Supplementary Information

Mapping the biosynthetic pathway of a hybrid polyketide-nonribosomal peptide in a metazoan

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a Thioesterase (TE) domains

```
PKS-1_TE1    .....DLKYPSNDLRELAHFYAEEIAA...HAGNKRIFVMGHSMGGIMSREIVAELKIWG
NRPS-1_TE2   ..........GDTVDEVAKLYRLQIEESAENIETSKLVFIGASSAGTFAFSTSQLFADDD
Pik_TE       GTGTGTALLPADLDTALDAQARAILR....AAGDAPVVLLGHSGGALLAHELAFRLERAH
Sur_TE       NQ.TS....AIEDLEELTDLYKQELN....LRPDRPFVLFGHSMGGMITFRLAQKLEREG
Ybt_TE       LRHLE....PLRSITQLAALLANELEA..SVSPDTPLLLAGHSMGAQVAFETCRLLEQRG
Rif_TE       RRHEP....PVDSIGGLTNRLLEVLR....PFGDRPLALFGHSMGAIIGYELALRMPEAG
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b Condensation (C) domains

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NRPS-1_C2    ............EKSSVSNPLSAI...QCSKLSPETLQLIFHHISIDGRSLAIFYQQFK
NRPS-1_C3    ............PSGASRPKDQFSIQLWMSSKNKLLTISIHHLICDGRSLQILEHQLQ
NRPS-1_C4    IR.LLCDEPINV....LEGSPMIRASFI..SSPEKHVAFLHLHHLISDARSTQLTNSTMK
PKS-1_C1     .........NHLFE..IGKSTPLRVRVAEDCDNSRIHIVFNQHHILTDGWSMTVLSDTVS
ArfA-C2      FSARR....Y...RLDVSQAPLMRLVYARDPALDRVVGILLFHHLAMDHIALEV....MR
VibH_C       QIEQDLQRSSTLIDAPITSHQVYRLSHS......EHLIYTRAHHIVLDGYGMMLFEQRLS
CDA_C1       WMDRDRATPLPLDRPGLSSHALFTLGGG......RHLYYLGVHHIVIDGTSMALFYERLA
                                               *    *
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c Acyl-carrier protein (ACP) domains

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PKS-1_ACP3   ....KTLQMAVRHKVCLAVGDVIESGLDIDESQLSTGFSE.LGIDSLATVDLLNRLNQKY
PKS-1_ACP4   ESDATVDRTEIRRKVSLAVFDLATETLSAEDLQ.SKGFTE.LGMDSLSIVDFVNRLNDKY
PKS-1_ACP6   ....KVKEEIKKKSLNFEEIFFEIVGITDISSKLNIPFMD.LGIDSLCMENLRYSLNKN.
Bac_ACP      ..........ADTLERVTKIIVDRLGVDEADVKLEASFKEDLGADSLDVVELVMELEDE.
PKS-1_ACP5   ...MNFSVEDEEEVLELIKEKVSSILMCSPTKLKNNKNIMDMGLDSKLIVEFLNFINST.
NRPS-1_ACP7  .....CTARPSRSMEILISILKDQMKLLSTSHEVETTPLPYLGIDSLRLAELEYHVASH.
PKS-1_ACP1   .NIVDEQTNSSLSDAEIESTVRTIVKQFLDIEEDDINLLETGAVDSLTSIEMVEAFGTAV
PKS-1_ACP2   .KITKKVENEDQKRASKNMLHVWFEENFGWTDIDNTTGFFDLGLTSIQAVKLRNAIKSN.
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d Adenylation (A) domains

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EntE_A       FFALLKLGVAPVL..ALFSHQRSELNAYASQIEPALLIADRQH....ALFSGDDFLNTFV
PKS-1_A1     LLACVFLGLPYAPIDPTWPEPRQLFVKSKVSFTLE....N..............CF...
SidN_A3      IVGIMKSGNTYVPIEAGLPNDRKSFLLRDSRAAMAFVCDN.........NFDGVELPP...
Grs_A        ILAVLKAGGAYVPIDIEYPKERIQYILDDSQARMLLTQKHLVHLIHNIQFNGQVEIF...
NRPS-1_A2    VLAAWEAGLYPVPMHKDSKEAQIEKTLEALGIEEA..............FDSKDLCQ...
NRPS-1_A3    LAVQFT.GAAYLPIDASYPEERKKTILKDSVFNFE..............YNGRVDQE...

EntE_A       TEHSSIRVVQLLNDSGEHNLQDAINHPAEDFTATPSPADEVAYFQLSGGTTGTPKLIPRT
PKS-1_A1     ........................SCNLKLRNFNSRTQFGSIYSIFTSGSTGVPKGVLMA
SidN_A3      .ET...KV...LD.TKNQSFIENLSTQDTSDILNNYPENLDAYLLYTSGSTGTPKGVRVS
Grs_A        .EE...DT...IK............IREGTNLHVPSKSTDLAYVIYTSGTTGNPKGTMLE
NRPS-1_A2    ........................TKLRIFNKSILYDLAYVTSTSGSTGTPKLVGTS
NRPS-1_A3    ........................PRHRHFAISTDYCLSYIITTSGTTGTPKSVAIG
                                                    *

EntE_A       HNDYYYSVRRSVE........ICQFTQQTRYLCAIPAAHNYAMSSPGSLGVFLAGGTVVL
PKS-1_A1     EQSVSSFMTSASK........QCMFRSNIRVLDSVKQVFD..VSVSNIIGSVLNGGVLIS
SidN_A3      RHNLSSFSDAWGKLIGNVAPKSLELGGVGKFLCLASRAFD..VHIGEMFLAWRFGLCAVT
Grs_A        HKGISNLKVFFEN.........SLNVTEKDRIGQFASISFD..ASVWEMFMALLTGASLYI
NRPS-1_A2    FEGHSNLARQYTT.........TYQISSRDTVGQVVDPSFD..IFFADIVK......TLVN
NRPS-1_A3    AKSLLNLFLSSTL.........TMKCSSSSRTYQFTNFVFD..NSVLEVSMSIASQGTLVY
```

e Peptidyl-carrier protein (PCP) domains

```
NRPS-1_PCP3  .LPHPTLPNSLESDLSSIWTSLLNCPEPSPSDH.FFLIGGHSLLLVRLRHLIETKLGISL
NRPS-1_PCP1  KAAISIARLSLTSSLKHWLEHYSNLEIPSDDDD.IFTLGVDSIAVMLAMQKLRSEN.IEI
PKS-1_PCP2   KREIVVMKNSLEEKVINVFSKILGR.NVAPTDK.FESIGGNSLNAIQIAHRLAEELKIEI
NRPS-1_PCP2  KLLGLVEVRKKLELIIAAFKKFLDNTDVTKSTD.FFQAGGHSLTAMRLIDHLSDLLEVEI
Yer_PCP1     .AEADLPQGDIEKQVAALWQQLLSTGNVTRETD.FFQQGGDSLLATRLTGQLHQAG.YEA
PKS-1_PCP1   ESLKLPKSTSCEFVIAEIWKETLGISILNDANPNFFSLGGDSLSALQVVWKVQKKTDRIV
                                                 *
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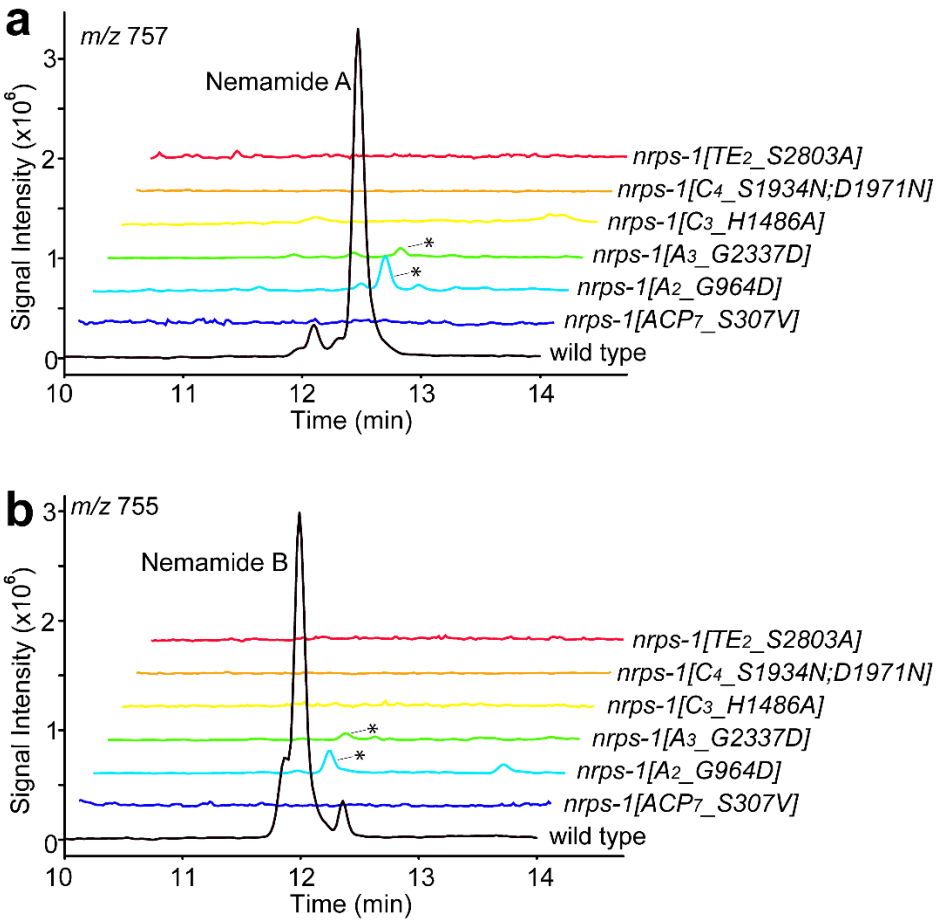
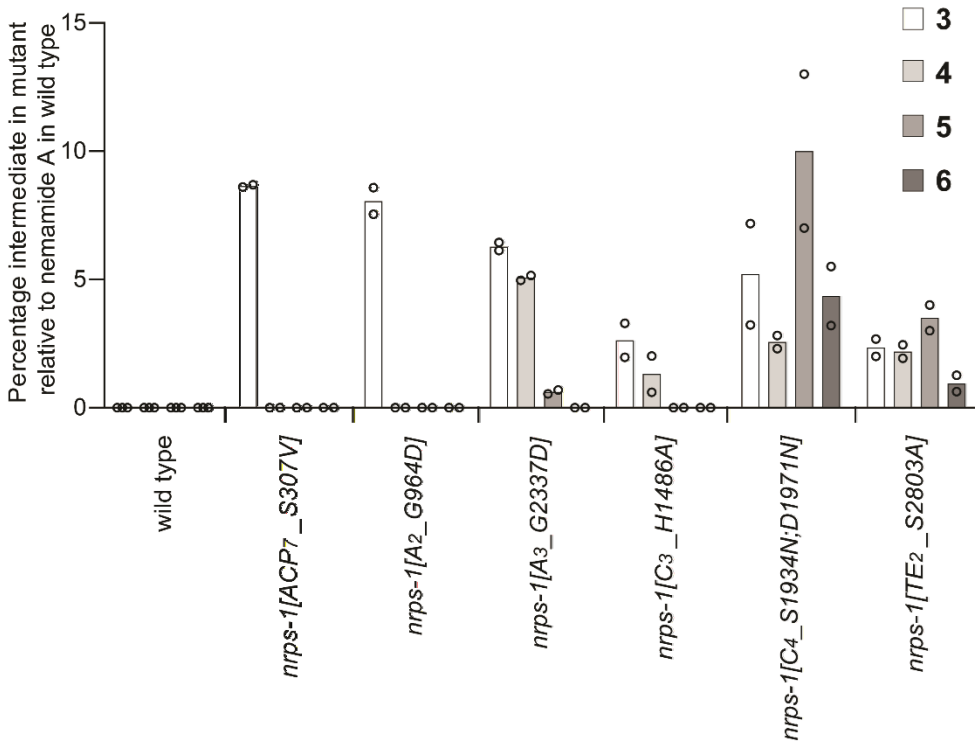
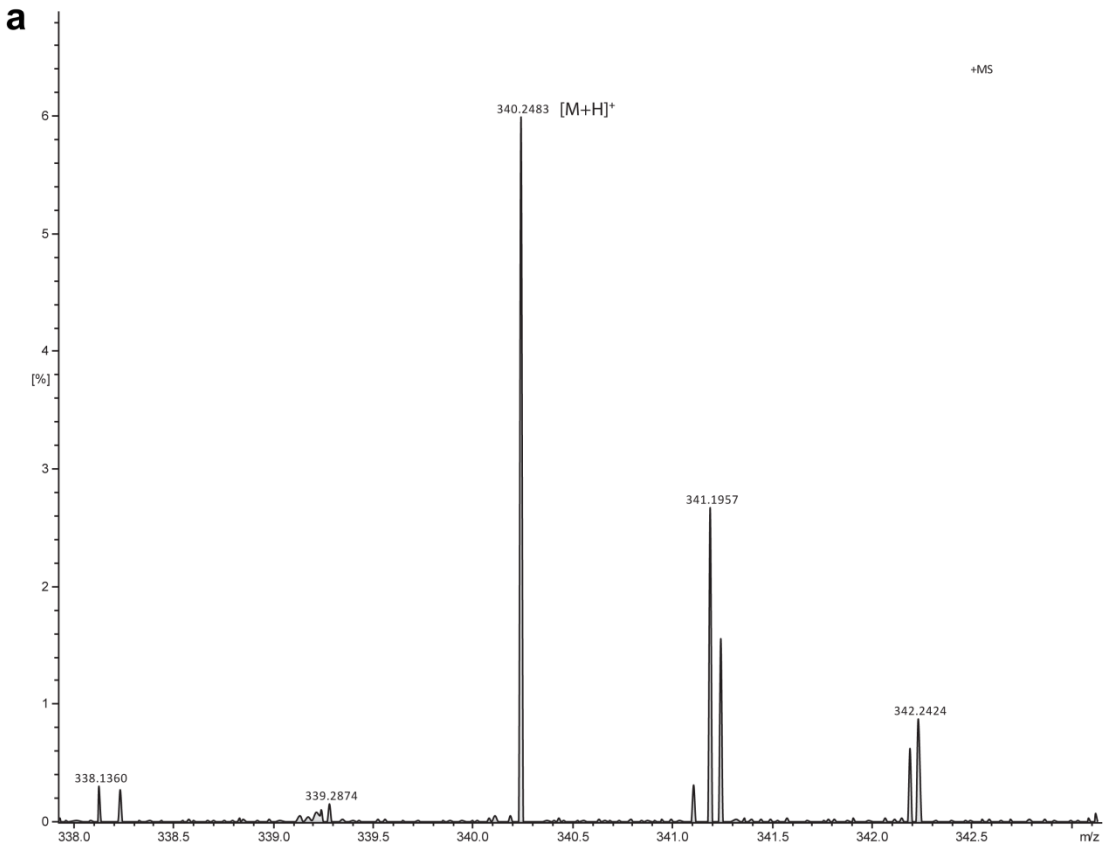
Supplementary Figure 1. Domain sequence alignments of PKS-1 and NRPS-1 with known functional domains. **a** Sequence alignment of TE domains with Pik_TE (Pikromycin TE domain, PDB ID: 2H7Y), Sur_TE (Surfactin TE domain, PDB ID: 2RON), Yer_TE (Yersiniabactin TE domain, PDB ID: 6BA8), and Rif_TE (Rifamycin TE domain, PDB ID: 3FLA). Sequences used for TE domains are PKS-1_TE_1 (7559-7610) and NRPS-1_TE_2 (2771-2820). The catalytic serine residue is labeled with an asterisk. **b** Sequence alignment of C domains with ArfA_C_2 (Arthrofactin module A C_2 domain), VibH_C (Vibriobactin free-standing C domain VibH, PDB ID: 1L5A), CDA_C_1 (Calcium-dependent antibiotic synthase C_1 domain, PDB ID: 4JN3). Sequences used for C domains are PKS-1_C_1 (6653-6701), NRPS-1_C_2 (520-563), NRPS-1_C_3 (1457-1502), and NRPS-1_C_4 (1895-1947). In the HHxxxDG motif, the second histidine (asterisk) serves as a catalytic base, and the aspartate (asterisk) is critical for the structural integrity of the active site. **c** Sequence alignment of NRPS-1_ACP_7 with PKS-1_ACP domains and *Bacillus subtilis* ACP (GenBank accession no. P80643, PDB ID: 1HY8). The conserved active site serine is marked with asterisk. Protein sequences used are PKS-1_ACP_1 (719-776), PKS-1_ACP_2 (1776-1833), PKS-1_ACP_3 (2792-2846), PKS-1_ACP_4 (2940-2997), PKS-1_ACP_5 (3471-3526), PKS-1_ACP_6 (5154-5207), and NRPS-1_ACP_7 (267-320). **d** Sequence alignment of A domains with EntE_A (Enterobactin module E, PDB ID: 3RG2), SidN_A_3 (A_3 domain in Siderophore N synthetase, PDB ID: 3ITE), Grs_A (Gramicidin S, PDB ID: 1AMU). Sequences used for A domains are PKS-1_A_1 (7044-7167), NRPS-1_A_2 (900-1019), and NRPS-1_A_3 (2274-2398). The conserved glycine residue in the flexible loop involved in the interaction with the pyrophosphate leaving group during amino acid loading is marked with an asterisk.[1] **e** Sequence alignment of PKS-1_PCP domains, NRPS-1_PCP domains, and Yer_PCP_1 (Yersiniabactin synthetase PCP1 domain, GenBank accession no. Q7CI41, PDB ID: 5U3H). The serine residue for phosphopantetheinyl postranslational modification is located in the conserved PCP motif DXFFXLGGDSL and is marked with an asterisk. Protein sequences are PKS-1_PCP_1 (6462-6521), PKS-1_PCP_2 (7424-7481), NRPS-1_PCP_3 (1289-1346), NRPS-1_PCP_4 (1700-1758), and NRPS-1_PCP_5 (2648-2705). Alignment was generated by Clustal Omega 1.2.4 and ESPript 3.0.[2]



Supplementary Figure 2. Nemamide production in wild-type and *nrps-1* domain mutants. Extracted ion chromatograms for nemamide A (**a**) and nemamide B (**b**) in wild type, *nrps-1(reb8[ACP$_7$_S307V])*, *nrps-1(reb32[A$_2$_G964D])*, *nrps-1(reb31[A$_3$_G2337D])*, *nrps-1(reb10[C$_3$_H1486A])*, *nrps-1(gk186409[C$_4$_S1934N]; gk186410[C$_4$_D1971N]),* and *nrps-1(reb12[TE$_2$_S2803A])* worms. The *nrps-1(gk186409[C$_4$_S1934N]; gk186410[C$_4$_D1971N])* mutant was obtained from the Caenorhabditis Genetics Center and backcrossed four times with wild type. As indicated by the asterisks (*), both the *nrps-1(reb32[A$_2$_G964D])* and the *nrps-1(reb31[A$_3$_G2337D])* strains produced small amounts of nemamides; the *nrps-1(reb32[A$_2$_G964D])* strain produced 15.7±3.7% nemamide A relative to wild type, and the *nrps-1(reb31[A$_3$_G2337D])* strain produced 2.7±0.8% nemamide A relative to wild type, suggesting that the A domains in these mutants have some residual activity. Note that the retention times of the nemamides in the Supplementary Information are different than in the main text due to the fact that the samples were analyzed on different columns.



Supplementary Figure 3. Production of intermediates in wild type and *nrps-1* domain mutants. The production of nemamide A (**1**) and intermediates **3**, **4**, **5**, and **6** in wild type, *nrps-1(reb8[ACP$_7$_S307V])*, *nrps-1(reb32[A$_2$_G964D])*, *nrps-1(reb31[A$_3$_G2337D])*, *nrps-1(reb10[C$_3$_H1486A])*, *nrps-1(gk186409[C$_4$_S1934N]; gk186410[C$_4$_D1971N]),* and *nrps-1(reb12[TE$_2$_S2803A])* worms. The *nrps-1(gk186409[C$_4$_S1934N]; gk186410[C$_4$_D1971N])* mutant was obtained from the Caenorhabditis Genetics Center and backcrossed four times with wild type. Percentage for each intermediate in each strain was determined by comparing the amount of the intermediate (as gauged by UV at 280 nm) relative to the mean amount of nemamide A (as gauged by UV at 280 nm) in wild type. Data represent the mean of data points (indicated as open circles), n = 3 independent experiments for wild type and n = 2 independent experiments for all mutants. In each experiment, the worm strain was grown in large-scale culture, and the worms were collected for extraction of nemamide (for wild type) and intermediates (for wild type and mutants). Source data are provided as a Source Data file.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341.1957
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338.1360
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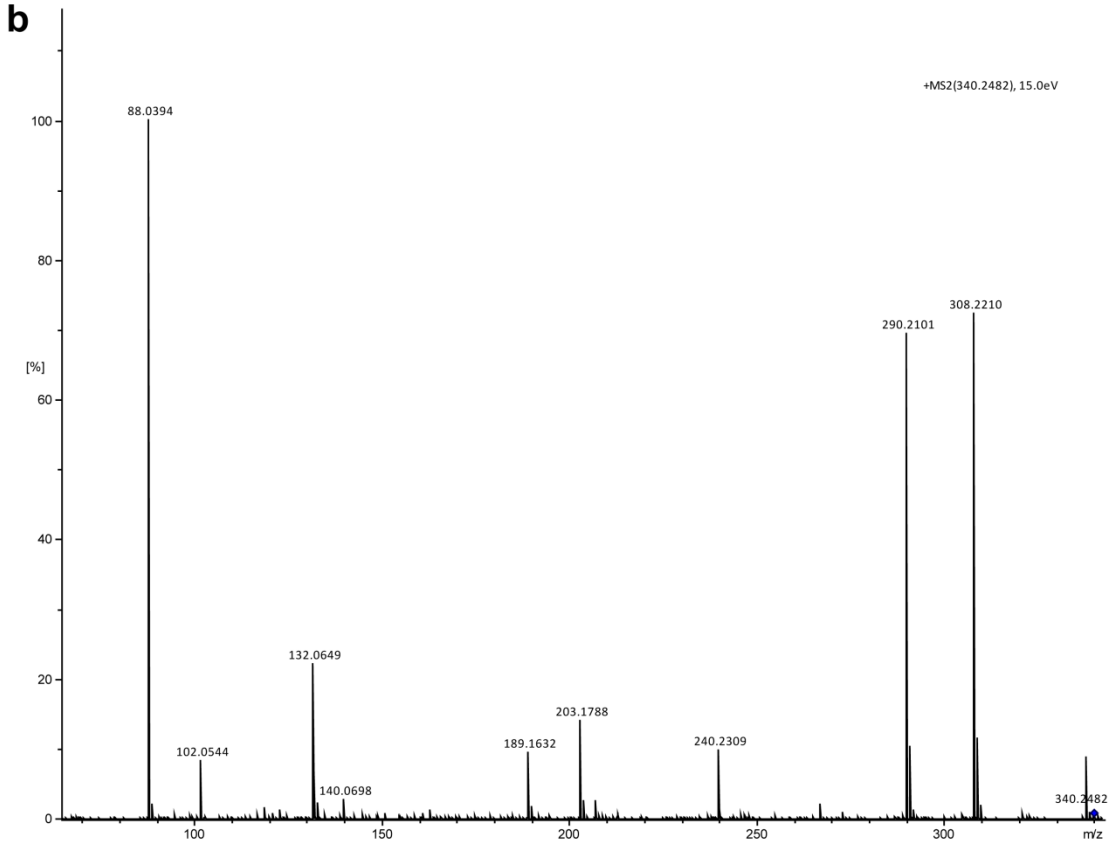
m/z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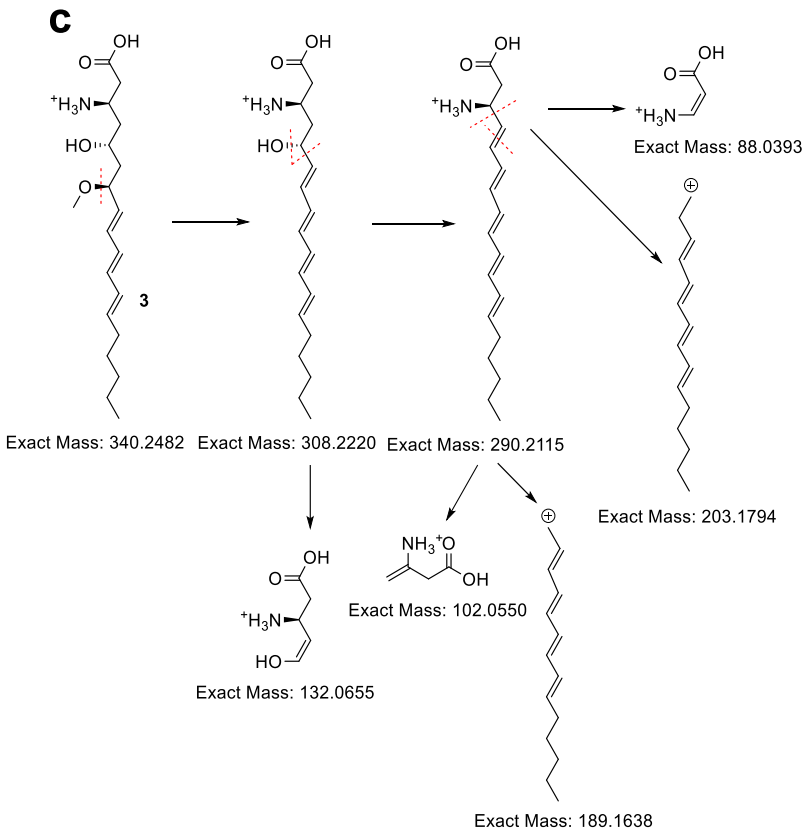
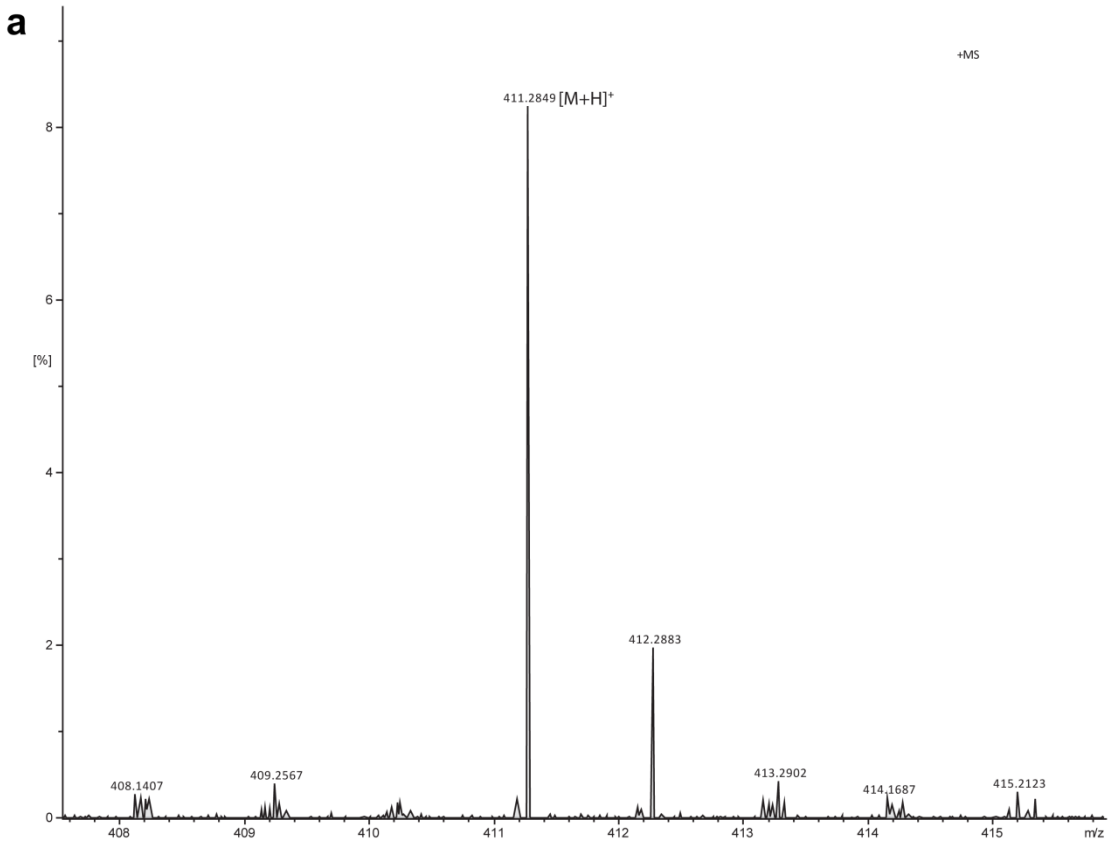


Supplementary Figure 4. Mass spectrometry analysis of intermediate 3. **a**,**b** HR-LC-MS/MS of intermediate **3**. **c** Analysis of the fragmentation patterns.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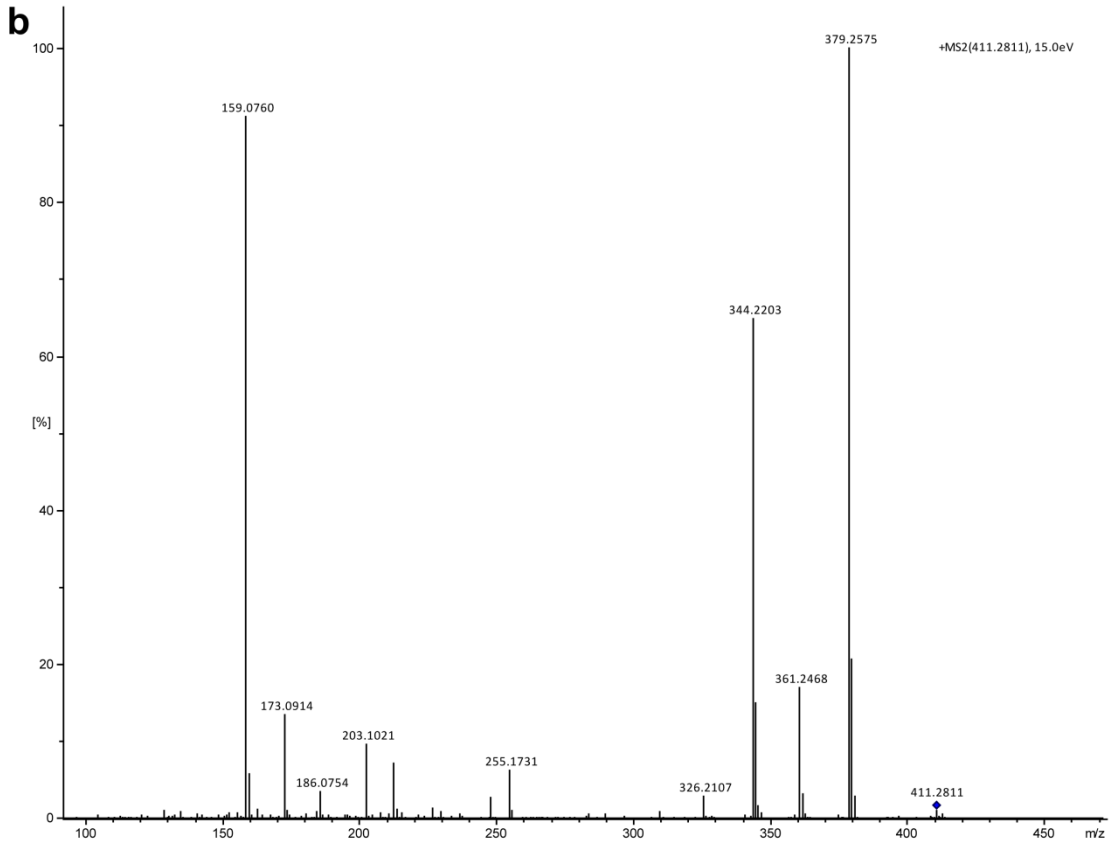
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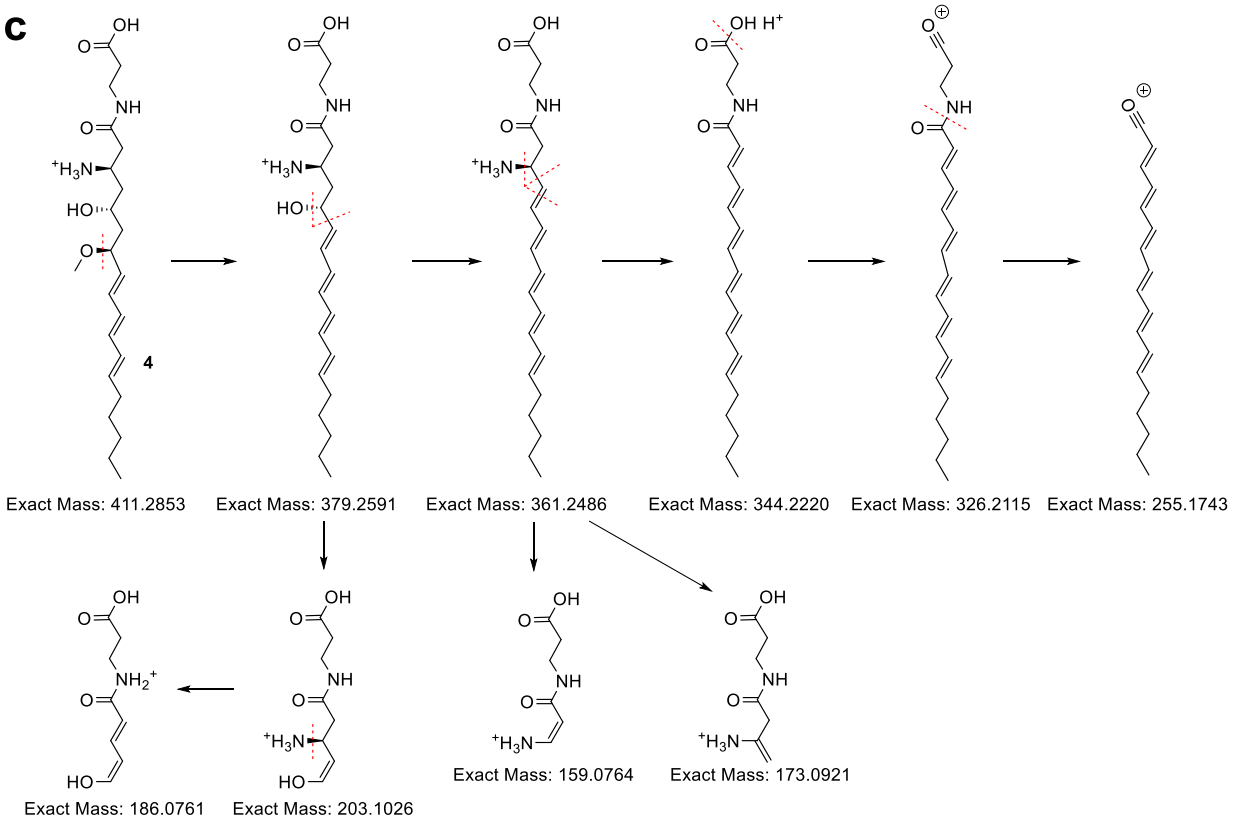
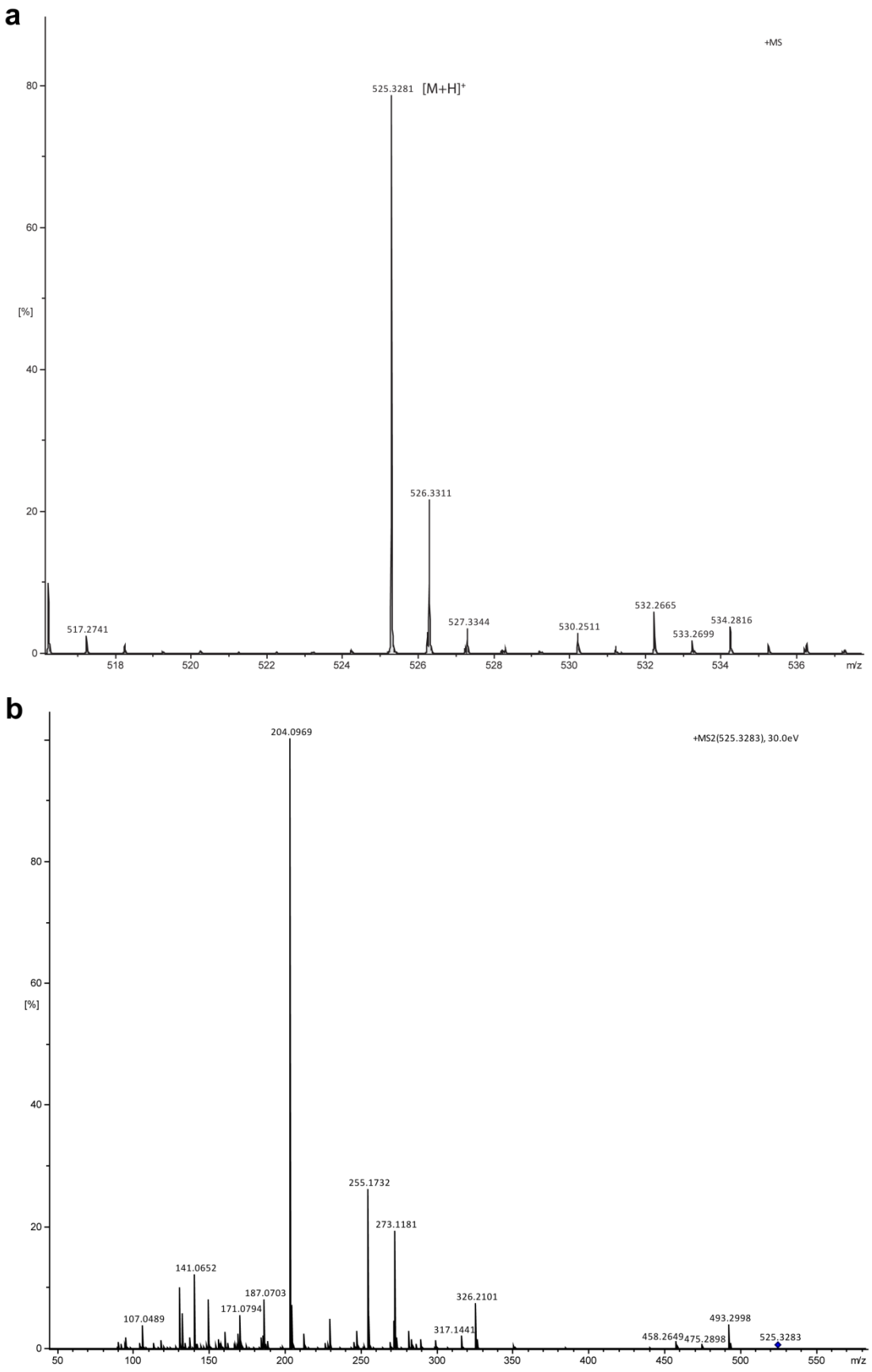
m/z



Supplementary Figure 5. Mass spectrometry analysis of intermediate 4. **a**,**b** HR-LC-MS/MS of intermediate **4**. **c** Analysis of the fragmentation patterns.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m/z
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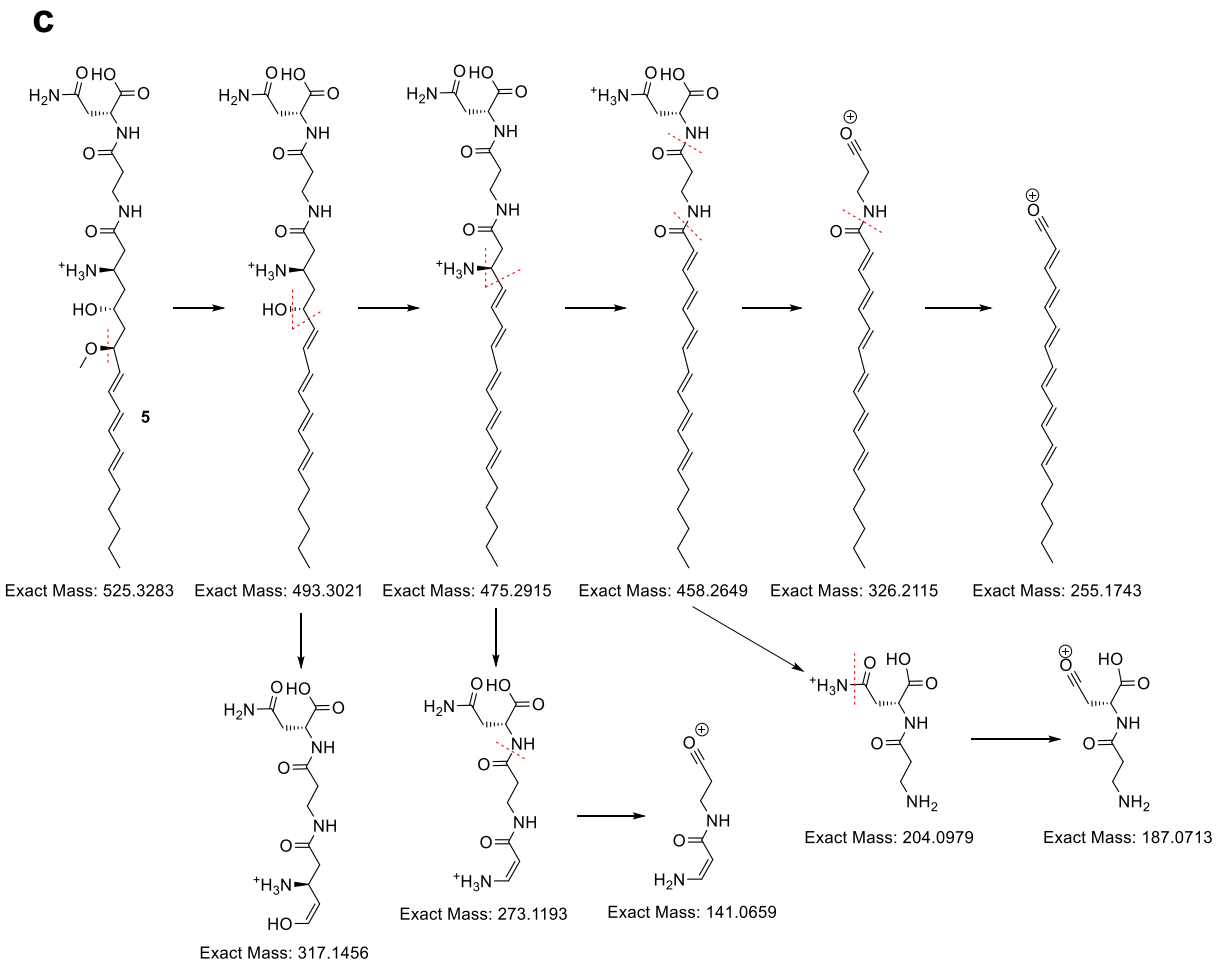
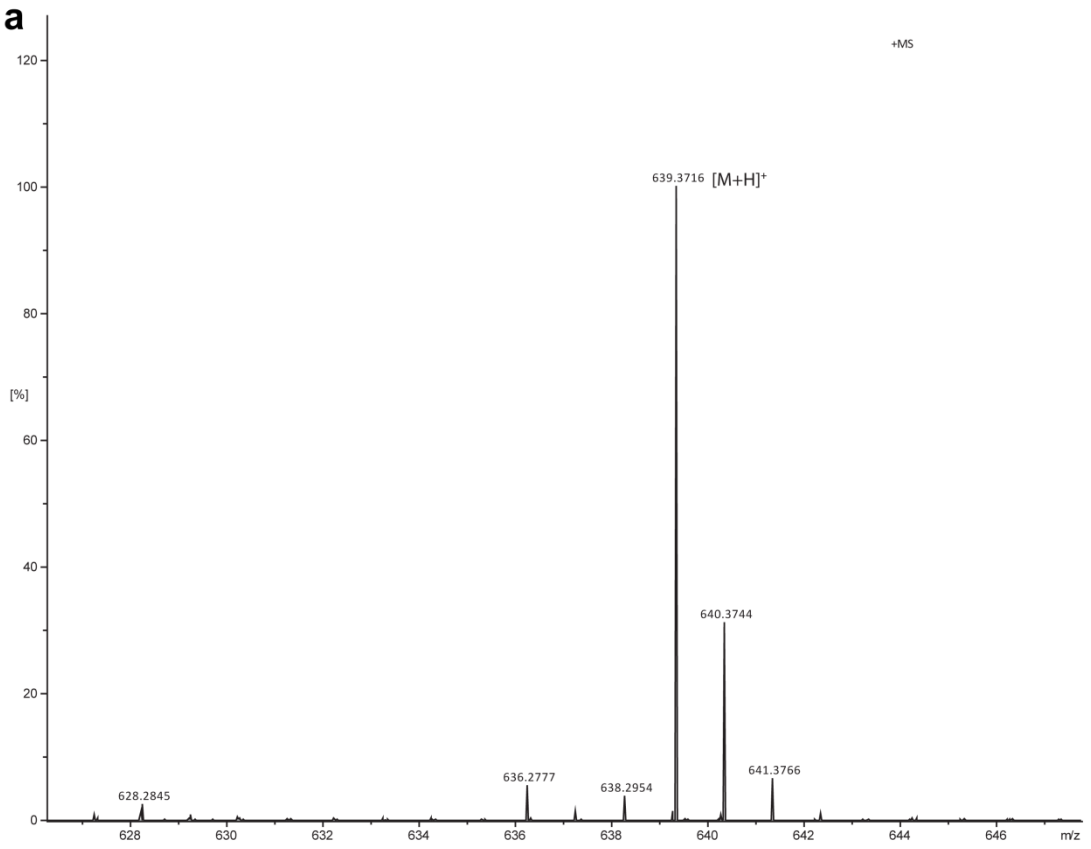


Supplementary Figure 6. Mass spectrometry analysis of intermediate 5. **a**,**b** HR-LC-MS/MS of intermediate **5**. **c** Analysis of the fragmentation patterns.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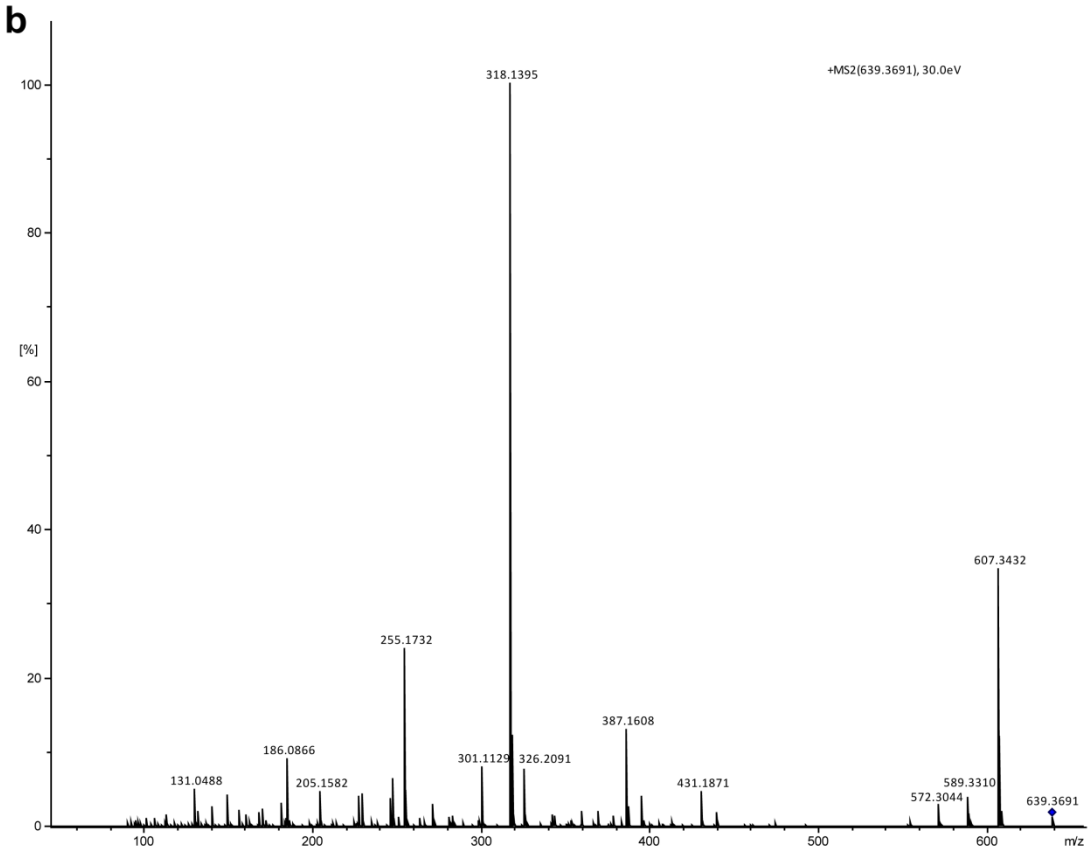
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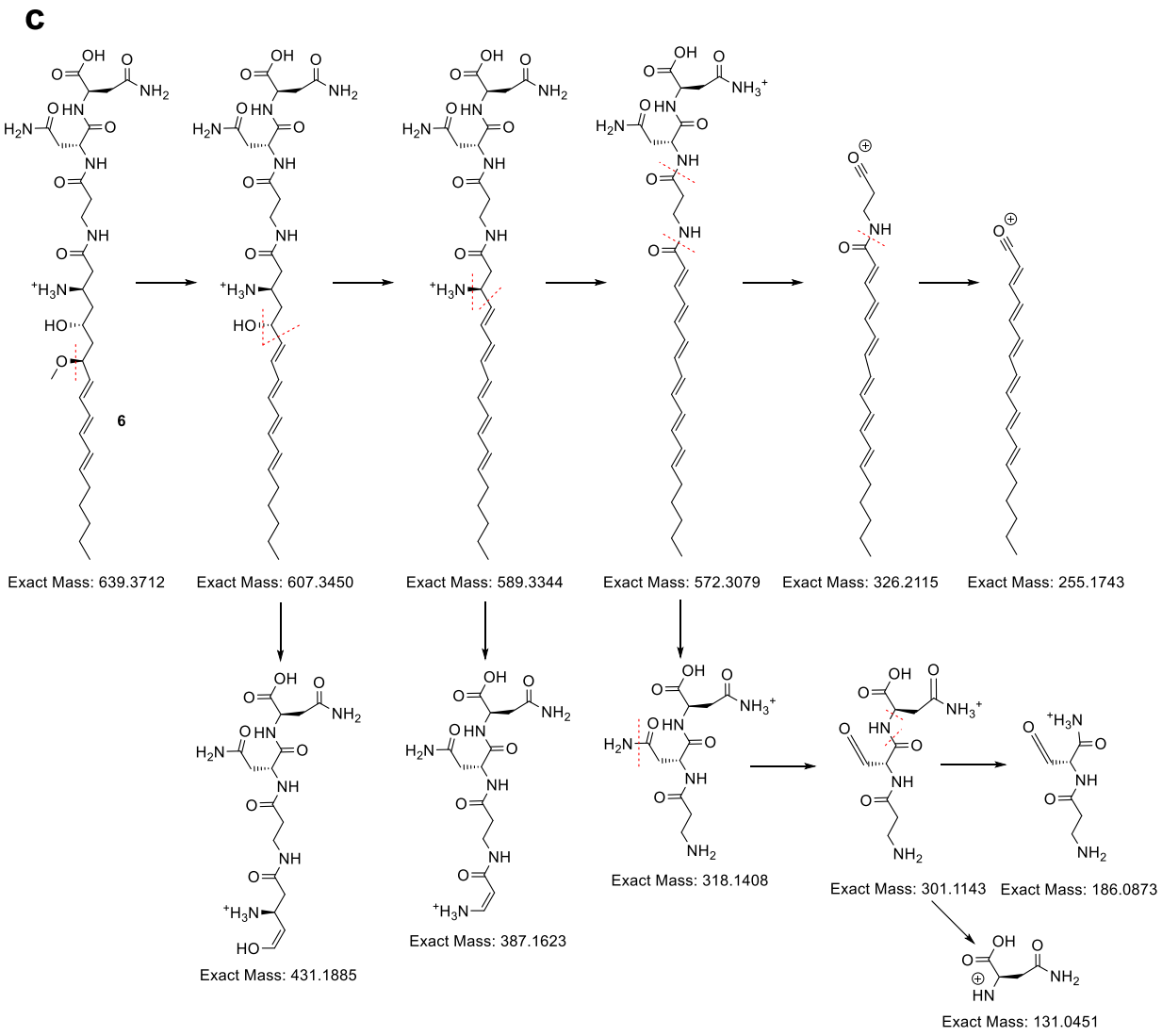
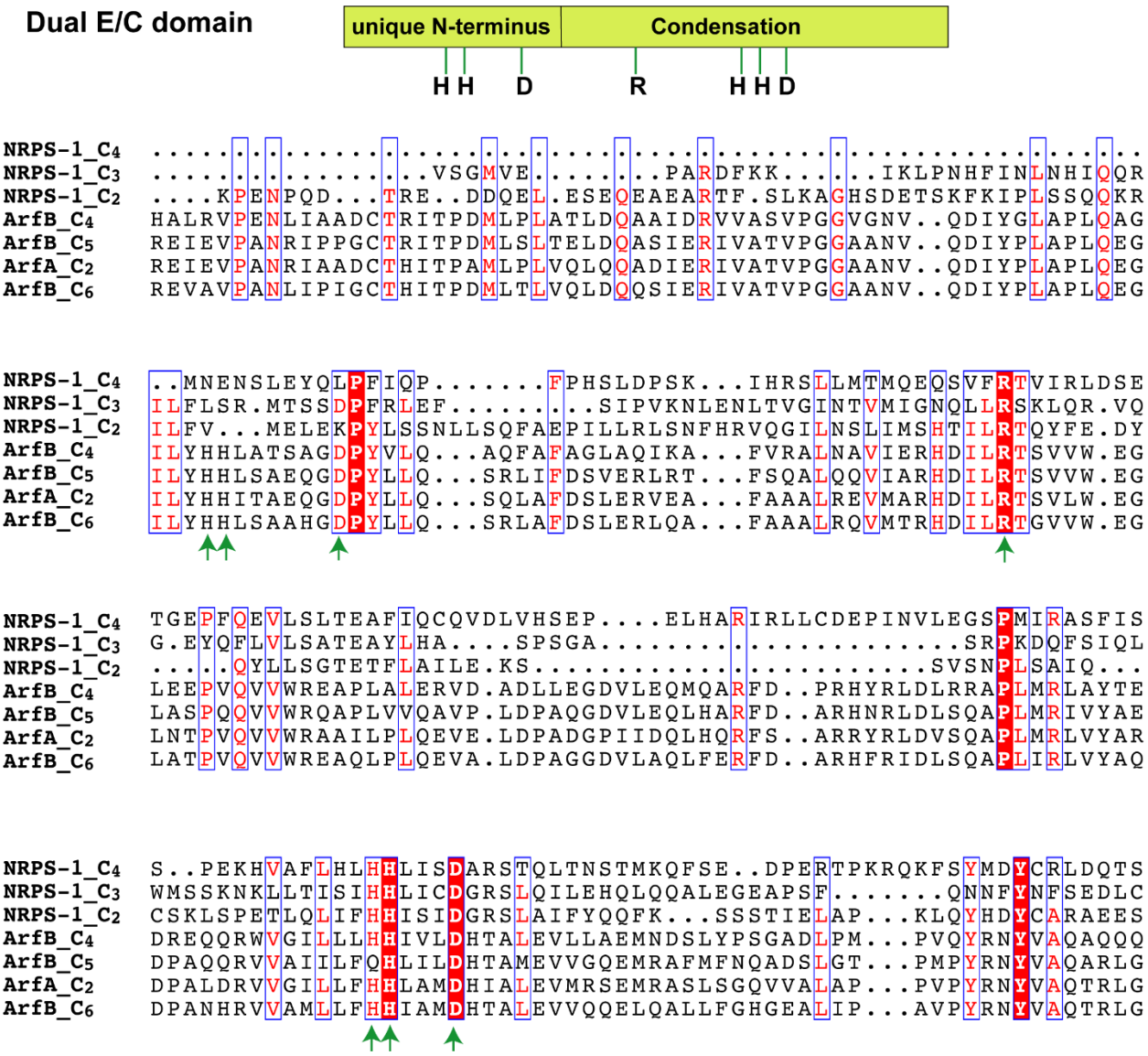
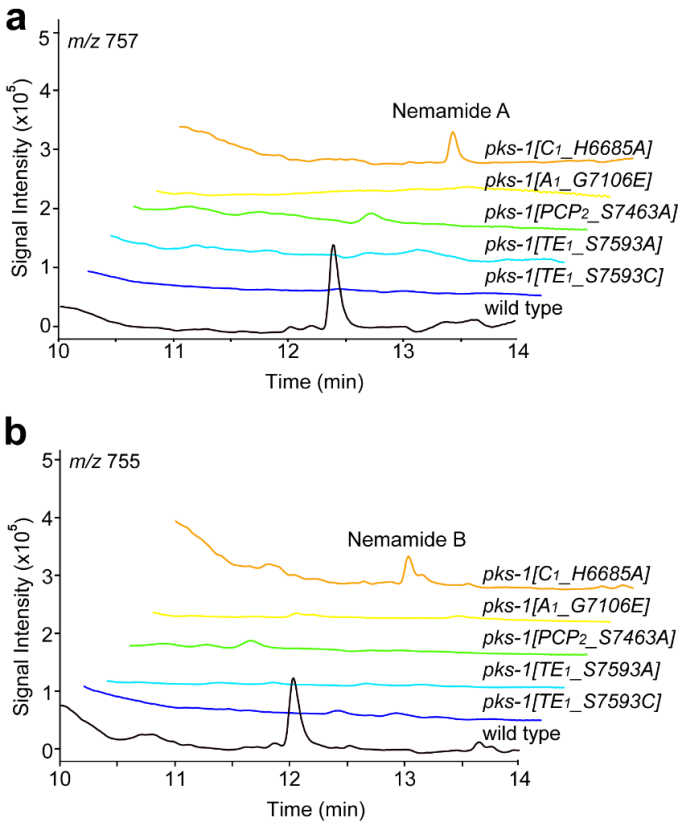


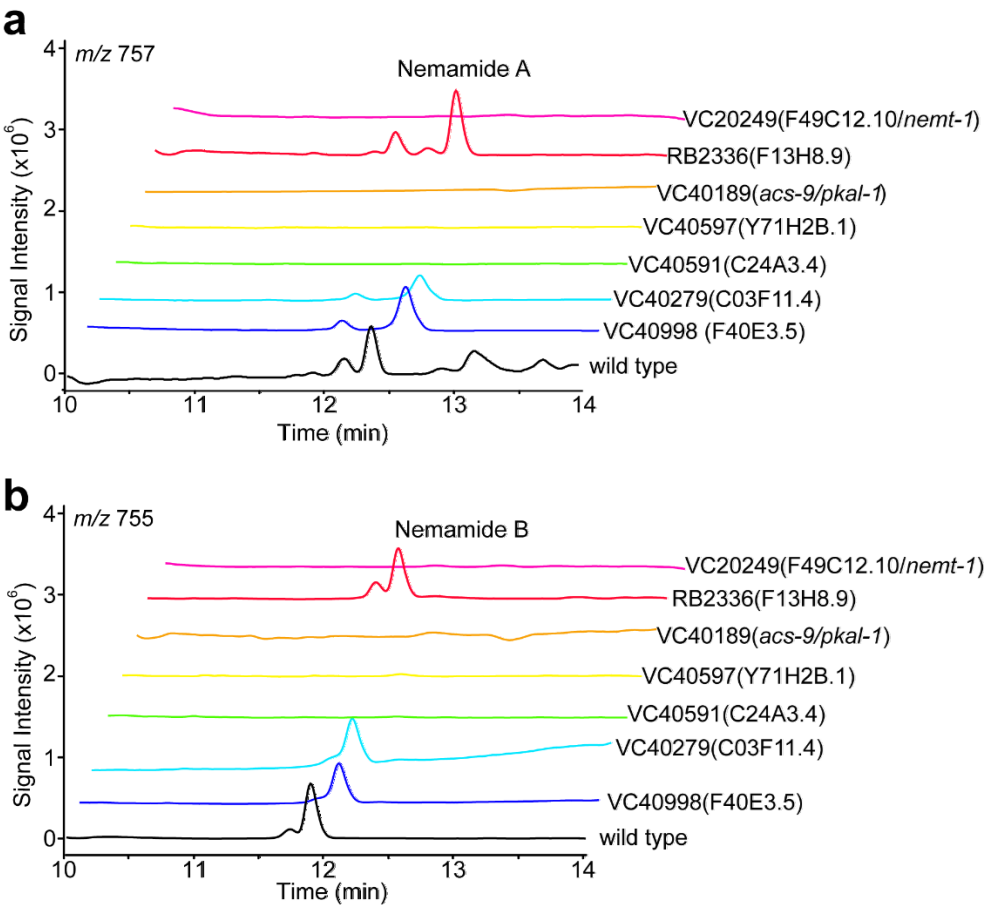
Supplementary Figure 7. Mass spectrometry analysis of intermediate 6. **a**,**b** HR-LC-MS/MS of intermediate **6**. **c** Analysis of the fragmentation patterns.



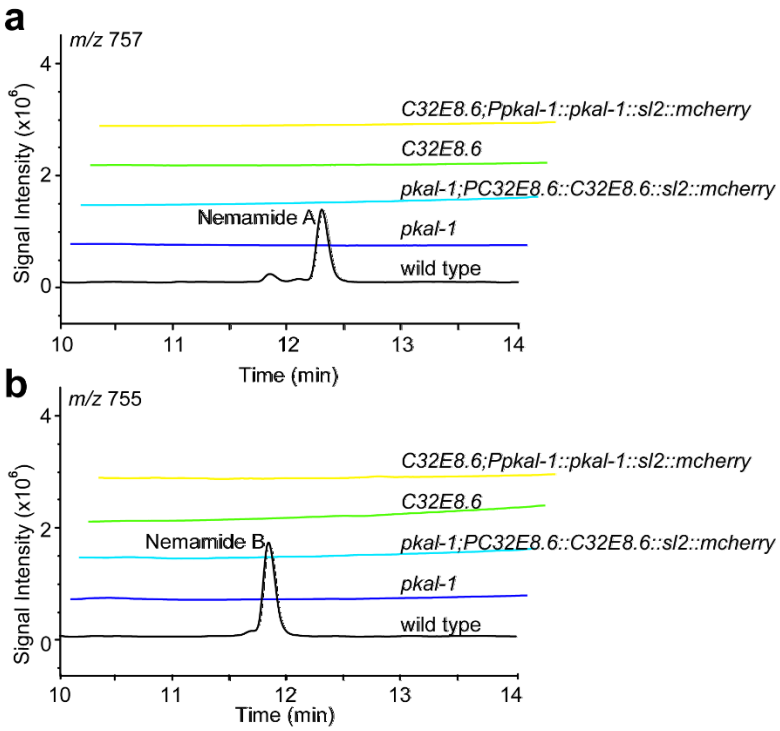
Supplementary Figure 8. Domain sequence alignments of the C domains in NRPS-1 with the dual epimerase (E)/C domains from the arthrofactin biosynthetic pathway. Dual E/C domains have a unique N-terminus with catalytic residues HH-D, followed by a common C domain with a catalytic motif R-HHXXXD.[3] The sequences of the C_2 domain from NRPS-1 (residues 401-586), the C_3 domain from NRPS-1 (residues 1361-1524), and the C_4 domain from NRPS-1 (residues 1815-1974) were compared to the sequences of the C domains of ArfB from the arthrofactin biosynthetic pathway. Essential residues for catalysis are indicated by arrows. The C domains in NRPS-1 are missing the catalytic residues in the N-terminus that are characteristic of dual E/C domains.



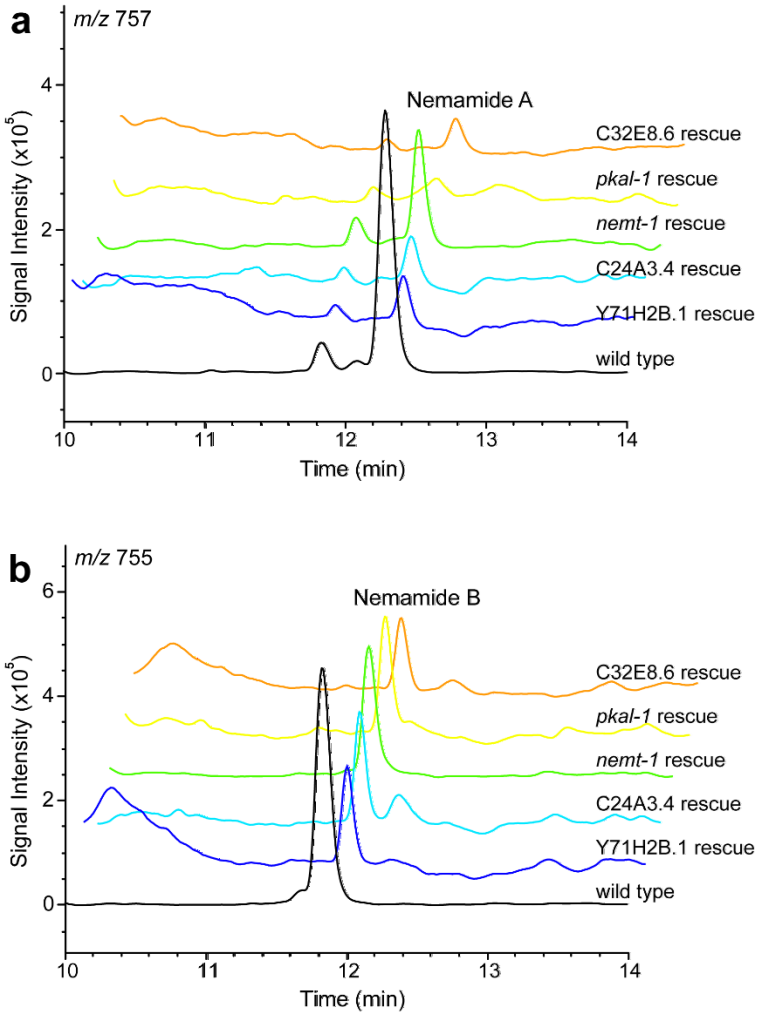
Supplementary Figure 9. Nemamide production in wild type and *pks-1* mutant strains. Extracted ion chromatogram for nemamide A (**a**) and nemamide B (**b**) in wild type and *pks-1(reb29[PCP$_2$_S7463A])*, *pks-1(reb22[A$_1$_G7106E])*, *pks-1(reb9[C$_1$_H6685A])*, *pks-1(reb11[TE$_1$_S7593A]),* and *pks-1(reb13[TE$_1$_S7593C])* mutant worms, containing mutations in the C-terminal NRPS module of PKS-1. Note that the nemamides could not be detected in the crude extracts of small-scale cultures of *pks-1(reb22[A$_1$_G7106E])* (as shown here), but they could be detected in very small amounts in the partially purified extracts of large-scale cultures of *pks-1(reb22[A$_1$_G7106E])* (as shown in Fig. 3). Note that the retention times of the nemamides in the Supplementary Information are different than in the main text due to the fact that the samples were analyzed on different columns.



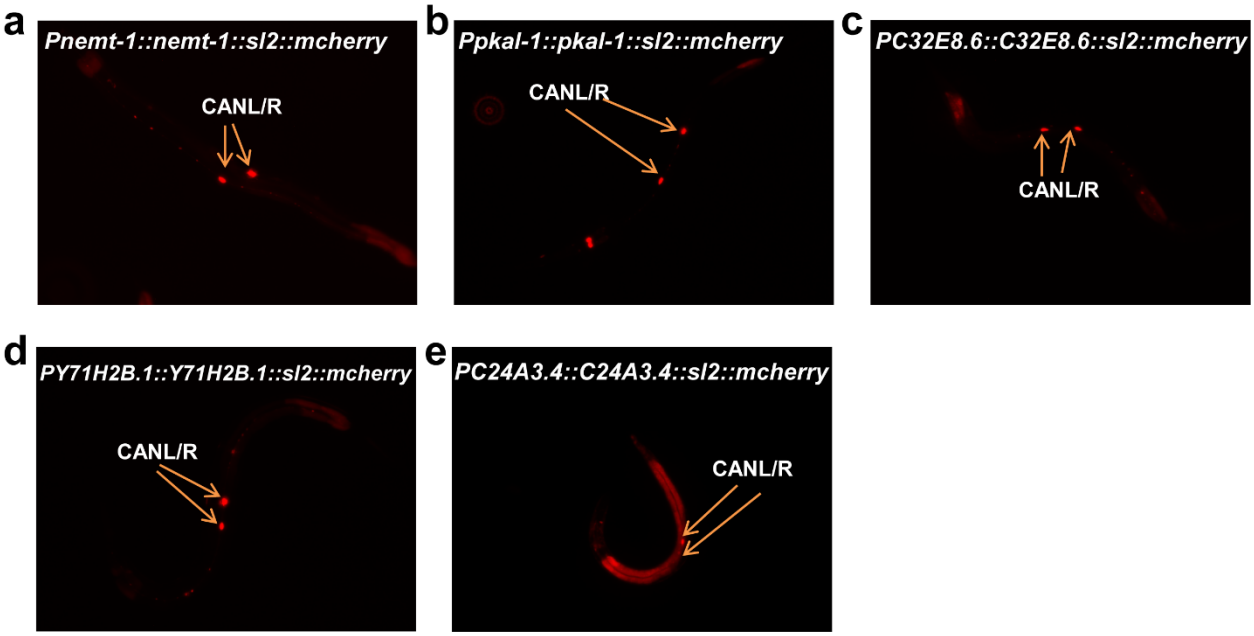
Supplementary Figure 10. Initial screen of available mutants for nemamide production. Extracted ion chromatogram for nemamide A (**a**) and nemamide B (**b**) in wild-type and the indicated mutant strains from Supplementary Table 2. Note that the retention times of the nemamides in the Supplementary Information are different than in the main text due to the fact that the samples were analyzed on different columns.



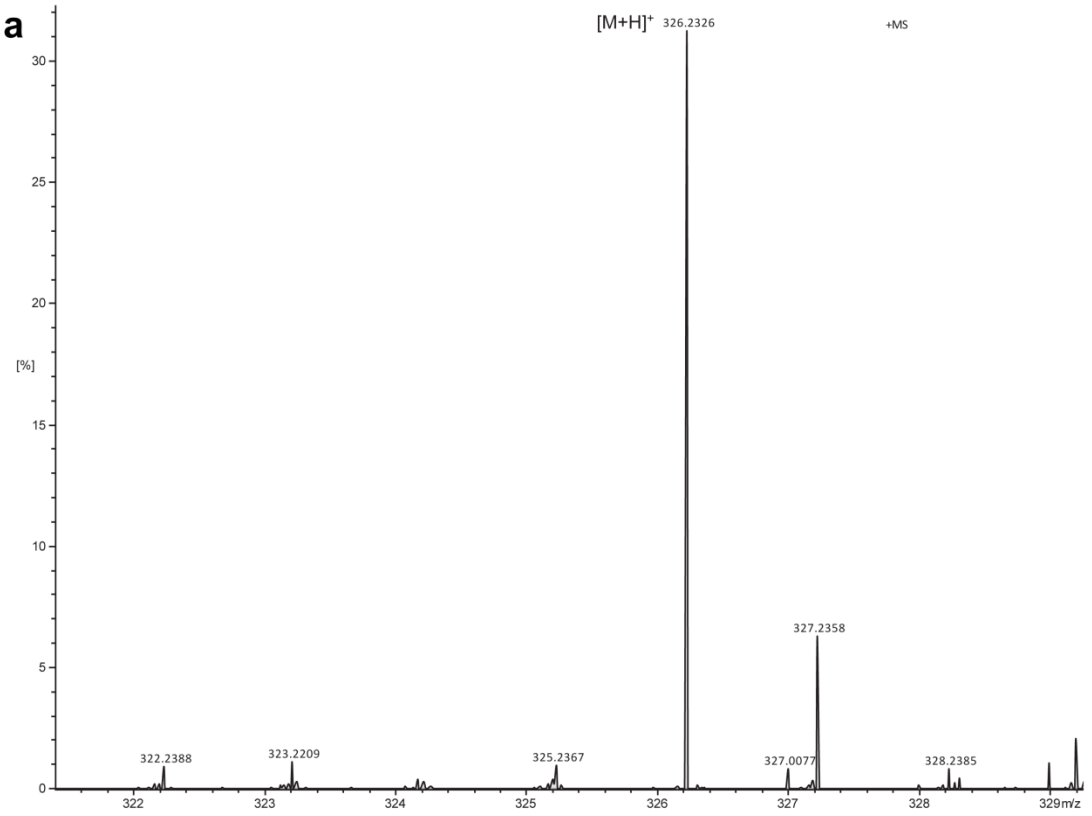
Supplementary Figure 11. Failure of T20F7.7 (*pkal-1*) and C32E8.6 to rescue each other. Extracted ion chromatogram for nemamide A (**a**) and nemamide B (**b**) in wild-type, the *pkal-1* mutant, the *pkal-1* mutant in which C32E8.6 was expressed under the control of its own promoter, the C32E8.6 mutant, and the C32E8.6 mutant in which *pkal-1* was expressed under the control of its own promoter. Note that the retention times of the nemamides in the Supplementary Information are different than in the main text due to the fact that the samples were analyzed on different columns.



Supplementary Figure 12. Nemamide production in wild type and mutant rescue strains. Extracted ion chromatograms for nemamide A (**a**) and nemamide B (**b**). Mutants were rescued by complementing with *sl2::mCherry* plasmids under control of gene promoters::genes. Note that the retention times of the nemamides in the Supplementary Information are different than in the main text due to the fact that the samples were analyzed on different columns.



Supplementary Figure 13. Imaging of translational reporter strains. Analysis of in translational reporter worm strains *Pnemt-1::nemt-1::sl2::mcherry* (**a**), *Ppkal-1::pkal-1::sl2::mcherry* (**b**), *PC32E8.6::C32E8.6::sl2::mcherry* (**c**), *PY71H2B.1::Y71H2B.1::sl2::mcherry* (**d**), and *PC24A3.4::C24A3.4::sl2::mcherry* (**e**) demonstrated expression of nemamide biosynthetic genes primarily in the CANs.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[M+H]+ 326.2326
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[%]
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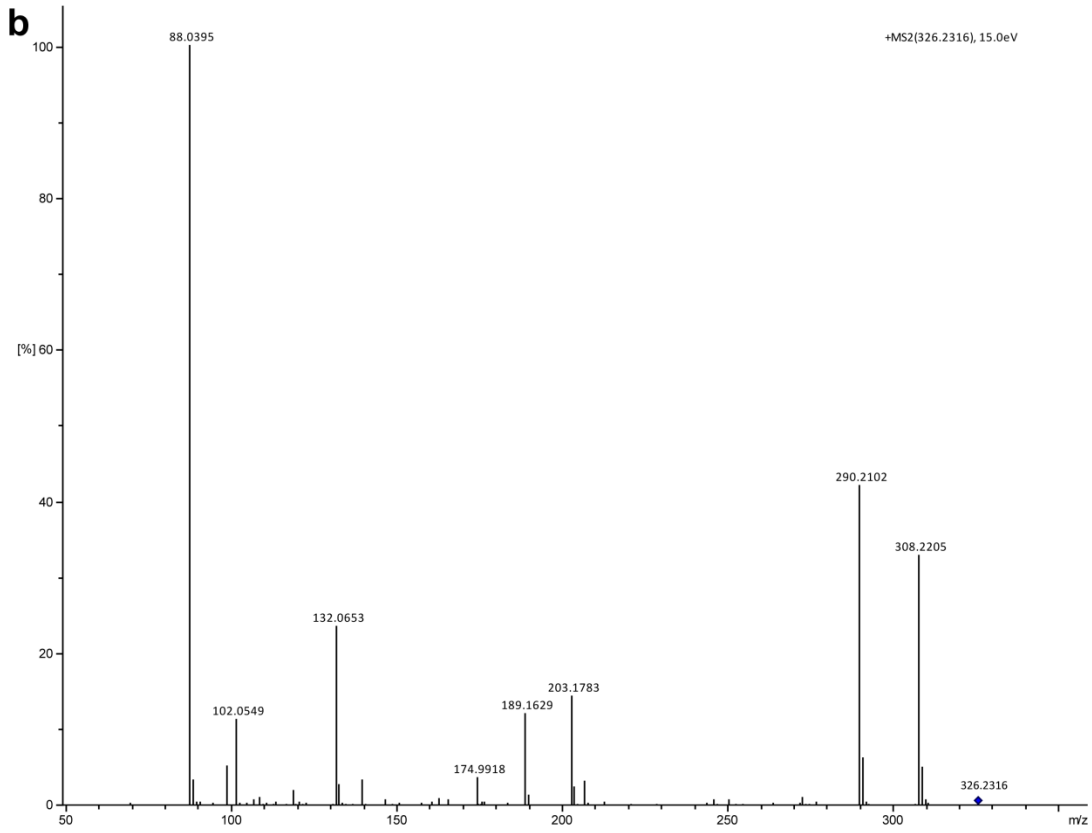
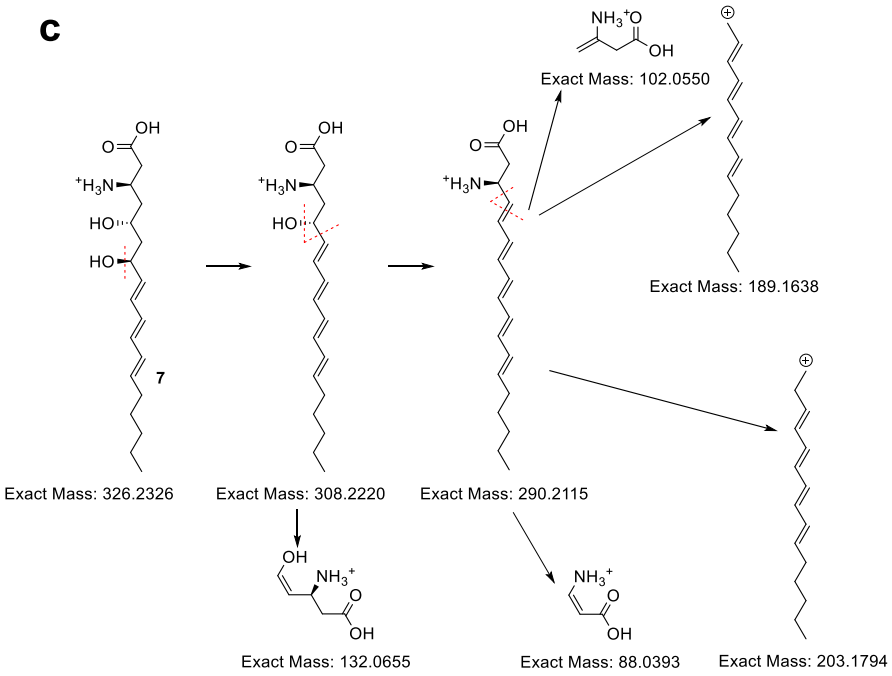
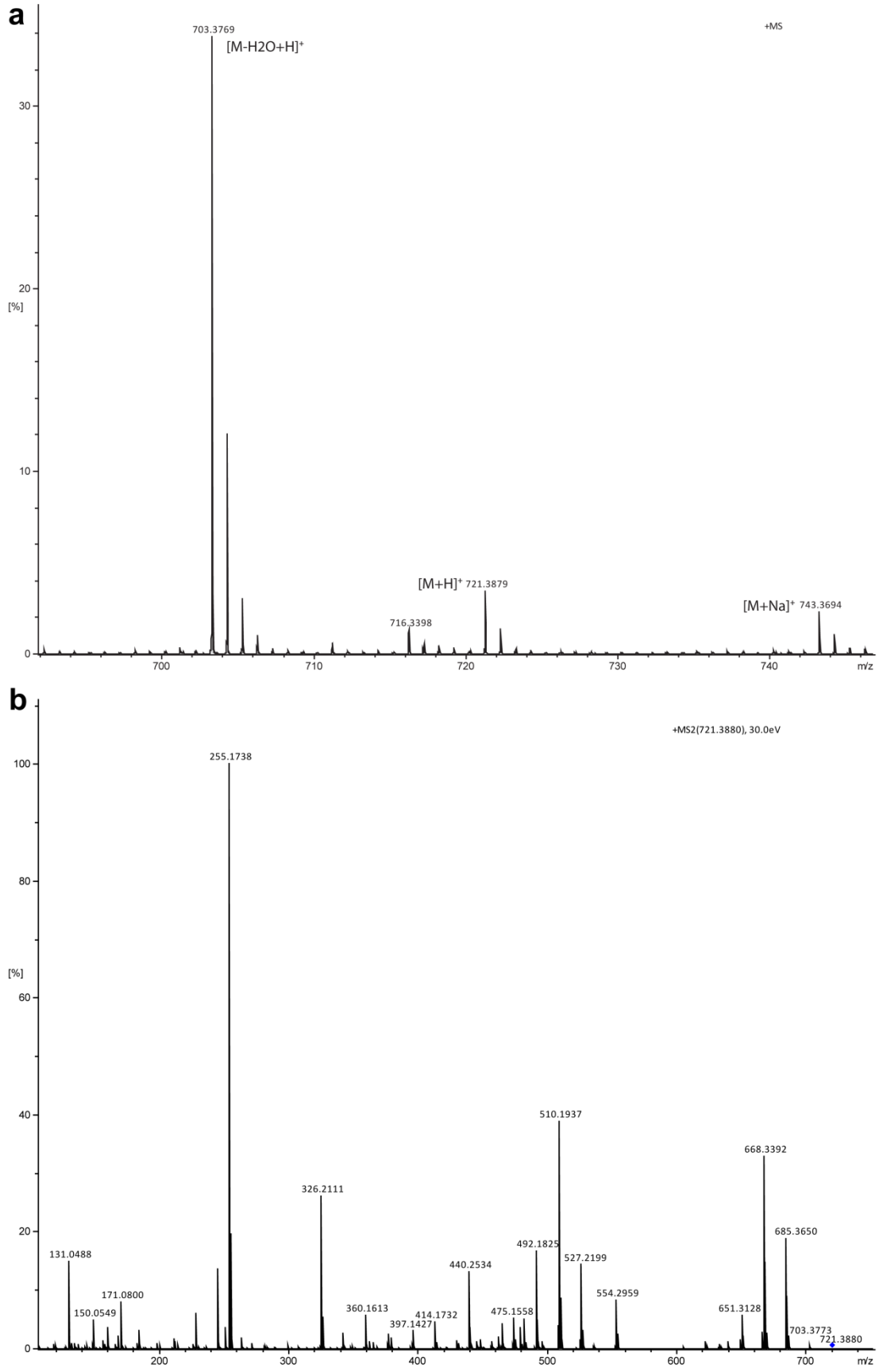
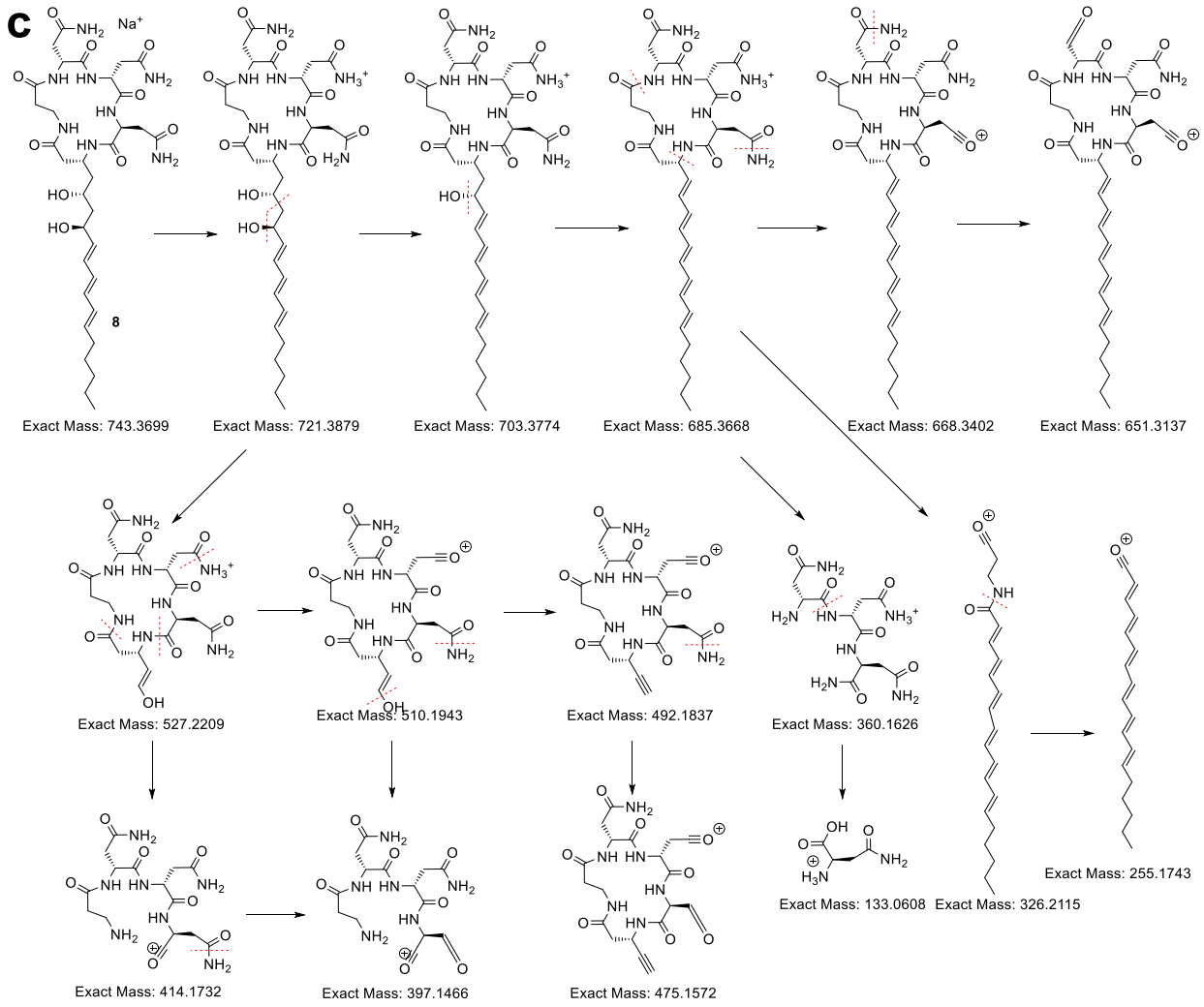
323.2209
325.2367
327.0077
328.2385
322
323
324
325
326
327
328
329m/z


b
88.0395
+MS2(326.2316), 15.0eV
[%]
290.2102
308.2205
132.0653
203.1783
189.1629
102.0549
174.9918
326.2316
50
100
150
200
250
300
m/z

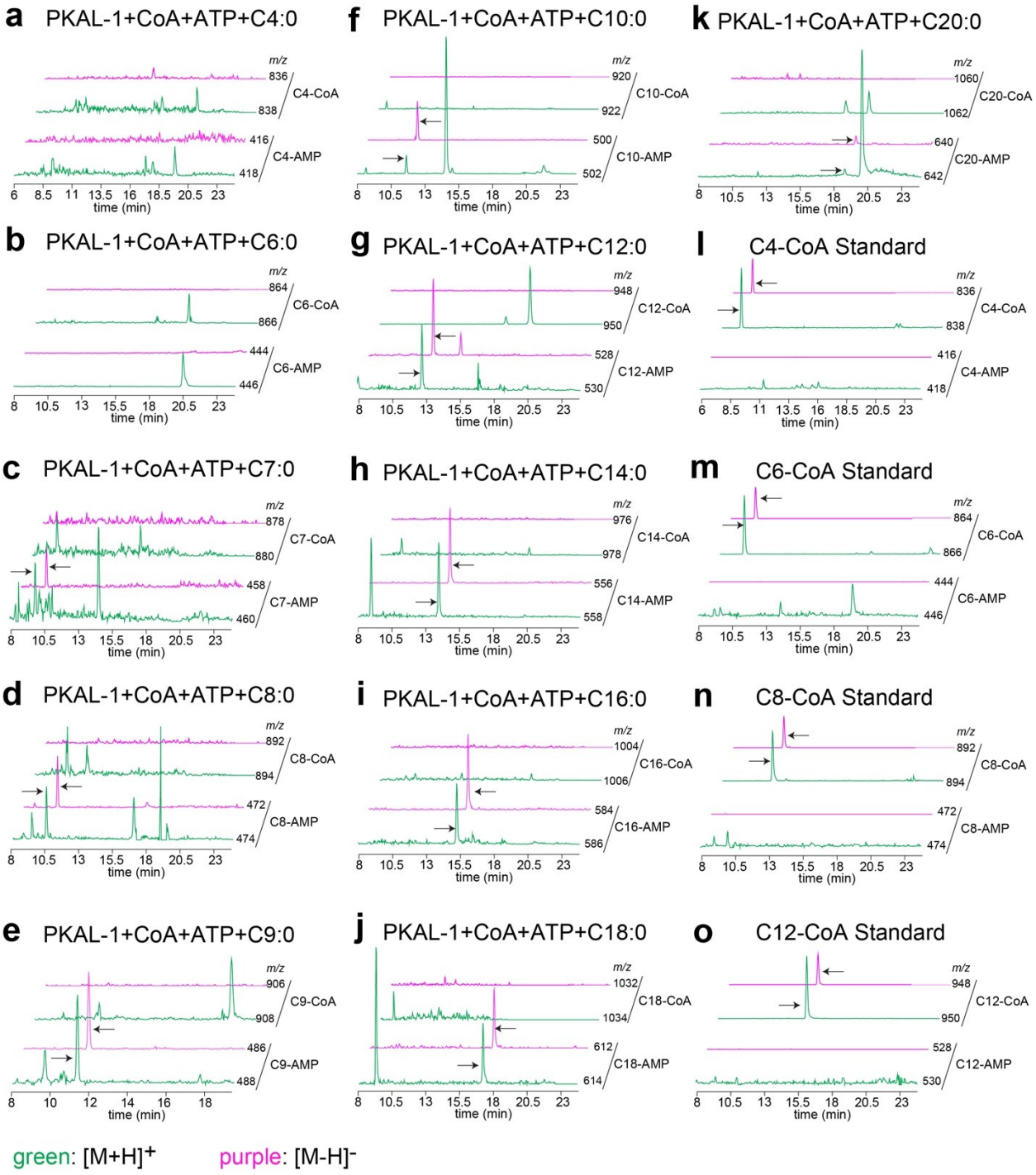


Supplementary Figure 14. Mass spectrometry analysis of intermediate 7. **a**,**b** HR-LC-MS/MS of intermediate **7**. **c** Analysis of the fragmentation patterns.

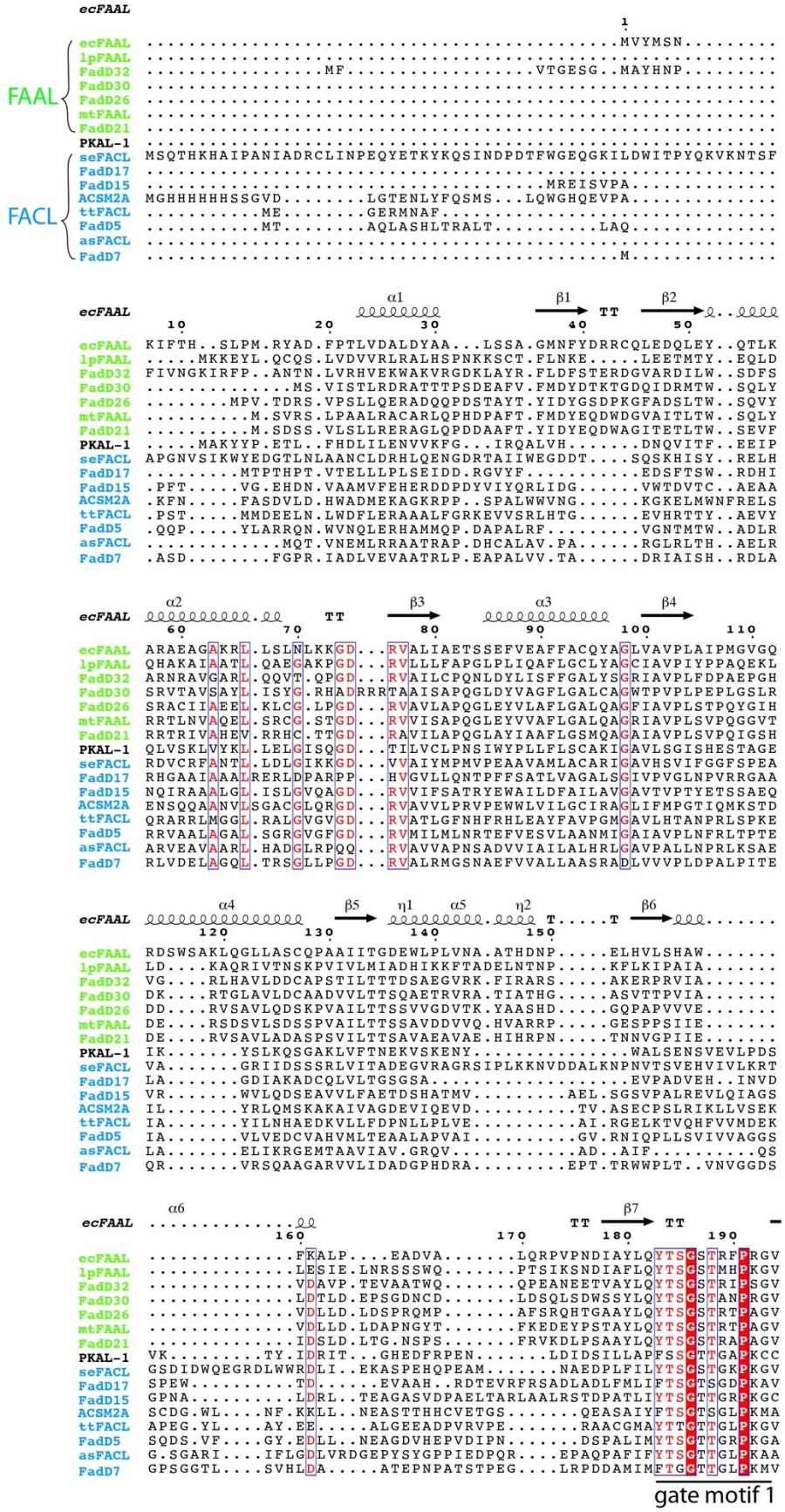

a
703.3769
[M-H2O+H]+
+MS
[%]
[M+H]+ 721.3879
716.3398
[M+Na]+ 743.3694
m/z
b
+MS2(721.3880), 30.0eV
255.1738
[%]
510.1937
668.3392
326.2111
685.3650
492.1825
527.2199
131.0488
440.2534
554.2959
171.0800
150.0549
360.1613
414.1732
397.1427
475.1558
651.3128
703.3773
721.3880
m/z

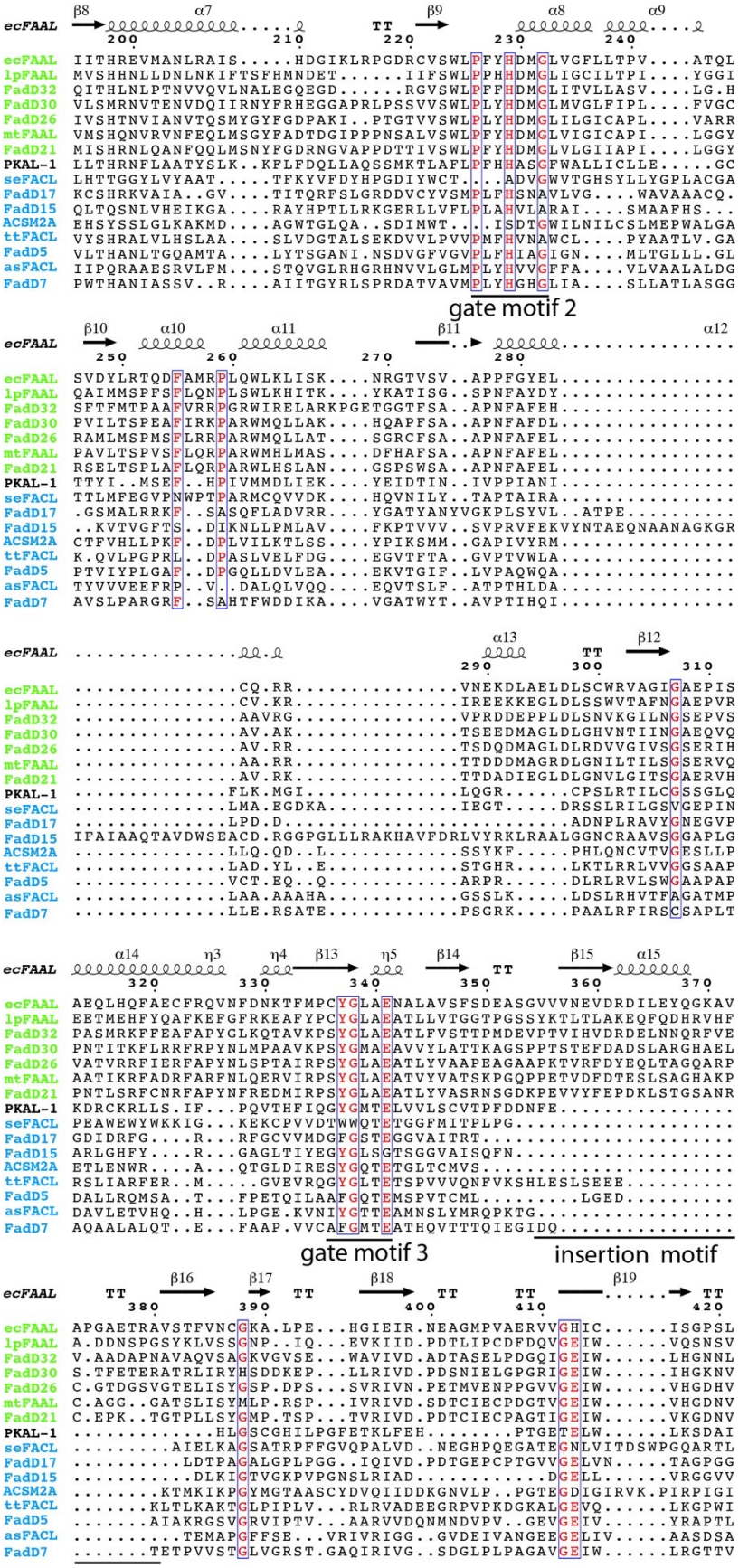


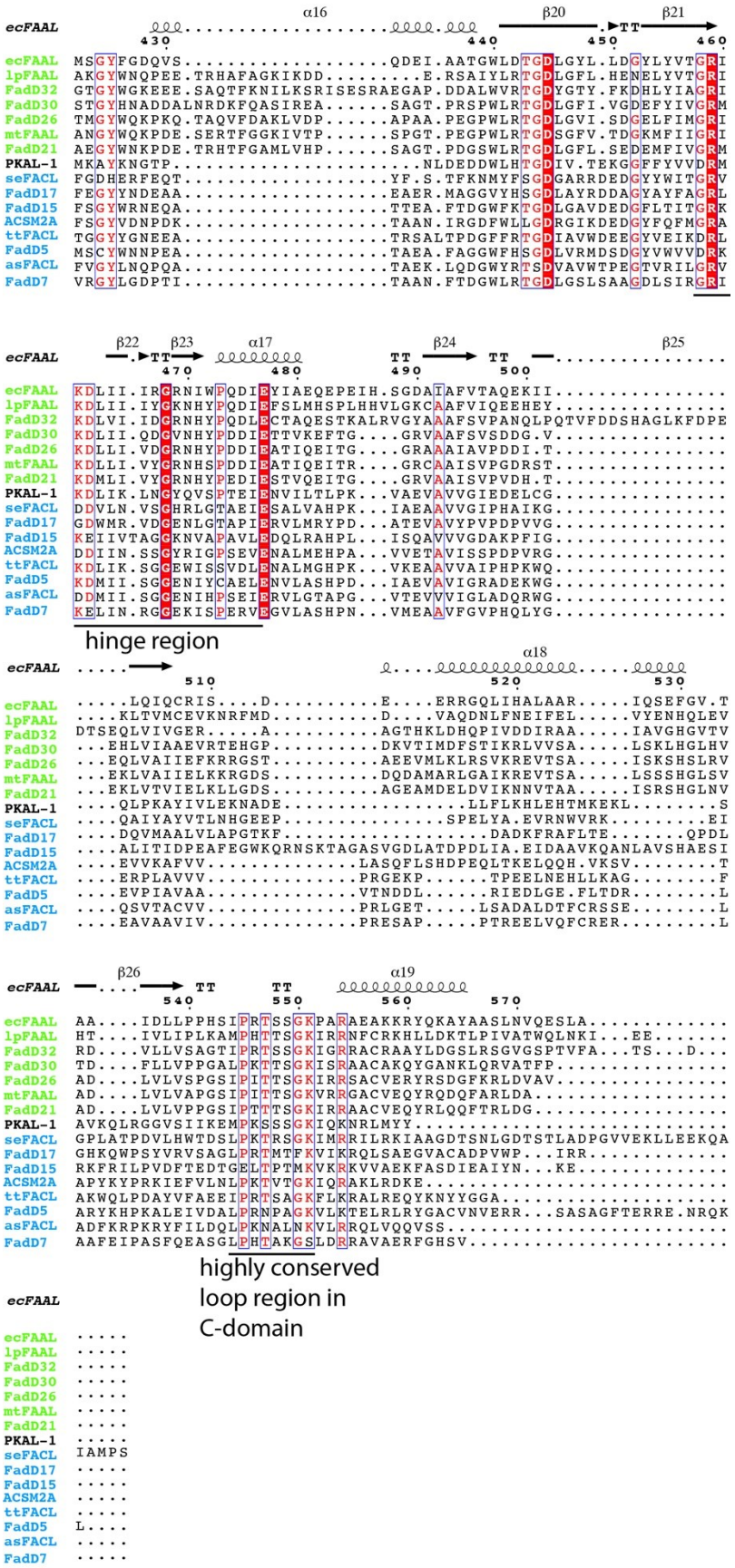
Supplementary Figure 15. Mass spectrometry analysis of desmethyl-nemamide, 8. **a**,**b** HR-LC-MS/MS of desmethyl-nemamide, **8**. **c** Analysis of the fragmentation patterns.



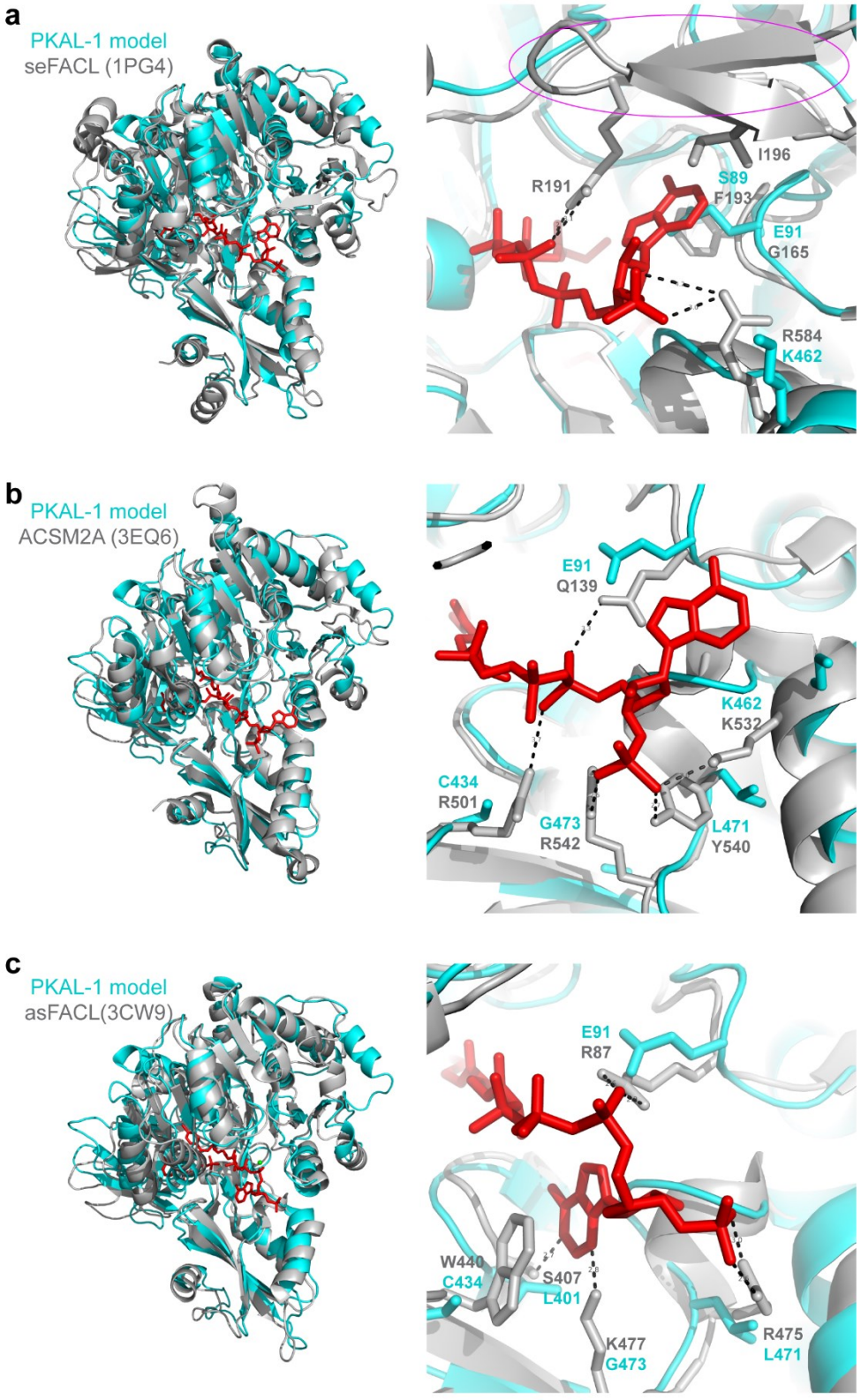
Supplementary Figure 16. PKAL-1 is not an FACL. Reaction of PKAL-1 with fatty acids of various lengths, ATP, and CoA did not result in the corresponding fatty acyl-CoAs. Several fatty acyl-CoAs were used as standards.


FAAL
FACL
gate motif 1


gate motif 2
gate motif 3
insertion motif

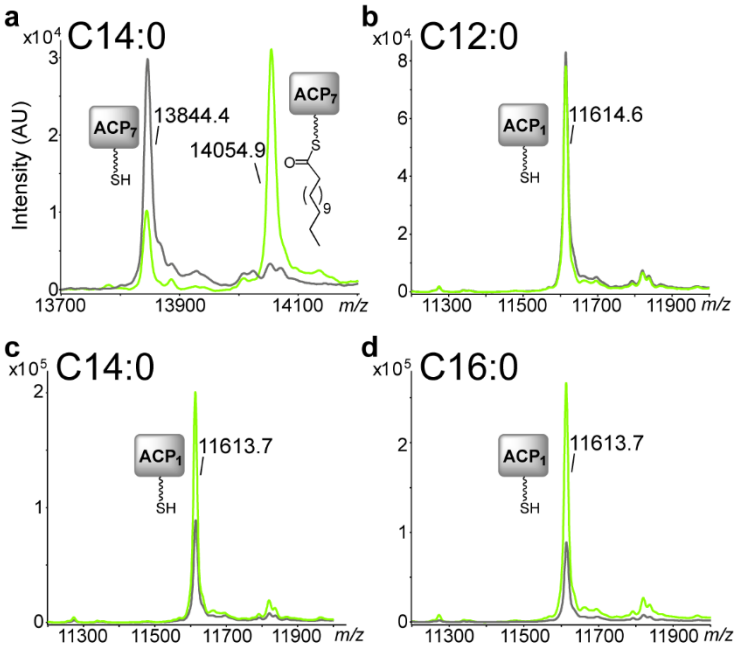

α16
β20
β21
ecFAAL
430
440
450
460
ecFAAL MSGYFGDQVS.....QDEI.AATGWLDTGDLGYL.LDGYLYVTGRI
lpFAAL AKGYWNQPEE.TRHAFAGKIKDD.....E.RSAIYLRTGDLGFL.HENELYVTGRI
FadD32 GTGYWGKEEE.SAQTFKNILKSRISESRAEGAP.DDALWVRTGDYGTY.FKDHLYIAGRI
FadD30 STGYHNADDALNRDKFQASIREA.....SAGT.PRSPWLRTGDLGFI.VGDEFYIVGRM
FadD26 TMGYWQKPKQ.TAQVFDAKLVDP.....APAA.PEGPWLRTGDLGVI.SDGELFIMGRI
mtFAAL ANGYWQKPDE.SERTFGGKIVTP.....SPGT.PEGPWLRTGDSGFV.TDGKMFIIGRI
FadD21 AEGYWNKPDE.TRHTFGAMLVHP.....SAGT.PDGSWLRTGDLGFL.SEDEMFIVGRM
PKAL-1 MKAYKNGTP.....NLDEDDWLHTGDIV.TEKGGFFYVVDRM
seFACL FGDHERFEQT.....YF.S.TFKNMYFSGDGARRDEDGYYWITGRV
FadD17 FEGYYNDEAA.....EAER.MAGGVYHSGDLAYRDDAGYAYFAGRL
FadD15 FSGYWRNEQA.....TTEA.FTDGWFKTGDLGAVDEDGFLTITGRK
ACSM2A FSGYVDNPDK.....TAAN.IRGDFWLLGDRGIKDEDGYFQFMGRA
ttFACL TGGYYGNEEA.....TRSALTPDGFFRTGDIAVWDEEGYVEIKDRL
FadD5 MSCYWNNPEA.....TAEA.FAGGWFHSGDLVRMDSDGYVWVVDRK
asFACL FVGYLNQPQA.....TAEK.LQDGWYRTSDVAVWTPEGTVRILGRV
FadD7 VRGYLGDPTI.....TAAN.FTDGWLRTGDLGSLSAAGDLSIRGRI
β22
β23
α17
β24
β25
ecFAAL
470
480
490
500
ecFAAL KDLII.IRGRNIWPQDIEYIAEQEPEIH.SGDAIAFVTAQEKII.....
lpFAAL KDLII.IYGKNHYPQDIEFSLMHSPLHHVLGKCAAFVIQEEHEY.....
FadD32 KDLVI.IDGRNHYPQDLECTAQESTKALRVGYAAAFSVPANQLPQTVFDDSHAGLKFDPE
FadD30 KDLII.QDGVNHYPDDIETTVKEFTG....GRVAAFSVSDDG.V.....
FadD26 KDLLI.VDGRNHYPDDIEATIQEITG....GRAAAIAVPDDI.T.....
mtFAAL KDLLI.VYGRNHSPDDIEATIQEITR....GRCAAISVPGDRST.....
FadD21 KDMLI.VYGRNHYPEDIESTVQEITG....GRVAAISVPVDH.T.....
PKAL-1 KDLIK.LNGYQVSPTEIENVILTLPK...VAEVAVVGIEDELCG.....
seFACL DDVLN.VSGHRLGTAEIESALVAHPK...IAEAAVVGIPHAIKG.....
FadD17 GDWMR.VDGENLGTAPIERVLMRYPD...ATEVAVYPVPDPVVG.....
FadD15 KEIIVTAGGKNVAPAVLEDQLRAHPL...ISQAVVVGDAKPFIG.....
ACSM2A DDIIN.SSGYRIGPSEVENALMEHPA...VVETAVISSPDPVRG.....
ttFACL KDLIK.SGGEWISSVDLENALMGHPK...VKEAAVVAIPHPKWQ.....
FadD5 KDMII.SGGENIYCAELENVLASHPD...IAEVAVIGRADEKWG.....
asFACL DDMII.SGGENIHPSEIERVLGTAPG...VTEVVVIGLADQRWG.....
FadD7 KELIN.RGGEKISPERVEGVLASHPN...VMEAAVFGVPHQLYG.....
hinge region
α18
ecFAAL
510
520
530
ecFAAL .....LQIQCRIS....D.....E....ERRGQLIHALAAR.....IQSEFGV.T
lpFAAL ....KLTVMCEVKNRFMD.....D....VAQDNLFNEIFEL.....VYENHQLEV
FadD32 DTSEQLVIVGER.....A.....AGTHKLDHQPIVDDIRAA.....IAVGHGVTV
FadD30 ...EHLVIAAEVRTEHGP.....DKVTIMDFSTIKRLVVSA.....LSKLHGLHV
FadD26 ...EQLVAIIEFKRRGST.....AEEVMLKLRSVKREVTSA.....ISKSHSLRV
mtFAAL ...EKLVAIIELKKRGDS.....DQDAMARLGAIKREVTSA.....LSSSHGLSV
FadD21 ...EKLVTVIELKLLGDS.....AGEAMDELDVIKNNVTAA.....ISRSHGLNV
PKAL-1 ....QLPKAYIVLEKNADE.....LLFLKHLEHTMKEKL.....S
seFACL ....QAIYAYVTLNHGEEP.....SPELYA.EVRNWVRK.....EI
FadD17 ....DQVMAALVLAPGTKF.....DADKFRAFLTE.....QPDL
FadD15 ....ALITIDPEAFEGWKQRNSKTAGASVGDLATDPDLIA.EIDAAVKQANLAVSHAESI
ACSM2A ....EVVKAFVV.....LASQFLSHDPEQLTKELQQH.VKSV.....T
ttFACL ....ERPLAVVV.....PRGEKP.....TPEELNEHLLKAG.....F
FadD5 ....EVPIAVAA.....VTNDDL.....RIEDLGE.FLTDR.....L
asFACL ....QSVTACVV.....PRLGET.....LSADALDTFCRSSE.....L
FadD7 ....EAVAAVIV.....PRESAP.....PTREELVQFCRER.....L
β26
α19
ecFAAL
540
550
560
570
ecFAAL AA....IDLLPPHSIPRTSSGKPARAEAKKRYQKAYAASLNVQESLA.....
lpFAAL HT....IVLIPLKAMPHTTSGKIRRNFCRKHLLDKTLPIVATWQLNKI...EE.....
FadD32 RD....VLLVSAGTIPRTSSGKIGRRACRAAYLDGSLRSGVGSPTVFA...TS...D...
FadD30 TD....FLLVPPGALPKTTSGKISRAACAKQYGANKLQRVATFP.....
FadD26 AD....LVLVSPGSIPITTSGKIRRSACVERYRSDGFKRLDVAV.....
mtFAAL AD....LVLVAPGSIPITTSGKVRRGACVEQYRQDQFARLDA.....
FadD21 AD....LVLVPPGSIPTTTSGKIRRAACVEQYRLQQFTRLDG.....
PKAL-1 AVKQLRGGVSIIKEMPKSSSGKIQKNRLMYY.....
seFACL GPLATPDVLHWTDSLPKTRSGKIMRRILRKIAAGDTSNLGDTSTLADPGVVEKLLEEKQA
FadD17 GHKQWPSYVRVSAGLPRTMTFKVIKRQLSAEGVACADPVWP...IRR.....
FadD15 RKFRILPVDFTEDTGELTPTMKVKRKVVAEKFASDIEAIYN...KE.....
ACSM2A APYKYPRKIEFVLNLPKTVTGKIQRAKLRDKE.....
ttFACL AKWQLPDAYVFAEEIPRTSAGKFLKRALREQYKNYYGGA.....
FadD5 ARYKHPKALEIVDALPRNPAGKVLKTELRLRYGACVNVERR...SASAGFTERRE.NRQK
asFACL ADFKRPKRYFILDQLPKNALNKVLRRQLVQQVSS.....
FadD7 AAFEIPASFQEASGLPHTAKGSLDRRAVAERFGHSV.....
highly conserved
loop region in
C-domain
ecFAAL
ecFAAL .....
lpFAAL .....
FadD32 .....
FadD30 .....
FadD26 .....
mtFAAL .....
FadD21 .....
PKAL-1 .....
seFACL IAMPS
FadD17 .....
FadD15 .....
ACSM2A .....
ttFACL .....
FadD5 L....
asFACL .....
FadD7 .....

Supplementary Figure 17. Sequence alignment of PKAL-1 with FAAL enzymes. The FAAL enzymes (in green) are ecFAAL from *Escherichia coli*, lpFAAL from *Legionella pneumophila*, mtFAAL from *Mycobacterium tuberculosis*, and FadD21, FadD26, FadD30, and FadD32 from *M. tuberculosis*, and FACL enzymes (in blue), including seFACL from *Salmonella enterica*, ttFACL from *Thermus thermophilus*, asFACL from *Alcaligenes sp.*, ACSM2A from human, and FadD5, FadD7, FadD15, and FadD17 from *M. tuberculosis*. The conserved motifs, including the insertion motif that is present in the FAAL enzymes, but missing in PKAL-1 and FACL enzymes, are indicated.

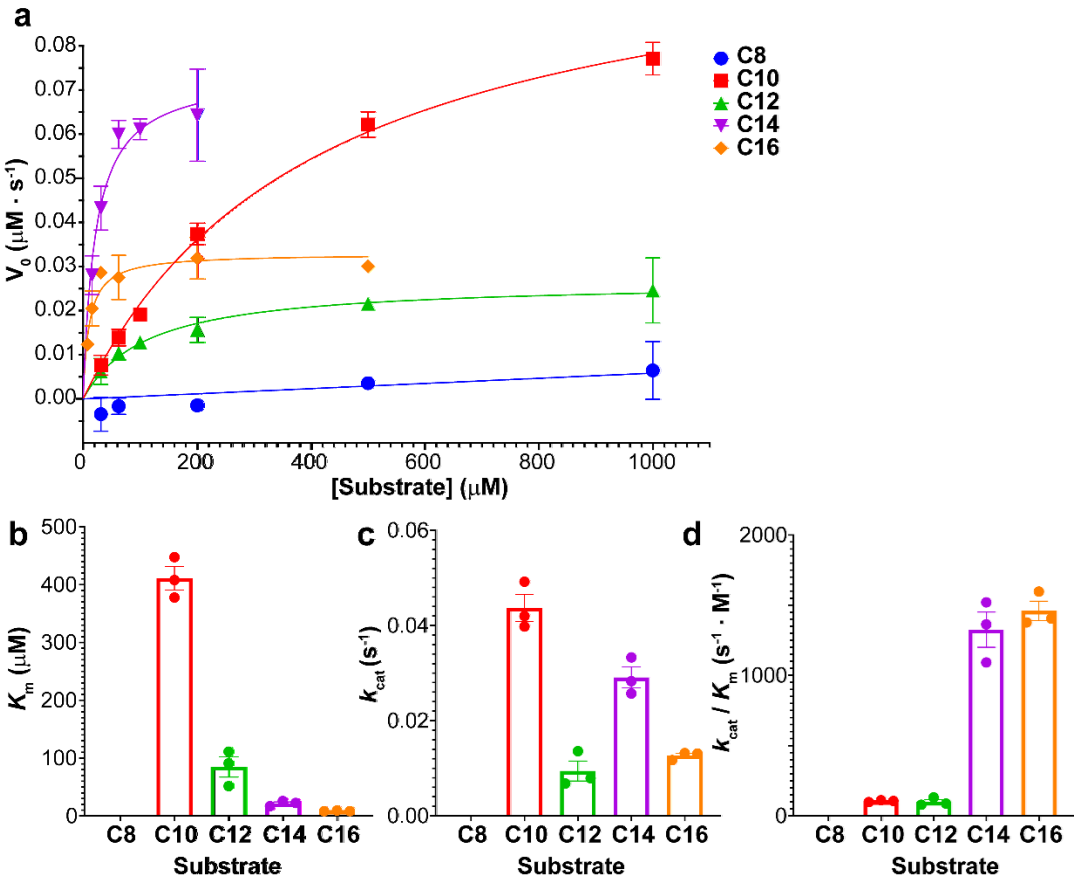


Supplementary Figure 18. Comparison of the modeled PKAL-1 structure to the structures of FACL enzymes bound to CoA substrates. PKAL-1 was modeled using Phyre2[4], and six

templates 5GXD, 6EQO, 6PLJ, 5ES8, 6MFZ, and 5U89 were selected for modeling, enabling 100% of residues to be modeled at >90% confidence. Using Pymol 2.3.3, the PKAL-1 structural model was then overlaid with the structures of three FACL enzymes in the thioester-forming conformation: (**a**) 1PG4, the structure of acetyl-CoA synthetase from *Salmonella enterica* (seFACL), bound to adenosine-5'-propylphosphate and CoA (in red), (**b**) 3EQ6, the structure of the human medium-chain acyl-CoA synthetase (ACSM2A) bound to AMP and butyryl-CoA (in red), and (**c**) 3CW9, the structure of 4-chlorobenzoate:CoA ligase from *Alcaligenes sp.* (asFACL) bound to 4-chlorophenyacyl-CoA (in red). In (**a**), the seFACL structure has a beta hairpin (circled in pink), which contains R191 that binds CoA and which is missing in the PKAL-1 model. A hydrophobic pocket for the adenine ring of CoA that is formed by I196 in the beta hairpin and F193 and G165 is also missing in the PKAL-1 model. In (**b**), the ACSM2A structure has several residues, including R501, R542, Y540, and Q139, which are important for binding of the CoA substrate and which are replaced with C434, G473, L471, and E91, respectively, in the PKAL-1 model. In (**c**), the asFACL structure has several residues, including W440, S407, K477, R475, and R87, which are important for binding of the CoA substrate and which are replaced with C434, L401, G473, L471, and E91, respectively, in the PKAL-1 model.



Supplementary Figure 19. Loading of PKS-1_ACP$_1$ or NRPS-1_ACP$_7$ by PKAL-1. To determine the carrier protein specificity of PKAL-1, PKAL-1 was incubated with (**a**) holo-ACP$_7$ (positive control), ATP, and C14:0 fatty acid, or with (**b**-**d**) holo-ACP$_1$ from PKS-1, ATP, and C12:0 fatty acid (**b**), C14:0 fatty acid (**c**), or C16:0 fatty acid (**d**). Samples were analyzed by MALDI to determine if the fatty acid substrates were loaded onto the respective carrier proteins.



Supplementary Figure 20. Kinetic data for PKAL-1 against various fatty acid substrates. **a** PKAL-1 was analyzed in an enzyme-coupled continuous kinetic assay using C8:0, C10:0, C12:0, C14:0, and C16:0 fatty acids as substrates. Kinetic constants, including K_m (**b**), k_{cat} (**c**), and k_{cat} / K_m (**d**), were obtained using GraphPad software. Data represent the mean ± SEM of three independent experiments. Source data are provided as a Source Data file.

Supplementary Table 1. A domain selectivity codes for the PKS-1 A_1 domain from various nematode species.

Species	235	236	239	278	299	301	322	330	331	517
C. elegans	D	V	S	F	T	G	I	I	W	K
C. angaria	D	V	S	F	T	G	I	I	W	K
C. japonica	D	V	A	F	T	G	I	V	W	K
C. brenneri	D	V	S	F	T	G	I	I	W	K
C. remanei	D	V	S	F	T	G	I	V	W	K
C. briggsae	D	V	S	F	T	G	I	V	W	K
C. tropicalis	D	V	S	F	T	G	I	I	W	K
A. suum	D	V	M	Y	F	G	I	I	W	K
T. canis	D	V	M	F	F	G	I	I	W	K
D. immitis	D	V	M	F	Y	G	I	I	W	K
O. volvulus	D	V	V	F	Y	G	I	V	W	K
L. loa	D	V	V	F	Y	G	I	V	W	K
B. malayi	D	V	M	F	F	G	I	I	W	K
P. pacificus	D	V	F	F	I	G	I	I	W	K
P. exspectatus	D	V	F	F	I	G	I	I	W	K
S. carpocapsae	D	V	F	F	Y	G	I	I	W	K
B. xylophilus	D	V	F	F	I	G	I	I	W	K
A. ceylanicum	D	V	M	F	F	G	I	V	W	K
A. duodenale	D	V	M	F	L	G	I	I	W	K
O. dentatum	D	V	L	F	F	G	I	V	W	K
N. americanus	D	V	F	F	V	G	I	V	W	K
H. bacteriophora	D	V	V	F	F	G	I	V	W	K
H. contortus	D	V	F	F	F	G	I	V	W	K

Supplementary Table 2. Genes with enriched expression (> 5-fold) in the CANs.

Enriched genes	Predicted function from WormBase	Initial strain used for screening	Defective in nemamide production?
C24A3.4	Predicted to have CoA-transferase activity; homolog of human AMACR (alpha-methylacyl-CoA racemase)	VC40591	Yes
srv-1	Serpentine receptor, class V		
T22F3.12	Predicted to have peptidyl-prolyl cis-trans isomerase activity; ortholog of PPIAL4A (peptidylprolyl isomerase A like 4A); PPIAL4D (peptidylprolyl isomerase A like 4D); and PPIL6 (peptidylprolyl isomerase like 6)		
F49C12.10 (*nemt-1*)	Predicted to have methyltransferase activity	VC20249	Yes
dhhc-5	Predicted to have protein-cysteine S-palmitoyltransferase activity; homolog of human ZDHHC21 (zinc finger DHHC-type palmitoyltransferase 21)		
cyk-1	Predicted to have Rho GTPase binding activity and actin binding activity		
C32E8.6	Contains AMP-dependent synthetase domain	No strain available (RAB60, RAB61 generated)	Yes
T05A1.5	Ortholog of human SLC22A9 (solute carrier family 22 member 9)		
pks-1	Polyketide synthase	N/A	Yes
C08G5.6	No homology to known protein domains		
C03F11.4	No homology to known protein domains; located in the genome near *pks-1*	VC40279	No
ZK112.6	Weak homology to acetyl-CoA synthetase		
Y46H3C.7	No homology to known protein domains		
C41A3.2	No homology to known protein domains; enriched in coelomocyte, germ line, head mesodermal cell, and sensory neurons; located in the genome near *pks-1*		
aexr-1	Predicted to have neuropeptide receptor activity		
R04A9.6	No homology to known protein domains; enriched in AVE and hypodermis		
acy-2	Predicted to have adenylate cyclase activity; involved in adenylate cyclase-modulating G protein-coupled receptor signaling pathway and nematode larval development		
C23H5.11	No homology to known protein domains		
F27C8.2	Contains an acyl-CoA N-acyltransferases domain; enriched in GABAergic neurons, body wall muscle cell, excretory cell, and seam cell		
pqn-52	Contains prion-like (Q/N-rich) domain; enriched in cholinergic neurons, coelomocyte, germ line, germline precursor cell, and head mesodermal cell		

pak-1	Member of P21-activated kinase family; exhibits GTP binding activity and protein kinase activity; involved in hemidesmosome assembly, inductive cell migration, and motor neuron axon guidance		
T12G3.4	Ortholog of human APMAP (adipocyte plasma membrane-associated protein)		
Y54E5A.2	No homology to known protein domains; enriched in males; involved in spermatogenesis		
nrps-1	Nonribosomal peptide synthetase	N/A	Yes
F27C8.3	No homology to known protein domains		
ace-3	Exhibits acetylcholinesterase activity; involved in hatching and regulation of backward locomotion; expressed in body wall musculature, neurons, pharyngeal muscle cell, and vulva		
ZK112.5	No homology to known protein domains; enriched in coelomocyte, male-specific tissues, and pharynx		
F13H8.9	Ortholog of human SCLY (selenocysteine lyase)	RB2336	No
lips-13	Predicted to have lipase activity		
F09C12.6	Predicted to have G-protein coupled receptor activity		
moc-1	Predicted to have molybdopterin adenylyltransferase activity and molybdopterin molybdotransferase activity; expressed in tail; ortholog of human GPHN (gephyrin)		
F42C5.6	Contains SUP-1-like domain; expressed in AVE		
K10C2.12	Contains ubiquitin-like domain; expressed in AVG, RIM, and command interneuron		
acbp-6	Predicted to have fatty-acyl-CoA-binding activity		
acs-9 (pkal-1)	Member of fatty acyl-CoA synthetase family; predicted to have catalytic activity	VC40189	Yes
swt-1	Exhibits sugar transmembrane transporter activity; localizes to golgi and plasma membrane; ortholog of human SLC50A1 (solute carrier family 50 member 1)		
Y71H2B.1	Predicted to have fatty-acyl-CoA binding activity	VC40597	Yes
F40E3.5	Predicted to have protein serine/threonine phosphatase activity	VC40998	No

Genes were identified using the dataset of Cao *et al.* and GExplore, applying an enrichment ratio of 5-fold in the CANs and a false detection rate of 0.05.[5,6] Enrichment ratio reflects the ratio of gene expression in the cell type where the gene is most highly expressed versus gene expression in the cell type where the gene is next most highly expressed. Genes were selected for screening for involvement in nemamide production based on the putative enzymatic role of the encoded protein and the availability of a corresponding loss-of-function mutant. C03F11.4 was selected based on its proximity to *pks-1* in the genome.

Supplementary Table 3. Strains used in this study.

Strain	Genotype	Mutation	Background	Resource
N2	wild type			CGC
VC40591	C24A3.4(*gk961371*) X	Large deletion		CGC
VC20249	F49C12.10(*gk208727*) IV	R176Opal		CGC
VC40279	C03F11.4(*gk551362*) X	R144Opal		CGC
RB2336	F13H8.9(*ok3172*) II	754bp deletion with 28 bp insertion		CGC
VC40597	Y71H2B.1*(gk712674)* III	W136Opal		CGC
VC40998	F40E3.5(gk930567) I	Q47Ochre		CGC
VC40189	*acs-9(gk504580) X*	Q436Amber		CGC
RAB43	*nrps-1(gk186409[C_4_S1934N]; gk186410[C_4_D1971N])* III	Located in NRPS-1_C_4	VC20469 (4X outcrossing)	CGC
RAB45	Y71H2B.1*(gk712674)* III	W136Opal	VC40597(2X outcrossing)	CGC
RAB51	*nrps-1(reb8[ACP_7_S307V])* III	NRPS-1_ACP_7	N2	CRISPR-Cas9
RAB52	*pks-1(reb9[C_1_H6685A])* X	PKS-1_C_1	N2	CRISPR-Cas9
RAB53	*nrps-1(reb10[C_3_H1486A])* III	NRPS-1_C_3	N2	CRISPR-Cas9
RAB54	*pks-1(reb11[TE_1_S7593A])* X	PKS-1_TE_1	N2	CRISPR-Cas9
RAB55	*nrps-1(reb12[TE_2_S2803A])* III	NRPS-1_TE_2	N2	CRISPR-Cas9
RAB56	*pks-1(reb13[TE_1_S7593C]; reb14[TE_1_G7596A])* X	PKS-1_TE_1	N2	CRISPR-Cas9
RAB57	*nemt-1(reb15)* IV	306 bp deletion with 27 bp insertion	N2	CRISPR-Cas9
RAB58	*pkal-1(reb21)* X	154 bp deletion with single base 'T' insertion	N2	CRISPR-Cas9
RAB59	*pkal-1(reb28)* X	552 bp deletion	N2	CRISPR-Cas9
RAB60	C32E8.6*(reb23)* I	991 bp deletion with 10 bp insertion	N2	CRISPR-Cas9
RAB61	C32E8.6*(reb24)* I	993 bp deletion	N2	CRISPR-Cas9
RAB62	C24A3.4*(reb16)* X	1361 bp deletion	N2	CRISPR-Cas9
RAB67	*pkal-1(reb28)*; *pks-1(reb11[TE_1_S7593A])*			Outcrossing
RAB68	*pkal-1(reb28)*; *nemt-1(reb15)*			Outcrossing
RAB69	*nemt-1(reb15); pks-1(reb11[TE_1_S7593A])*			Outcrossing
RAB72	*rebEx15 (Pnemt-1::gfp, 50 ng/µL; CAN::mcherry, 50 ng/µL)*		N2	Transgenesis
RAB73	*rebEx16 (Ppkal-1::gfp, 50 ng/µL; CAN::mcherry, 50 ng/µL)*		N2	Transgenesis
RAB74	*rebEx17 (PC32E8.6::gfp, 50 ng/µL; CAN::mcherry, 50 ng/µL)*		N2	Transgenesis

RAB76	*nemt-1(reb15); rebEx19(Pnemt-1::nemt-1::sl2::mcherry, 50 ng/µL)*			Transgenesis
RAB77	*pkal-1(reb28); rebEx20(Ppkal-1::pkal-1::sl2::mcherry, 50 ng/µL)*			Transgenesis
RAB78	*C32E8.6(reb24); rebEx21(PC32E8.6::C32E8.6::sl2::mcherry, 50 ng/µL)*			Transgenesis
RAB79	*C32E8.6(reb24); rebEx20(Ppkal-1::pkal-1::sl2::mcherry, 50 ng/µL)*			Transgenesis
RAB80	*pkal-1(reb28); rebEx21(PC32E8.6::C32E8.6::sl2::mcherry, 50 ng/µL)*			Transgenesis
RAB81	Y71H2B.1*(gk712674); rebEx22(P*Y71H2B.1*::*Y71H2B.1*::sl2::mcherry, 50 ng/µL)*			Transgenesis
RAB82	C24A3.4(*reb16); rebEx23(PC24A3.4::C24A3.4::sl2::mcherry, 50 ng/µL)*			Transgenesis
RAB89	*pks-1(reb22[A_1_G7106E])* X	PKS-1_A_1	N2	CRISPR-Cas9
RAB103	*pks-1(reb29[PCP_2_S7463A])* X	PKS-1_PCP_2	N2	CRISPR-Cas9
RAB109	*nrps-1(reb31[A_3_ G2337D])* III	NRPS-1_A_3	N2	CRISPR-Cas9
RAB110	*nrps-1(reb32[A_2_ G964D])* III	NRPS-1_A_2	N2	CRISPR-Cas9

Supplementary Table 4. Single worm PCR primers for mutant strains in this study.

Strain	Genotype	Sequence
RAB43	*nrps-1 (gk186409[C_4_S1934N]; gk186410[C_4_D1971N])* III	Forward: CTGAAGCCTTTATTCAGTGCCAAG Reverse: CTTGCACTGCTAGAGCTAAGCTTC
RAB45	Y71H2B.1*(gk712674)* III	Forward: GGAAAGCACGGAGATTTTGAAG Reverse: AGTGATGGGAATGGTCTCTGTT
RAB51	*nrps-1(reb8[ACP_7_S307V])* III	Forward: GAAGGAGCAGCAAACATCGAGAA Reverse: ATCTGAGTGACCTGCTTTCAGAG
RAB52	*pks-1(reb9[C_1_H6685A])* X	Forward: CATCTGTAAACCCTGCAGATATTGC Reverse: CGGCATCGCAGAAAACTGATAATGC
RAB53	*nrps-1(reb10[C_3_H1486A])* III	Forward: GAAGCTGGTGGAGTTGTCCAATGCT Reverse: GAAACTGTATCCCAGTTCTCTGGAG
RAB54	*pks-1(reb11[TE_1_S7593A])* X	Forward: GGTGATTAAATCTGGAGTAC Reverse: TAGTCCAGAGAAGACGTACT
RAB55	*nrps-1(reb12[TE_2_S2803A])* III	Forward: TCGAGACCAAACTCGGAATC Reverse: TCTGAGAAAATGTTCACCGG
RAB56	*pks-1(reb13[TE_1_S7593C]; reb14[TE_1_G7596A])* X	Forward: GAGGTGATTAAATCTGGAGTACGGC Reverse: TCACTATCCGGTAGTCCAGAGAAG
RAB57	*nemt-1(reb15)* IV	Forward: AGTGGCTTTGCCTTTCCTCCTT Reverse: AGCCCTCAACTACTTCATCAGTG
RAB58	*pkal-1(reb21)* X	Forward: GAGCTCGGGATTTCTCAAGGT Reverse: CAATTCTGCAACACAGAATGTCG
RAB59	*pkal-1(reb28)* X	Forward: GAGCTCGGGATTTCTCAAGGT Reverse: CAATTCTGCAACACAGAATGTCG
RAB60	*C32E8.6(reb23)* I	Forward: GCTTCAACTCCAGAGAATCAGG Reverse: CAACGGCTCTCCGCTCTTAAG
RAB61	*C32E8.6 (reb24)* I	Forward: GCTTCAACTCCAGAGAATCAGG Reverse: CAACGGCTCTCCGCTCTTAAG
RAB62	C24A3.4*(reb16)* X	Forward: CTCTGCCGTACCAGTGATGTTCTA Reverse: CTATCCATGTGCTACCAAACTTGTC
RAB89	*pks-1(reb22[A_1_G7106E])* X	Forward: CACCACTATACCAATTCGAAGAACTG Reverse: AGTGACTTGTCAACTTTCCCACTTG
RAB103	*pks-1(reb29[PCP_2_S7463A])* X	Forward: GAGACTCACTGAGCAATGAAACTTG Reverse: TCCAGATTTAATCACCTCTTCAGC
RAB109	*nrps-1(reb31[A_3_G2337D])* III	Forward: CTACCAGCAATTCTTTACTGCTAATTC Reverse: CTTCTCAATTCTACAGACATCTCCA
RAB110	*nrps-1(reb32[A_2_G964D])* III	Forward: CCGTATCTCAAATCATAGGCC Reverse: CCTATGTCCTCGCACCTTCACCTG

Supplementary Table 5. Single-worm PCR information used to confirm genotype of wild-type and mutant worm strains used in this study.

Strain	Genotype	Wild type	Mutant	Enzyme digestion
RAB43	*nrps-1 (gk186409[C_4_S1934N]; gk186410[C_4_D1971N])* III	445 bp	445 bp	Wild type is cut by BtsIMutI to 265bp + 180bp; no cut for mutant
RAB45	Y71H2B.1*(gk712674)* III	700 bp	700 bp	Wild type is cut by HinfI into 450bp + 200bp + 50bp; mutant is cut by HinfI into 500bp + 200bp
RAB51	*nrps-1(reb8[ACP_7_S307V])* III	820 bp	820 bp	Mutant is cut by AatII into 438bp + 382bp
RAB52	*pks-1(reb9[C_1_H6685A])* X	1098 bp	1098 bp	Mutant is cut by SphI into 750bp + 348bp
RAB53	*nrps-1(reb10[C_3_H1486A])* III	993 bp	993 bp	Mutant is cut by AseI into 658bp + 335bp
RAB54	*pks-1(reb11[TE_1_S7593A])* X	562 bp	562 bp	Mutant is cut by SphI into 355bp + 207bp
RAB55	*nrps-1(reb12[TE_2_S2803A])* III	539 bp	539 bp	Mutant is cut by NheI into 318bp + 221bp
RAB56	*pks-1(reb13[TE_1_S7593C]; reb14[TE_1_G7596A])* X	574 bp	574 bp	Mutant is cut by KasI into 364bp + 210bp
RAB57	*nemt-1(reb15)* IV	981 bp	702 bp	
RAB58	*pkal-1(reb21)* X	1723 bp	1570 bp	
RAB59	*pkal-1(reb28)* X	1723 bp	1171 bp	
RAB60	*C32E8.6(reb23)* I	1405 bp	424 bp	
RAB61	*C32E8.6(reb24)* I	1405 bp	412 bp	
RAB62	C24A3.4*(reb16)* X	1863 bp	502 bp	
RAB89	*pks-1(reb22[A_1_G7106E])* X	1350 bp	1350 bp	Mutant is cut by SalI into 914bp + 436bp
RAB103	*pks-1(reb29[PCP_2_S7463A])* X	1057bp	1057bp	Mutant is cut by SalI into 914bp + 436bp
RAB109	*nrps-1(reb31[A_3_ G2337D])* III	1009bp	1009bp	Wild type cut by BspEI to 395bp + 614bp (mutant not cut)
RAB110	*nrps-1(reb32[A_2_ G964D])* III	1062bp	1062bp	Mutant cut by SalI to 380bp + 682bp

Supplementary Table 6. gRNA sequences used for CRISPR-Cas9 in this study.

Strain	Genotype (alleles)	DNA encoding sgRNA (20 bases+NGG, and vector for cloning) or crRNA
RAB51	*nrps-1(reb8[ACP_7_S307V])* III	CTCCAGCTCGGCGAGTCTTA AGG (pTM55-FE*)
RAB52	*pks-1(reb9[C_1_H6685A])* X	ATCATATTTTAACTGATGGT TGG (pTM55-FE*)
RAB53	*nrps-1(reb10[C_3_H1486A])* III	GGCTTCTACCATCGCAGATC AGG (pTM55-FE*)
RAB54	*pks-1(reb11[TE_1_S7593A])* X	TTCGTTATGGGGCACTCGAT GGG (pTM55)
		CTTCGTTATGGGGCACTCGA TGG (pTM55)
		GTTATGGGGCACTCGATGGG TGG (pTM55)
RAB55	*nrps-1(reb12[TE_2_S2803A])* III	ACCTCTAAATTGGTGTTCAT TGG (pTM55)
		TTCATTGGCGCCTCGTCTGC TGG (pTM55)
RAB56	*pks-1(reb13[TE_1_S7593C];*	TTCGTTATGGGGCACTCGAT GGG (pTM55)
	reb14[TE_1_G7596A]) X	CTTCGTTATGGGGCACTCGA TGG (pTM55)
		GTTATGGGGCACTCGATGGG TGG (pTM55)
RAB57	*nemt-1(reb15)* IV	TATTACTACAGTTATGGCTT TGG (pTM55-FE*)
		CGAGAAATATGGAACACAGG TGG (pTM55-FE*)
RAB58	*pkal-1(reb21)* X	AAACTATTGGGCACTTTCGG AGG (pTM55-FE*)
		CCCAGAATCCAGATGCATGG TGG (pTM55-FE*)
		TCTTGTGGACATATTCTGCC AGG (pTM55-FE*)
RAB59	*pkal-1(reb28)* X	AAACTATTGGGCACTTTCGG AGG (pTM55-FE*)
		CCCAGAATCCAGATGCATGG TGG (pTM55-FE*)
		TCTTGTGGACATATTCTGCC AGG (pTM55-FE*)
RAB60	C32E8.6*(reb23)* I	CTTCCATTCTTCCATGCGGG TGG (pTM55-FE*)
		GACGTGATCCGGAAAGTGGA GGG (pTM55-FE*)
RAB61	C32E8.6*(reb24)* I	CTTCCATTCTTCCATGCGGG TGG (pTM55-FE*)
		GACGTGATCCGGAAAGTGGA GGG (pTM55-FE*)
RAB89	*pks-1(reb22[A_1_G7106E])* X	AGGGACACCTGTTGAGCCAC TGG (pTM55-FE*)
RAB103	*pks-1(reb29[PCP_2_S7463A])* X	AGCAATTTGAATAGCATTGA GGG (pTM55-FE*)
RAB109	*nrps-1(reb31[A_3_ G2337D])* III	rCrUrUrCrGrGrUrGrUrArCrCrCrGrUrUrGrUrUrC
		rGrUrUrUrUrArGrArGrCrUrArUrGrCrU
RAB110	*nrps-1(reb32[A_2_ G964D])* III	rGrCrUrUrArCrGrUrCrArCrCrUrCrArArCrArUrC
		rGrUrUrUrUrArGrArGrCrUrArUrGrCrU

* pTM55-FE is a modified version of pTM55 (a gift of Patrick McGrath) and was mutated to enable a higher level of recognition efficiency[7] by Cas9.

Supplementary Table 7. Repair templates used for CRISPR-Cas9 in this study.

Strain	Genotype (alleles)	Repair template*
RAB51	*nrps-1(reb8[ACP_7_S307V])* III	ATGAAGTTGAAACCACTCCTCTACCATACCTCGGAATCG ACGTCTTAAGACTCGCCGAGCTGGAGTACCACGTGGCT AGT (underlined: AatII; S307V: TCC»GTC)
RAB52	*pks-1(reb9[C_1_H6685A])* X	TGATAACAGTCGAATTCACATCGTTTTCAATCAGCATGC AATTTTAACTGATGGTTGGTCAATGACTGTTCTTTCTGA CACTGT (underlined: SphI; H6685A: CAT»GCA)
RAB53	*nrps-1(reb10[C_3_H1486A])* III	CTGGATGAGTAGCAAAAATAAGTTATTGACAATTTCCATT CACGCATTAATCTGCGATGGTAGAAGCCTGCAGATTCTC GAG (underlined: AseI; H1486A: CAC»GCA)
RAB54	*pks-1(reb11[TE_1_S7593A])* X	TGCCGCACATGCCGGAAACAAGAGAATCTTCGTTATGGG GCATGCGATGGGTGGAATAATGAGTCGCGAAATAGTGGC TGAGCTCAAAAT (underlined: SphI; S7593A: TCG»GCG)
RAB55	*nrps-1(reb12[TE_2_S2803A])* III	GTGCTGAAAATATTGAAACCTCTAAATTGGTGTTCATTGG CGCCGCTAGCGCTGGTACTTTTGCATTTTCCACGTCACA ACTTTTTG (underlined: NheI; S2803A: TCG»GCT)
RAB56	*pks-1(reb13[TE_1_S7593C]; reb14[TE_1_G7596A])* X	TGCCGCACATGCCGGAAACAAGAGAATCTTCGTTATGGG ACATTGCATGGGCGCCATAATGAGTCGCGAAATAGTGGC TGAGCTCAAAAT (underlined: KasI; S7593C/G7596A: TCG» TGC/ GGA»GCC)
RAB89	*pks-1(reb22[A_1_G7106E])* X	AGAACTCAATTTGGAAGTATTTACTCCATATTCACCAGTGA GTCGACAGGTGTCCCTAAAGGAGTTTTGATGGCGGAACA GTCA(underlined: SalI; G7106E: GGC»GAG)
RAB103	*pks-1(reb29[PCP_2_S7463A])* X	GTGGCGCCGACAGATAAATTTGAAAGTATTGGTGGAAACG CGTTAAATGCTATTCAAATTGCTCATCGGTTGGCTGAAG AG(underlined: MluI; S7463A: TCC»GCG)
RAB109	*nrps-1(reb31[A_3_ G2337D])* III	CACCGACTATTGTCTCTCCTACATTATCACAACTAGTGATAC **T**AC**T**GGTACACCGAAGTCGGTAGCAATCGGAGCGAAATC (bold: changed for crRNA binding purposes; underlined: previous BspEI site; G2337D; GGA»GAT)
RAB110	*nrps-1(reb32[A_2_ G964D])* III	CAACAAATCCATCCTATACGACCTAGCTTACGTCACGTC GACAA**AG**TGACAGCACTGGGACCCCGAAACTGGTTGG AACCTCATTTG (bold: changed for crRNA binding purposes; underlined: SalI; G964D; GGA»GAC)

* The underlined bases indicate restriction sites designed for screening worms for the desired mutations, and the bases labeled in red code for the amino acid that was mutated. Additional silent base changes made because they were necessary for creating or removing the restriction sites have not been indicated.

Supplementary Table 8. Primers for construction of transcriptional and translational reporter strains.

Strain	Genotype	Name and Sequence*
RAB72	*rebEx15*	nemt-1p_SalI_Fwd
		GCGCGTCGACCGAAAATCTCAAGTCTTGTCTTAA**
		nemt-1p_NotI_Rev
		CATGGCGGCCGCAGTGAATATGTGTTTACGAGTAAATG**
RAB73	*rebEx16*	pkal-1p_SalI_Fwd
		GCGCGTCGACGCTATAAATGGGTACCTGGCCGTAA**
		pkal-1p_NotI_Rev
		CATGGCGGCCGCCGTAGAGAAGAAACTGTGACAGTTC**
RAB74	*rebEx17*	C32E8.6p_SalI_Fwd
		GCGCGTCGACGTACCGGGAATCGAAAAATTGTCC**
		C32E8.6p_NotI_Rev
		CATGGCGGCCGCTCTTATTGTACAGAATGTTTCTTTCC**
RAB76	*rebEx19*	nemt-1SL2_SalI_Fwd
		GCGCGTCGACCGAAAATCTCAAGTCTTGTCTTAA***
		nemt-1SL2_NotI_Rev
		CATGGCGGCCGCAGTGAATATGTGTTTACGAGTAAATG***
RAB77	*rebEx20*	pkal-1SL2_PstI_Fwd
		CATGCTGCAGGCTATAAATGGGTACCTGGCCGTAA***
		pkal-1SL2_NotI_Rev
		CATGGCGGCCGCTCAATAGTACATTAGCCTATTCTTTTG***
RAB78	*rebEx21*	C32E8.6SL2_SalI_Fwd
		GCGCGTCGACGTACCGGGAATCGAAAAATTGTCC***
		C32E8.6SL2_NotI_Rev
		CATGGCGGCCGCTCAATGCAACATATGACGGACTAG***
RAB81	*rebEx22*	Y71H2B.1SL2_PstI_Fwd
		CATGCTGCAGCCAGATGAAGAAAACGATGGAACT***
		Y71H2B.1SL2_NotI_Rev
		CATGGCGGCCGCTTAAACCCATCCCTCTGGAGGATT***
RAB82	*rebEx23*	C24A3.4SL2_SalI_Fwd
		GCAGGTCGACGGTCTCTACAGTGATGACACTCATT***
		C24A3.4SL2_NotI_Rev
		CATGGCGGCCGCTCACAGCTTGGACCGCGCCGCAAA***

*The underlined bases indicate restriction sites used for plasmid construction.

**Used to amplify gene promoter for insertion into pPD114.108.

***Used to promoter and gene from genomic DNA for insertion into pBS77-SL2-mCherry.

Supplementary Table 9. Primers for construction of protein expression plasmids.

Purpose	Name and sequence*
PKAL-1 expression vector	pkal-1_for CATGCCATGGGGGCGAAATATTATCCAGAAAC pkal-1_rev CATGGCGGCCGCATAGTACATTAGCCTATTC
PKS-1_ACP_1 expression vector	pks-1_acp1_for GCGCCCATGGGGCTTTCTGATGCGGAAATTGAGTC pks-1_acp1_rev CATGGCGGCCGCAGTTGTTGCTTTAGTAACTGGAAC
NRPS-1_ACP_7 expression vector	nrps-1_acp7_for CATGCCATGGGGAGTGAAGACTCCGATGAAGAAGT nrps-1_acp7_rev CATGGCGGCCGCTCCGGACCCCAGCGCTTTCTCAC
Mutagenesis of PKAL-1	pkal-1K488A_1 GTCAAGTGGGGCCATTCAAAAGAATAG pkal-1K488A_2 GATTTTGGCATCTCTTTTATAATTG
Mutagenesis of PKS-1_ACP_1	pks-1_acp1_1 ATATACCATGTACTGGTCTGATGCGGAAATTGAG pks-1_acp1_2 CTCCTTCTTAAAGTTAAACAAAATTATTTC
Mutagenesis of NRPS-1_ACP_7	nrps-1_acp7_1 GTACAGTGAAGACTCCGATGAAG nrps-1_acp7_2 CACATATATCTCCTTCTTAAAGTTAAAC

*The underlined bases indicate restriction sites used for plasmid construction.

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