELSE
EALGGVDASTLPVPTLCSYDGVDASVCMPCYPSYDD

TruLy1 with 3 heterocycles:
MGSSHHHHHHSSGLVPRGSHMNKKNILPQLGQPVIRLTAGQLSSQLAELSE
EALGGVDASTLPVPTL(Tz)SYDGVDASV(Tz)MP(Tz)YPSYDD
Tz=thiazoline
```

Table S2. Summary of FT-ICR data

Substrate	Product[a]	Expected *m/z*	Observed *m/z*	Δppm
6	cyclo[APMPP**y**P]	822.4219 ($M+H^+$)	822.4216	-0.36
7	APMPP**y**P	840.4324 ($M+H^+$)	840.4328	-0.47
9	cyclo[APMPPPAPMPP**y**P]	706.8589 ($M+2H^{2+}$)	706.8593	+0.56
10	cyclo[APMPP**y**PAPMPPYP]	788.3906 ($M+2H^{2+}$)	788.3912	+0.76
10	cyclo[APMPP**y**PAPMPP**y**P]	822.4219 ($M+2H^{2+}$)	822.4227	+0.97
11	cyclo[KKP**y**ILP]	454.8020 ($M+2H^{2+}$)	454.8019	-0.22
12	cyclo[KP**y**ILP]	780.5018 ($M+H^+$)	780.5013	-0.64
13	KP**y**ILP	798.5124 ($M+H^+$)	798.5137	+1.6
14	O- and C-prenyl boc-L-Tyr	350.1962 ($M+H^+$)	350.1966	+1.1

[a]'Y' denotes tyrosine, 'y' denotes prenylated tyrosine

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