Molecular Basis of Methylation and Chain-Length Programming in a Fungal Iterative Highly Reducing Polyketide Synthase

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Electronic Supplementary Information

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Contents

1. Mapping Swapped Fragments onto porcine mFAS structure (porcine mFAS numbering)

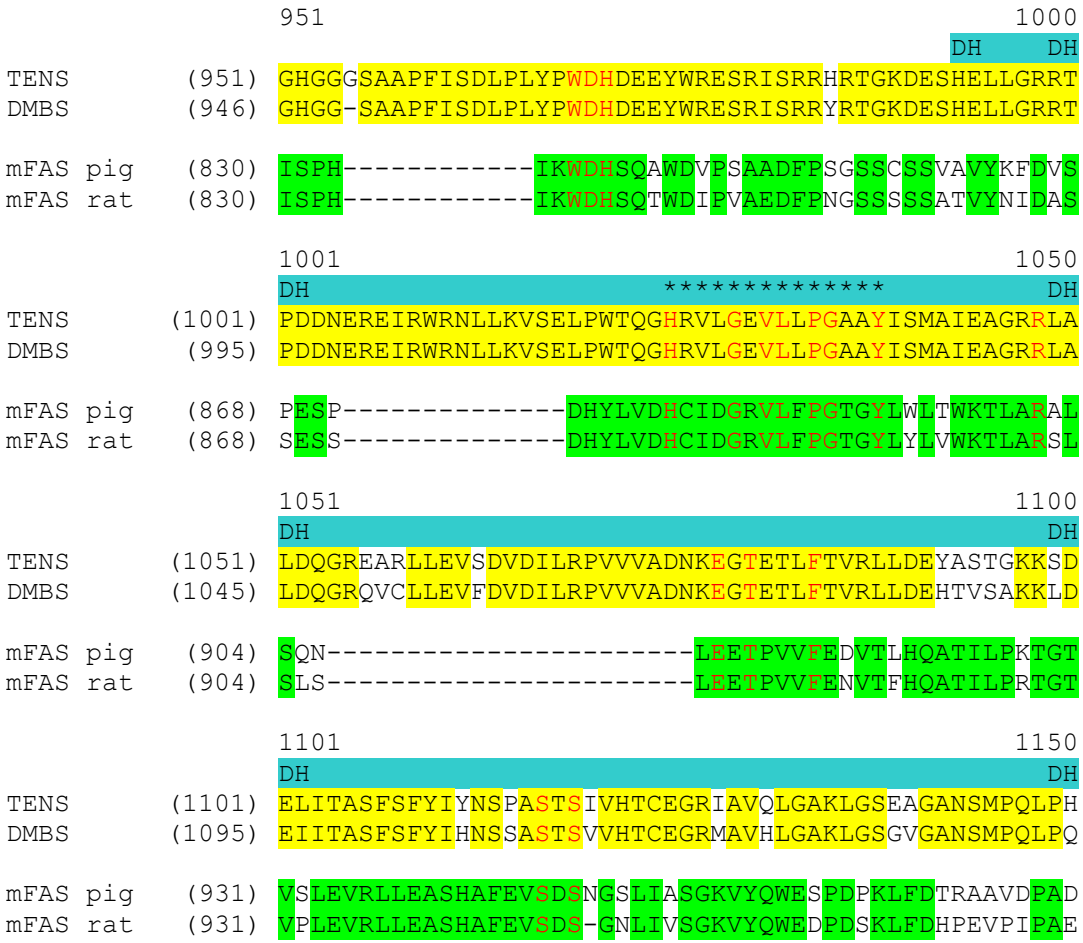
Fragment	Start	Stop	Size	Size	Size	Size
1A1	1042[2]	1121	79	142	227	479
1A2[1]	1122	1185	63			
1B1[1]	1186[3]	1216	30	85		
1B2	1217	1272	55			
2A1	1273	1303	30	78	140	
2A2	1304	1352	48			
2B1	1353	1393	40	62		
2B2	1394	1416	22			
3A	1417	1473	56	112	112	
3B	1474[4]	1530	56			
4A	1881	1953	73	143	281	281
4B	1953	2024	70			
5A	2024	2092	69	138		
5B	2092	2162	69			

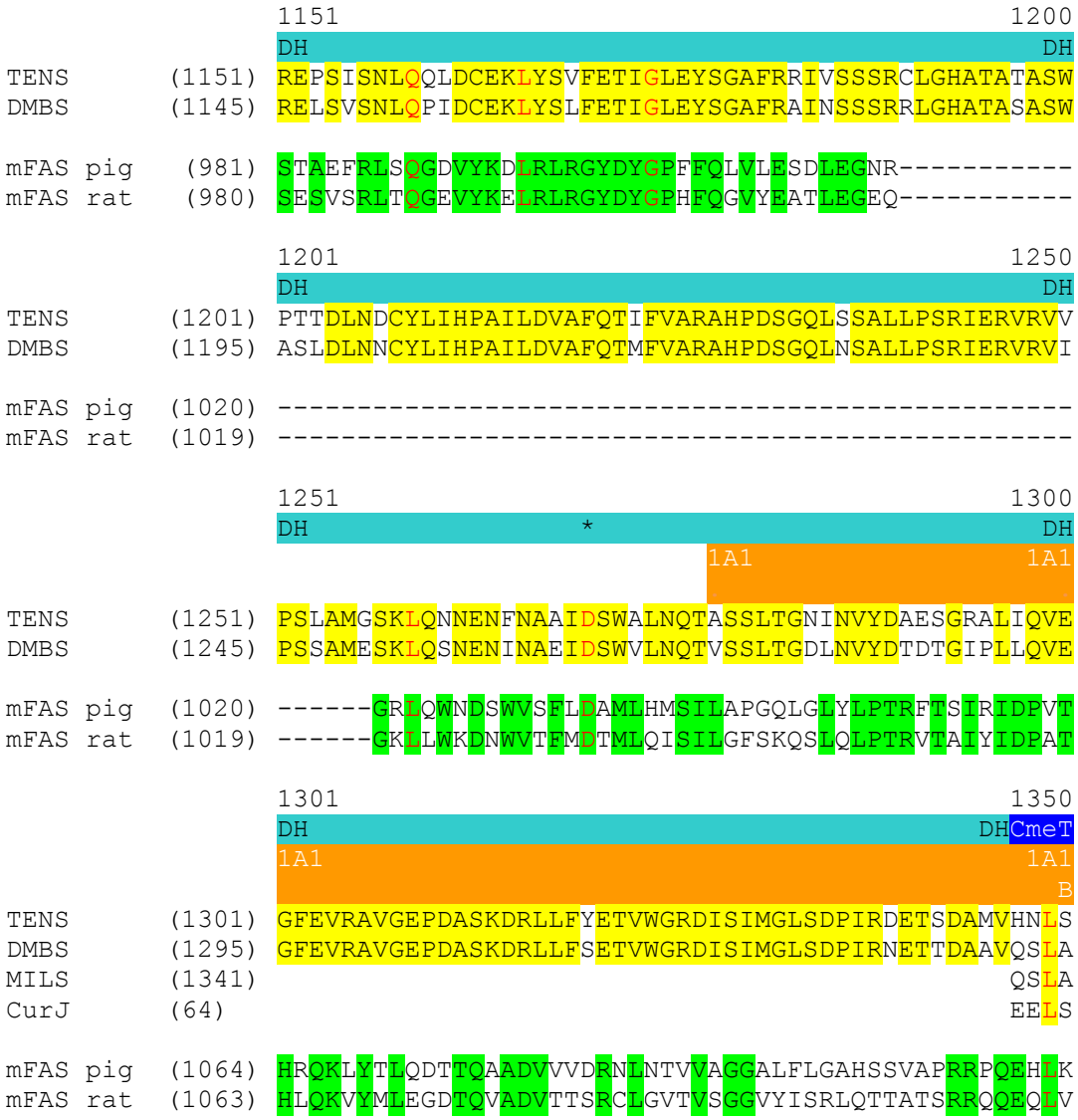
Table S1. Protein positions used for swaps relative to the porcine mFAS structure. Same colour code was used as in Table 1 in main paper.

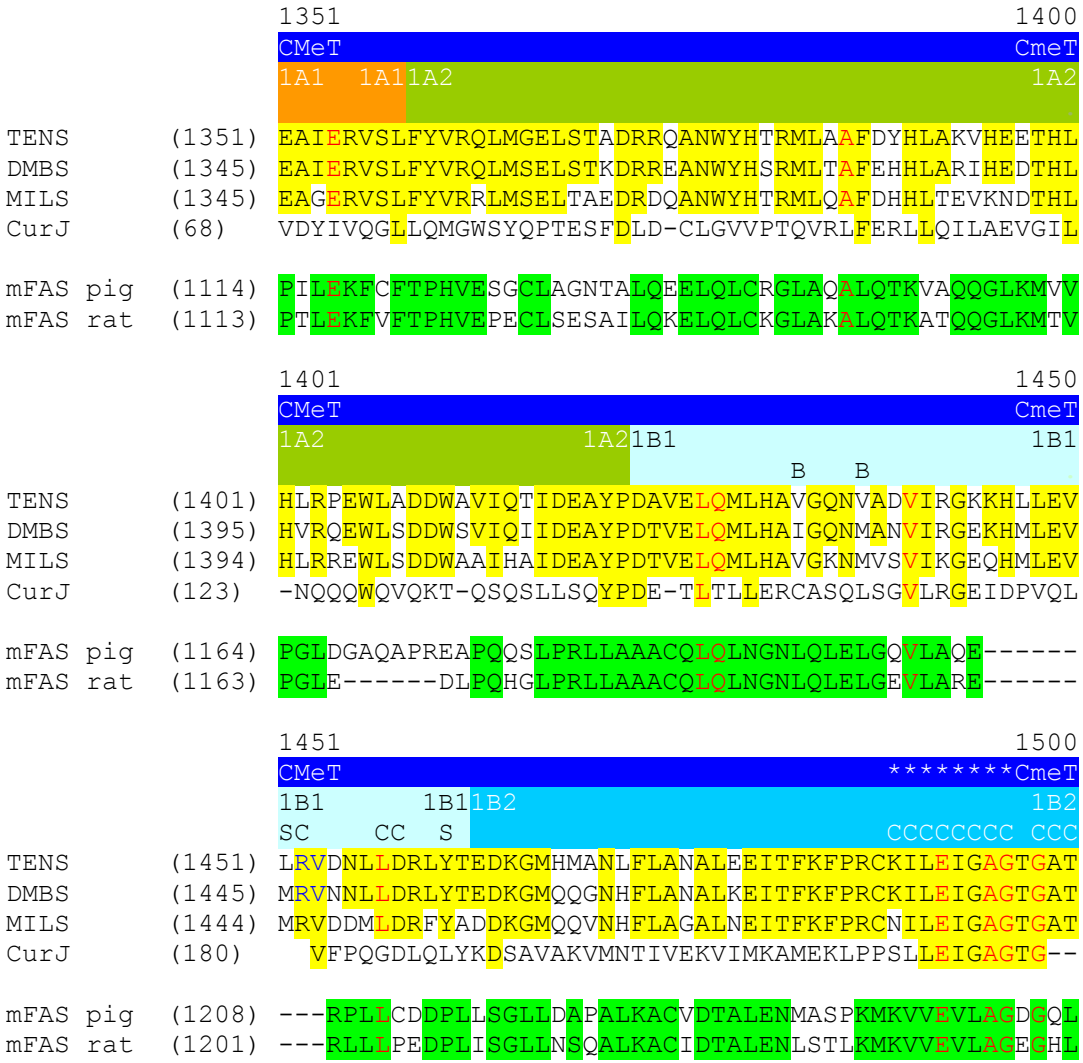
Notes

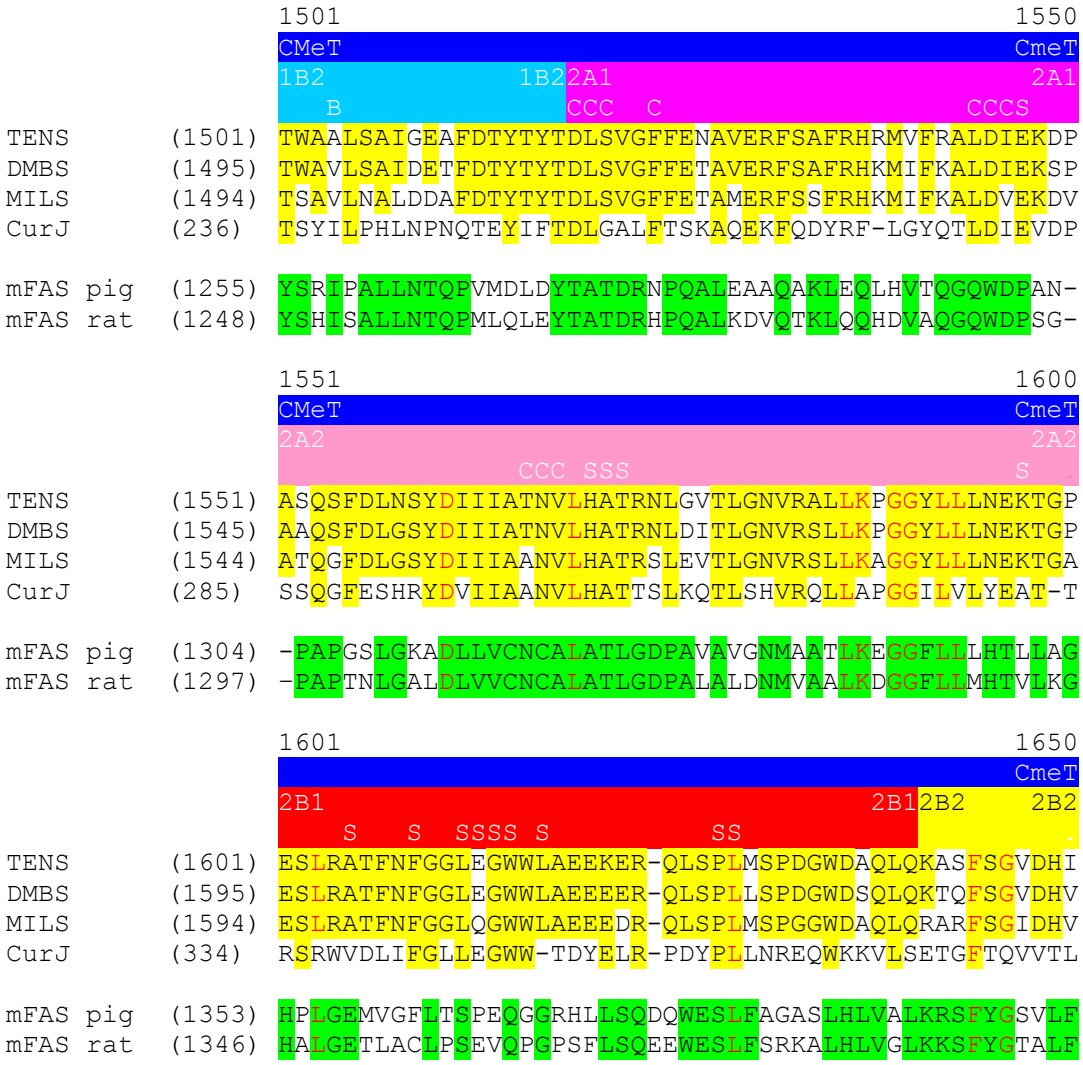
1. residues 1136 - 1215 (inc) are missing from the mFAS structure
2. 1042 - 1084 part of DH
3. 1085 - 1111 DH to CMeT linker
4. 1521 - 1530 ψKR-ER linker

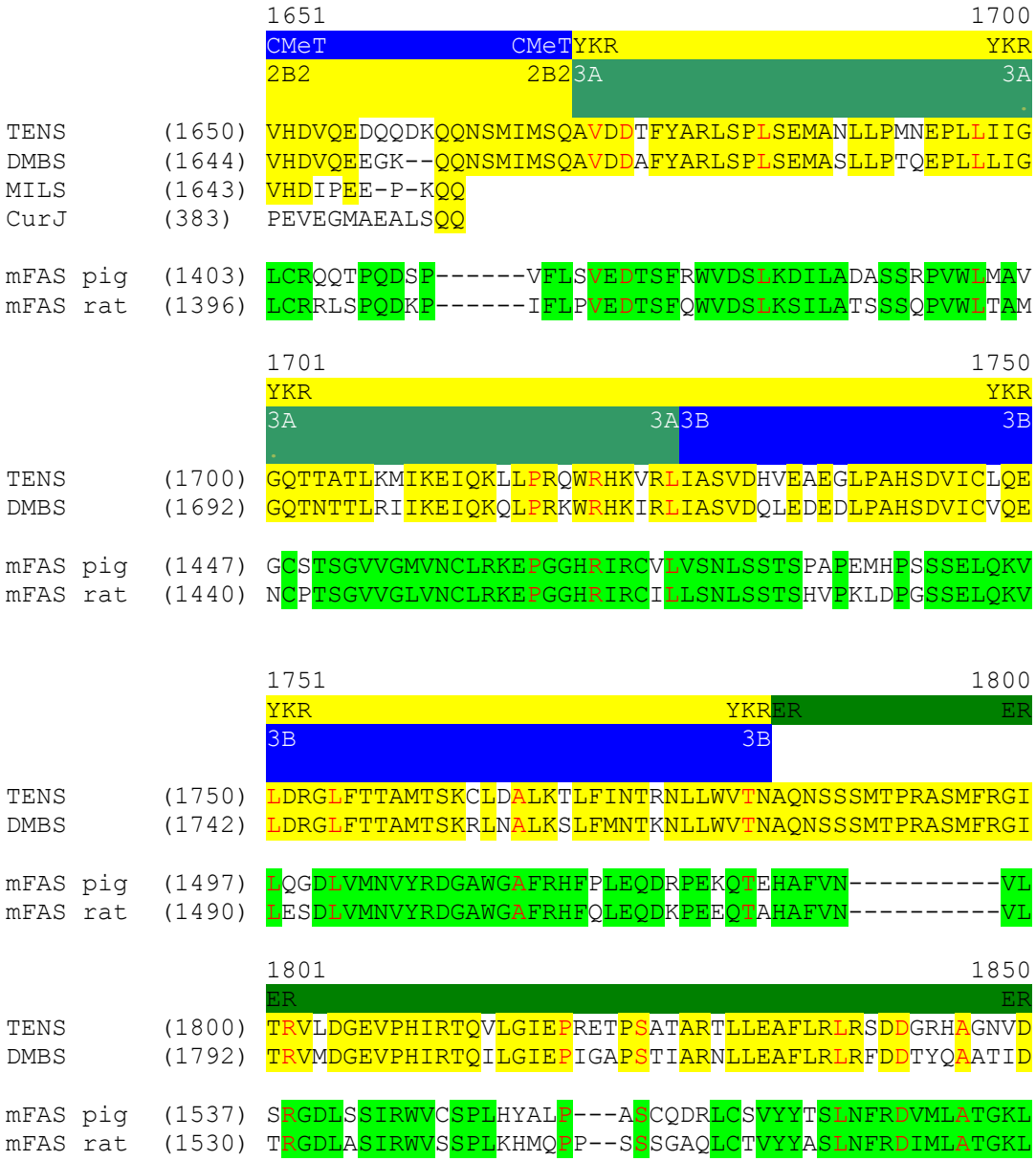
2. Multiple Alignment of β-Processing Domains.

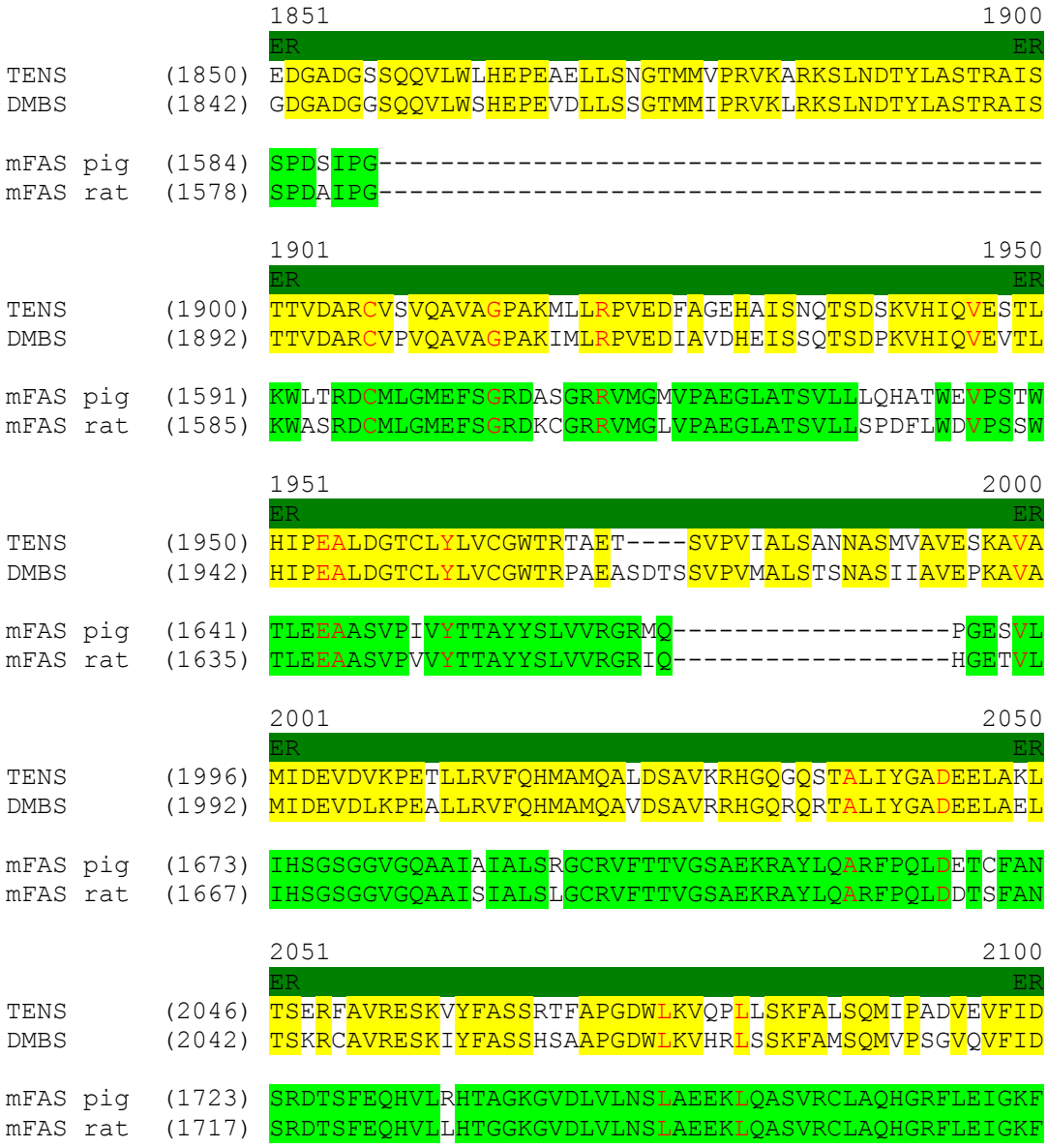


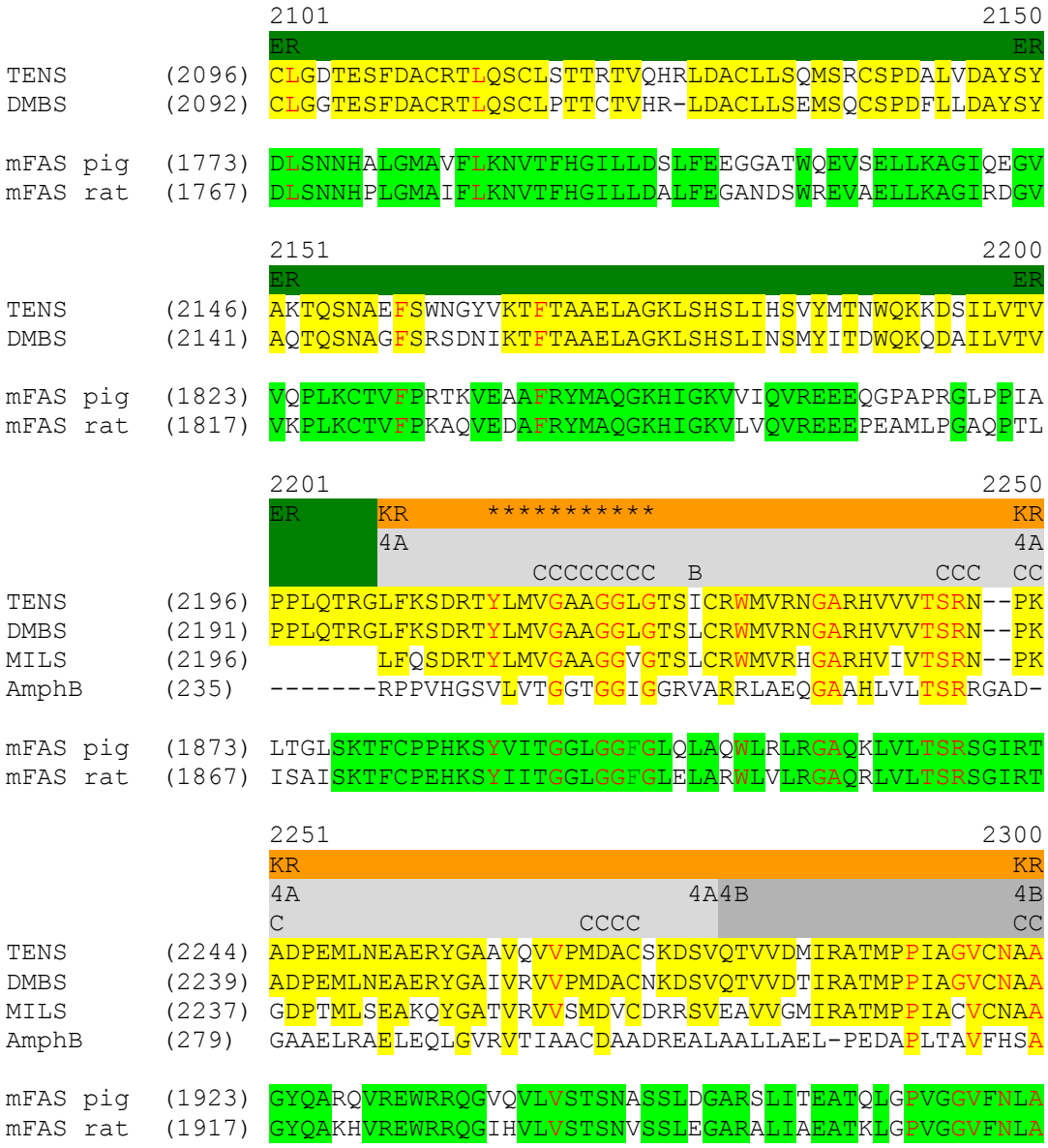

1151
1200
DH
DH
TENS (1151) REPSISNLQQLDCEKLYSVFETIGLEYSGAFRRIVSSSRCLGHATATASW
DMBS (1145) RELSVSNLQPIDCEKLYSLFETIGLEYSGAFRAINSSSRRLGHATASASW
mFAS pig (981) STAEFRLSQGDVYKDLRLRGYDYGPFFQLVLESDLEGNR----------
mFAS rat (980) SESVSRLTQGEVYKELRLRGYDYGPHFQGVYEATLEGEQ----------
1201
1250
DH
DH
TENS (1201) PTTDLNDCYLIHPAILDVAFQTIFVARAHPDSGQLSSALLPSRIERVRVV
DMBS (1195) ASLDLNNCYLIHPAILDVAFQTMFVARAHPDSGQLNSALLPSRIERVRVI
mFAS pig (1020) --
mFAS rat (1019) --
1251
1300
DH
*
DH
1A1
1A1
TENS (1251) PSLAMGSKLQNNENFNAAIDSWALNQTASSLTGNINVYDAESGRALIQVE
DMBS (1245) PSSAMESKLQSNENINAEIDSWVLNQTVSSLTGDLNVYDTDTGIPLLQVE
mFAS pig (1020) ------GRLQWNDSWVSFLDAMLHMSILAPGQLGLYLPTRFTSIRIDPVT
mFAS rat (1019) ------GKLLWKDNWVTFMDTMLQISILGFSKQSLQLPTRVTAIYIDPAT
1301
1350
DH
DH
CmeT
1A1
1A1
B
TENS (1301) GFEVRAVGEPDASKDRLLFYETVWGRDISIMGLSDPIRDETSDAMVHNLS
DMBS (1295) GFEVRAVGEPDASKDRLLFSETVWGRDISIMGLSDPIRNETTDAAVQSLA
MILS (1341) QSLA
CurJ (64) EELS
mFAS pig (1064) HRQKLYTLQDTTQAADVVVDRNLNTVVAGGALFLGAHSSVAPRRPQEHLK
mFAS rat (1063) HLQKVYMLEGDTQVADVTTSRCLGVTVSGGVYISRLQTTATSRRQQEQLV

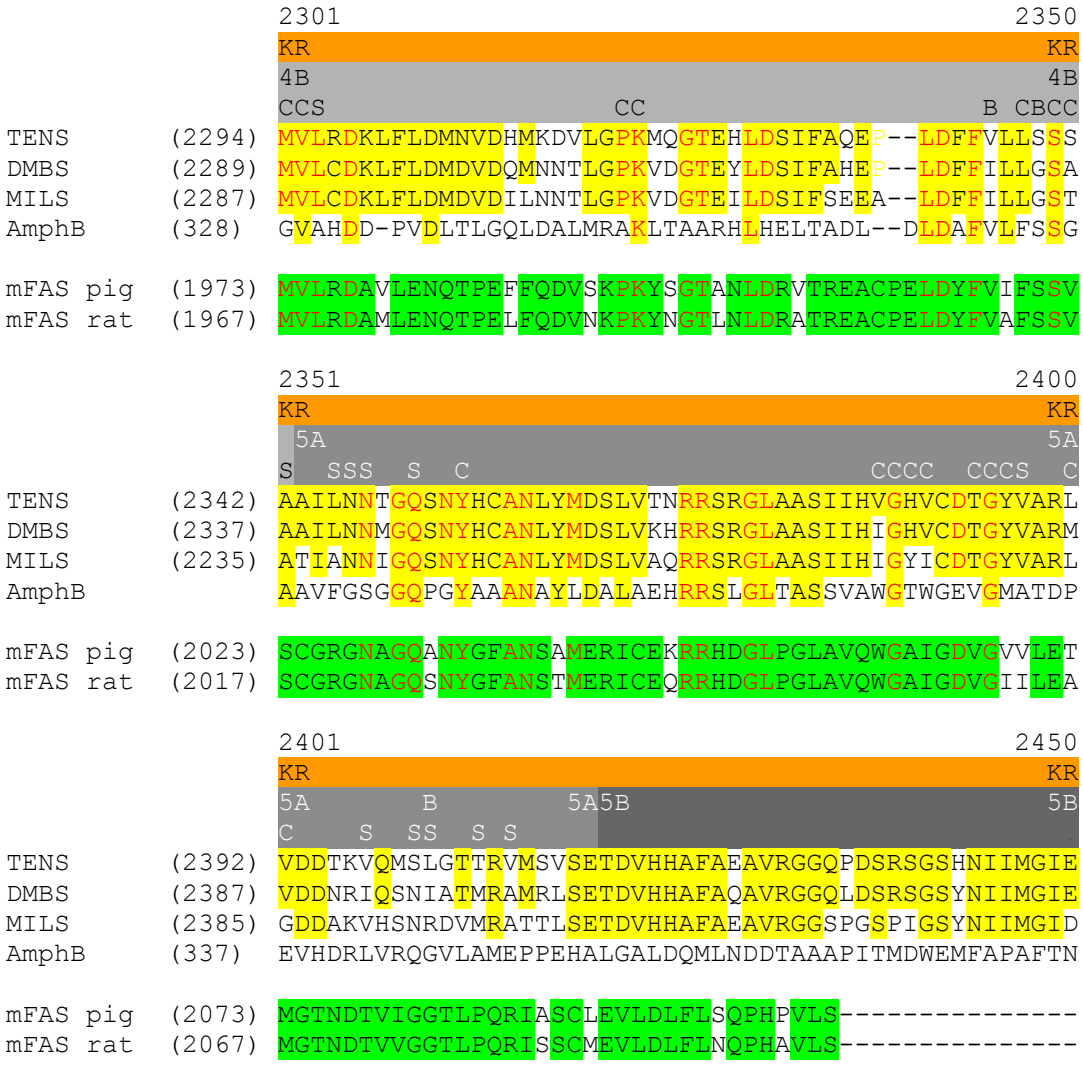

1351 1400
CMeT CmeT
1A1 1A1 1A2 1A2
TENS (1351) EAIERVSLFYVRQLMGELSTADRRQANWYHTRMLAAFDYHLAKVHEETHL
DMBS (1345) EAIERVSLFYVRQLMSELSTKDRREANWYHSRMLTAFEHHLARIHEDTHL
MILS (1345) EAGERVSLFYVRRLMSELTAEDRDQANWYHTRMLQAFDHHLTEVKNDTHL
CurJ (68) VDYIVQGLLQMGWSYQPTESFDLD-CLGVVPTQVRLFERLLQILAEVGIL
mFAS pig (1114) PILEKFCFTPHVESGCLAGNTALQEELQLCRGLAQALQTKVAQQGLKMVV
mFAS rat (1113) PTLEKFVFTPHVEPECLSESAILQKELQLCKGLAKALQTKATQQGLKMTV
1401 1450
CMeT CmeT
1A2 1A2 1B1 1B1
B B
TENS (1401) HLRPEWLADDWAVIQTIDEAYPDAVELQMLHAVGQNVADVIRGKKHLLEV
DMBS (1395) HVRQEWLSDDWSVIQIIDEAYPDTVELQMLHAIGQNMANVIRGEKHMLEV
MILS (1394) HLRREWLSDDWAAIHAIDEAYPDTVELQMLHAVGKNMVSVIKGEQHMLEV
CurJ (123) -NQQQWQVQKT-QSQSLLSQYPDE-TLTLLERCASQLSGVLRGEIDPVQL
mFAS pig (1164) PGLDGAQAPREAPQQSLPRLLAAACQLQLNGNLQLELGQVLAQE------
mFAS rat (1163) PGLE------DLPQHGLPRLLAAACQLQLNGNLQLELGEVLARE------
1451 1500
CMeT ********CmeT
1B1 1B1 1B2 1B2
SC CC S CCCCCCCC CCC
TENS (1451) LRVDNLLDRLYTEDKGMHMANLFLANALEEITFKFPRCKILEIGAGTGAT
DMBS (1445) MRVNNLLDRLYTEDKGMQQGNHFLANALKEITFKFPRCKILEIGAGTGAT
MILS (1444) MRVDDMLDRFYADDKGMQQVNHFLAGALNEITFKFPRCNILEIGAGTGAT
CurJ (180) VFPQGDLQLYKDSAVAKVMNTIVEKVIMKAMEKLPPSLLEIGAGTG--
mFAS pig (1208) ---RPLLCDDPLLSGLLDAPALKACVDTALENMASPKMKVVEVLAGDGQL
mFAS rat (1201) ---RLLLPEDPLISGLLNSQALKACIDTALENLSTLKMKVVEVLAGEGHL

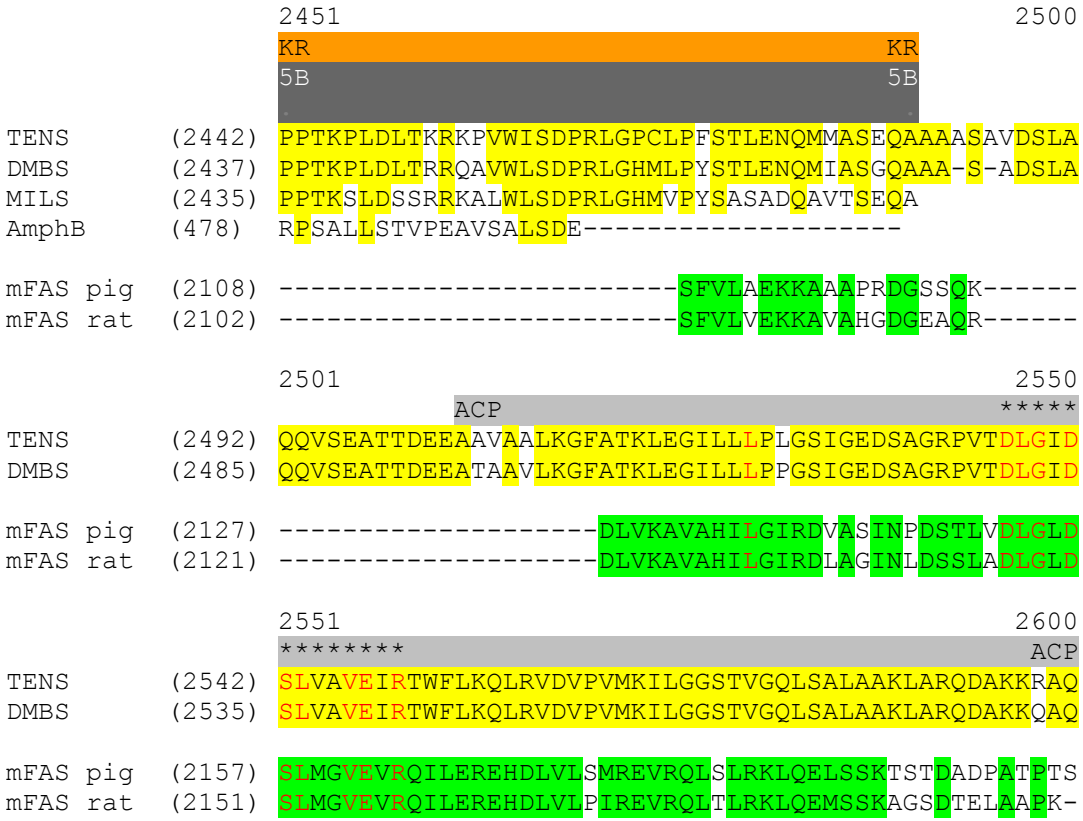

1501 1550
CMeT CmeT
1B2 1B2 2A1 2A1
B CCC C CCCS
TENS (1501) TWAALSAIGEAFDTYTYTDLSVGFFENAVERFSAFRHRMVFRALDIEKDP
DMBS (1495) TWAVLSAIDETFDTYTYTDLSVGFFETAVERFSAFRHKMIFKALDIEKSP
MILS (1494) TSAVLNALDDAFDTYTYTDLSVGFFETAMERFSSFRHKMIFKALDVEKDV
CurJ (236) TSYILPHLNPNQTEYIFTDLGALFTSKAQEKFQDYRF-LGYQTLDIEVDP
mFAS pig (1255) YSRIPALLNTQPVMDLDYTATDRNPQALEAAQAKLEQLHVTQGQWDPAN-
mFAS rat (1248) YSHISALLNTQPMLQLEYTATDRHPQALKDVQTKLQQHDVAQGQWDPSG-
1551 1600
CMeT CmeT
2A2 2A2
CCC SSS S
TENS (1551) ASQSFDLNSYDIIIATNVLHATRNLGVTLGNVRALLKPGGYLLLNEKTGP
DMBS (1545) AAQSFDLGSYDIIIATNVLHATRNLDITLGNVRSLLKPGGYLLLNEKTGP
MILS (1544) ATQGFDLGSYDIIIAANVLHATRSLEVTLGNVRSLLKAGGYLLLNEKTGA
CurJ (285) SSQGFESHRYDVIIAANVLHATTSLKQTLSHVRQLLAPGGILVLYEAT-T
mFAS pig (1304) -PAPGSLGKADLLVCNCALATLGDPAVAVGNMAATLKEGGFLLLHTLLAG
mFAS rat (1297) -PAPTNLGALDLVVCNCALATLGDPALALDNMVAALKDGGFLLMHTVLKG
1601 1650
CMeT
2B1 2B1 2B2 2B2
S S SSSS S SS
TENS (1601) ESLRATFNFGGLEGWWLAEEKER-QLSPLMSPDGWDAQLQKASFSGVDHI
DMBS (1595) ESLRATFNFGGLEGWWLAEEEER-QLSPLLSPDGWDSQLQKTQFSGVDHV
MILS (1594) ESLRATFNFGGLQGWWLAEEEDR-QLSPLMSPGGWDAQLQRARFSGIDHV
CurJ (334) RSRWVDLIFGLLEGWW-TDYELR-PDYPLLNREQWKKVLSETGFTQVVTL
mFAS pig (1353) HPLGEMVGFLTSPEQGGRHLLSQDQWESLFAGASLHLVALKRSFYGSVLF
mFAS rat (1346) HALGETLACLPSEVQPGPSFLSQEEWESLFSRKALHLVGLKKSFYGTALF


1651 1700
CMeT CMeTYKR YKR
2B2 2B2 3A 3A
TENS (1650) VHDVQEDQQDKQQNSMIMSQAVDDTFYARLSPLSEMANLLPMNEPLLIIG
DMBS (1644) VHDVQEEGK--QQNSMIMSQAVDDAFYARLSPLSEMASLLPTQEPLLLIG
MILS (1643) VHDIPEE-P-KQQ
CurJ (383) PEVEGMAEALSQQ
mFAS pig (1403) LCRQQTPQDSP------VFLSVEDTSFRWVDSLKDILADASSRPVWLMAV
mFAS rat (1396) LCRRLSPQDKP------IFLPVEDTSFQWVDSLKSILATSSSQPVWLTAM
1701 1750
YKR YKR
3A 3A 3B 3B
TENS (1700) GQTTATLKMIKEIQKLLPRQWRHKVRLIASVDHVEAEGLPAHSDVICLQE
DMBS (1692) GQTNTTLRIIKEIQKQLPRKWRHKIRLIASVDQLEDEDLPAHSDVICVQE
mFAS pig (1447) GCSTSGVVGMVNCLRKEPGGHRIRCVLVSNLSSTSPAPEMHPSSSELQKV
mFAS rat (1440) NCPTSGVVGLVNCLRKEPGGHRIRCILLSNLSSTSHVPKLDPGSSELQKV
1751 1800
YKR YKRER ER
3B 3B
TENS (1750) LDRGLFTTAMTSKCLDALKTLFINTRNLLWVTNAQNSSSMTPRASMFRGI
DMBS (1742) LDRGLFTTAMTSKRLNALKSLFMNTKNLLWVTNAQNSSSMTPRASMFRGI
mFAS pig (1497) LQGDLVMNVYRDGAWGAFRHFPLEQDRPEKQTEHAFVN----------VL
mFAS rat (1490) LESDLVMNVYRDGAWGAFRHFQLEQDKPEEQTAHAFVN----------VL
1801 1850
ER ER
TENS (1800) TRVLDGEVPHIRTQVLGIEPRETPSATARTLLEAFLRLRSDDGRHAGNVD
DMBS (1792) TRVMDGEVPHIRTQILGIEPIGAPSTIARNLLEAFLRLRFDDTYQAATID
mFAS pig (1537) SRGDLSSIRWVCSPLHYALP---ASCQDRLCSVYYTSLNFRDVMLATGKL
mFAS rat (1530) TRGDLASIRWVSSPLKHMQPP--SSSGAQLCTVYYASLNFRDIMLATGKL


1851 1900
ER ER
TENS (1850) EDGADGSSQQVLWLHEPEAELLSNGTMMVPRVKARKSLNDTYLASTRAIS
DMBS (1842) GDGADGGSQQVLWSHEPEVDLLSSGTMMIPRVKLRKSLNDTYLASTRAIS
mFAS pig (1584) SPDSIPG----------
mFAS rat (1578) SPDAIPG----------
1901 1950
ER ER
TENS (1900) TTVDARCVSVQAVAGPAKMLLRPVEDFAGEHAISNQTSDSKVHIQVESTL
DMBS (1892) TTVDARCVPVQAVAGPAKIMLRPVEDIAVDHEISSQTSDPKVHIQVEVTL
mFAS pig (1591) KWLTRDCMLGMEFSGRDASGRRVMGMVPAEGLATSVLLLQHATWEVPSTW
mFAS rat (1585) KWASRDCMLGMEFSGRDKCGRRVMGLVPAEGLATSVLLSPDFLWDVPSSW
1951 2000
ER ER
TENS (1950) HIPEALDGTCLYLVCGWTRTAET----SVPVIALSANNASMVAVESKAVA
DMBS (1942) HIPEALDGTCLYLVCGWTRPAEASDTSSVPVMALSTSNASIIAVEPKAVA
mFAS pig (1641) TLEEAASVPIVYTTAYYSLVVRGRMQ----------PGESVL
mFAS rat (1635) TLEEAASVPVVYTTAYYSLVVRGRIQ----------HGETVL
2001 2050
ER ER
TENS (1996) MIDEVDVKPETLLRVFQHMAMQALDSAVKRHGQGQSTALIYGADEELAKL
DMBS (1992) MIDEVDLKPEALLRVFQHMAMQAVDSAVRRHGQRQRTALIYGADEELAEL
mFAS pig (1673) IHSGSGGVGQAAIAIALSRGCRVFTTVGSAEKRAYLQARFPQLDETCFAN
mFAS rat (1667) IHSGSGGVGQAAISIALSLGCRVFTTVGSAEKRAYLQARFPQLDTSFAN
2051 2100
ER ER
TENS (2046) TSERFAVRESKVYFASSRTFAPGDWLKVQPLLSKFALSQMIPADVEVFID
DMBS (2042) TSKRCAVRESKIYFASSHSAAPGDWLKVHRLSSKFAMSQMVPSGVQVFID
mFAS pig (1723) SRDTSFEQHVLRHTAGKGVDLVLNSLAEEKLQASVRCLAQHGRFLEIGKF
mFAS rat (1717) SRDTSFEQHVLLHTGGKGVDLVLNSLAEEKLQASVRCLAQHGRFLEIGKF


2101 2150
ER ER
TENS (2096) CLGDTESFDACRTLQSCLSTTRTVQHRLDACLLSQMSRCSPDALVDAYSY
DMBS (2092) CLGGTESFDACRTLQSCLPTTCTVHR-LDACLLSEMSQCSPDFLLDAYSY
mFAS pig (1773) DLSNNHALGMAVFLKNVTFHGILLDSLFEEGGATWQEVSELLKAGIQEGV
mFAS rat (1767) DLSNNHPLGMAIFLKNVTFHGILLDALFEGANDSWREVAELLKAGIRDGV
2151 2200
ER ER
TENS (2146) AKTQSNAEFSWNGYVKTFTAAELAGKLSHSLIHSVYMTNWQKKDSILVTV
DMBS (2141) AQTQSNAGFSRSDNIKTFTAAELAGKLSHSLINSMYITDWQKQDAILVTV
mFAS pig (1823) VQPLKCTVFPRTKVEAAFRYMAQGKHIGKVVIQVREEEQGPAPRGLPPIA
mFAS rat (1817) VKPLKCTVFPKAQVEDAFRYMAQGKHIGKVLVQVREEEPEAMLPGAQPTL
2201 2250
ER KR ********** KR
4A 4A
CCCCCCCC B CCC CC
TENS (2196) PPLQTRGLFKSDRTYLMVGAAGGLGTSICRWMVRNGARHVVVTSRN--PK
DMBS (2191) PPLQTRGLFKSDRTYLMVGAAGGLGTSLCRWMVRNGARHVVVTSRN--PK
MILS (2196) LFQSDRTYLMVGAAGGVGTSLCRWMVRHGARHVIVTSRN--PK
AmphB (235) -------RPPVHGSVLVTGGTGGIGGRVARRLAEQGAAHLVLTSRRGAD-
mFAS pig (1873) LTGLSKTFCPPHKSYVITGGLGGFGLQLAQWLRLRGAQKLVLTSRSGIRT
mFAS rat (1867) ISAISKTFCPEHKSYIITGGLGGFGLELARWLVLRGAQRLVLTSRSGIRT
2251 2300
KR KR
4A 4A4B 4B
C CCCC CC
TENS (2244) ADPEMLNEAERYGAAVQVVPMDACSKDSVQTVVDMIRATMPPIAGVCNAA
DMBS (2239) ADPEMLNEAERYGAIVRVVPMDACNKDSVQTVVDTIRATMPPIAGVCNAA
MILS (2237) GDPTMLSEAKQYGATVRVVSMDVCDRRSVEAVVGMIRATMPPIACVCNAA
AmphB (279) GAAELRAELEQLGVRVTIAACDAADREALAALLAEL-PEDAPLTAVFHSA
mFAS pig (1923) GYQARQVREWRRQGVQVLVSTSNASSLDGARSLITEATQLGPVGGVFNLA
mFAS rat (1917) GYQAKHVREWRRQGIHVLVSTSNVSSLEGARALIAEATKLGPVGGVFNLA


2301 2350
KR KR
4B 4B
CCS CC B CBCC
TENS (2294) MVLRDKLFLDMNVDHMKDVLGPKMQGTEHLDSIFAQEP--LDFFVLLSSS
DMBS (2289) MVLCDKLFLDMDVDQMNNTLGPKVDGTEYLDSIFAHEP--LDFFILLGSA
MILS (2287) MVLCDKLFLDMDVDILNNTLGPKVDGTEILDSIFSEEA--LDFFILLGST
AmphB (328) GVAHDD-PVDLTLGQLDALMRAKLTAARHLHELTADL--DLDAFVLFSSG
mFAS pig (1973) MVLRDAVLENQTPEFFQDVSKPKYSGTANLDRVTREACPELDYFVIFSSV
mFAS rat (1967) MVLRDAMLENQTPELFQDVNKPKYNGTLNLDRATREACPELDYFVAFSSV
2351 2400
KR KR
5A 5A
S SSS S C CCCC CCCS C
TENS (2342) AAILNNTGQSNYHCANLYMDSLVTNRRSRGLAASIIHVGHVCDTGYVARL
DMBS (2337) AAILNNMGQSNYHCANLYMDSLVKHRRSRGLAASIIHIGHVCDTGYVARM
MILS (2235) ATIANNIGQSNYHCANLYMDSLVAQRRSRGLAASIIHIGYICDTGYVARL
AmphB AAVFGSGGQPGYAAANAYLDALAEHRRSLGLTASSVAWGTWGEVGMATDP
mFAS pig (2023) SCGRGNAGQANYGFANSAMERICEKRRHDGLPGLAVQWGAIGDVGVVLET
mFAS rat (2017) SCGRGNAGQSNYGFANSTMERICEQRRHDGLPGLAVQWGAIGDVGIILEA
2401 2450
KR KR
5A B 5A5B 5B
C S SS S S
TENS (2392) VDDTKVQMSLGTTRVMSVSETDVHHAFAEAVRGGQPDSRSGSHNIIMGIE
DMBS (2387) VDDNRIQSNIATMRAMRLSETDVHHAFAQAVRGGQLDSRSGSYNIIMGIE
MILS (2385) GDDAKVHSNRDVMRATTLSETDVHHAFAEAVRGGSPGSPIGSYNIIMGID
AmphB (337) EVHDRLVRQGVLAMEPPEHALGALDQMLNDDTAAAPITMDWEMFAPAFTN
mFAS pig (2073) MGTNDTVIGGTLPQRIASCLEVLDLFLSQPHPVLS----------
mFAS rat (2067) MGTNDTVVGGTLPQRISSCMEVLDLFLNQPHAVLS----------

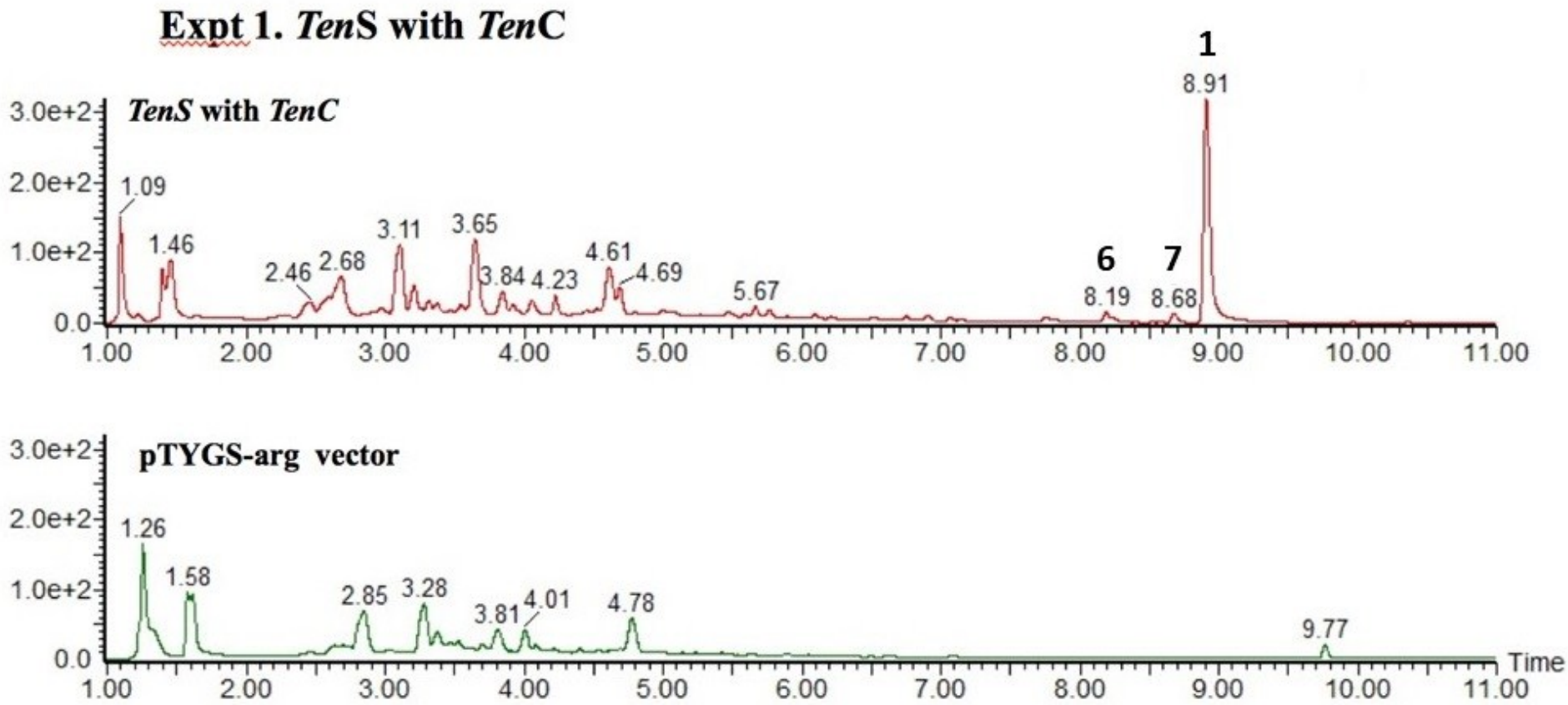


**** = 'active site'

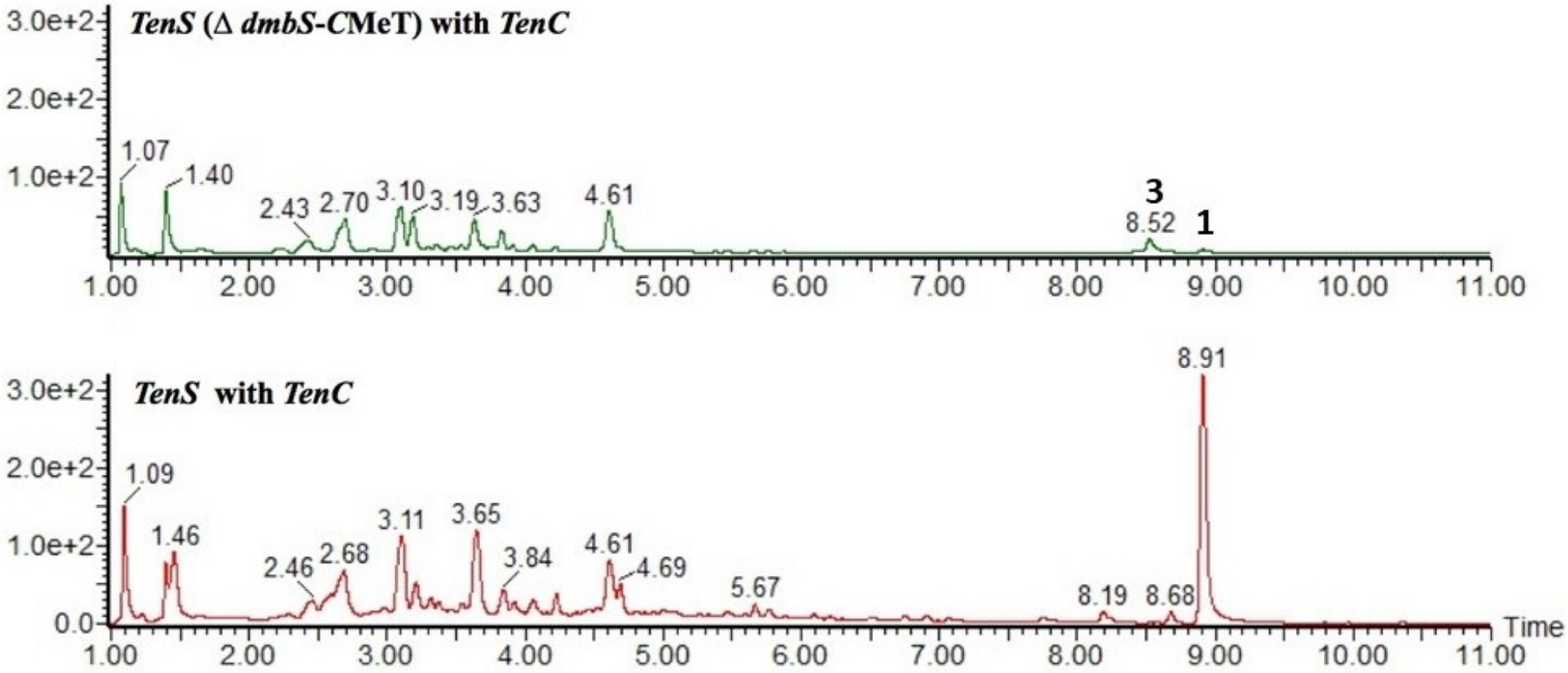
Identity within PKS-NRPS
Identity within mFAS
RED Identity between PKS-NRPS and mFAS

C = Cofactor binding
S = Substrate binding
B = Mutated Buried Residue

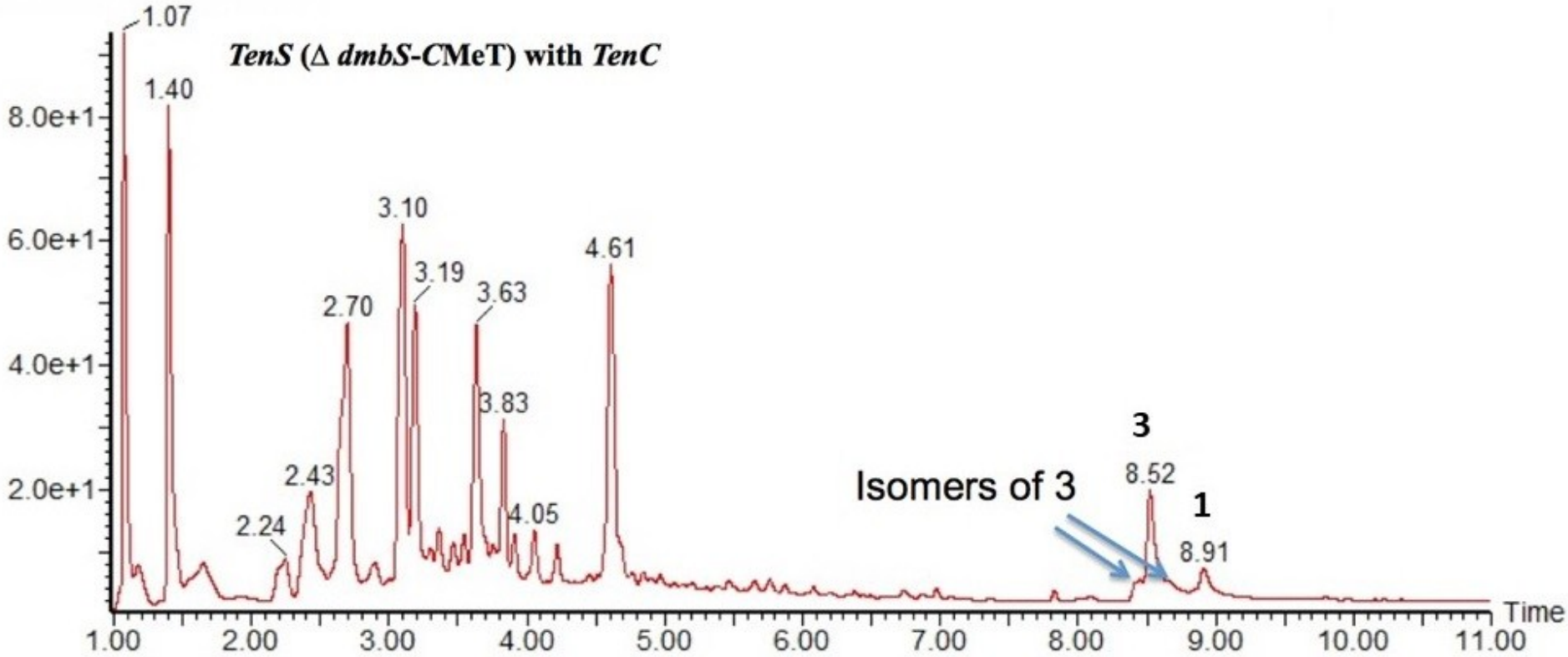
3. LCMS chromatograms



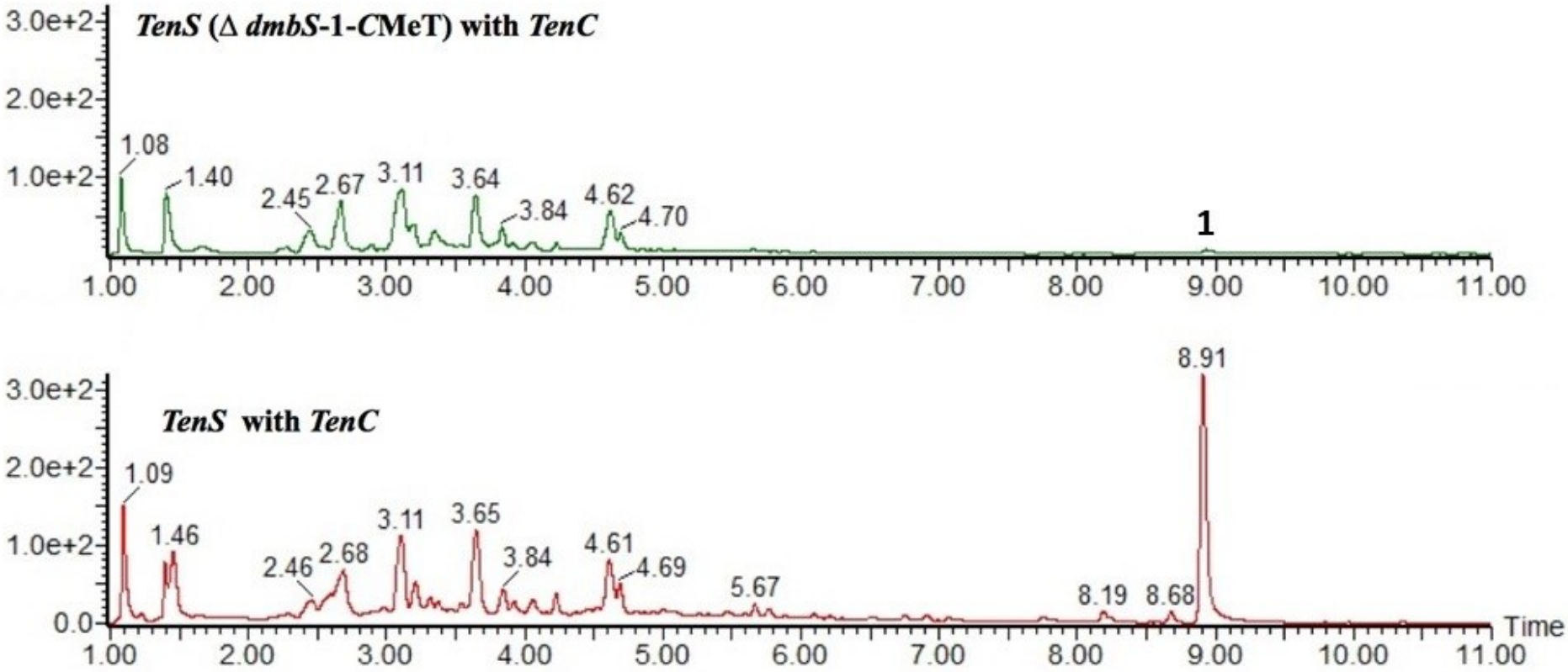
Expt 3. *Ten*S (Δ *dmb*S-*C*MeT) with *Ten*C



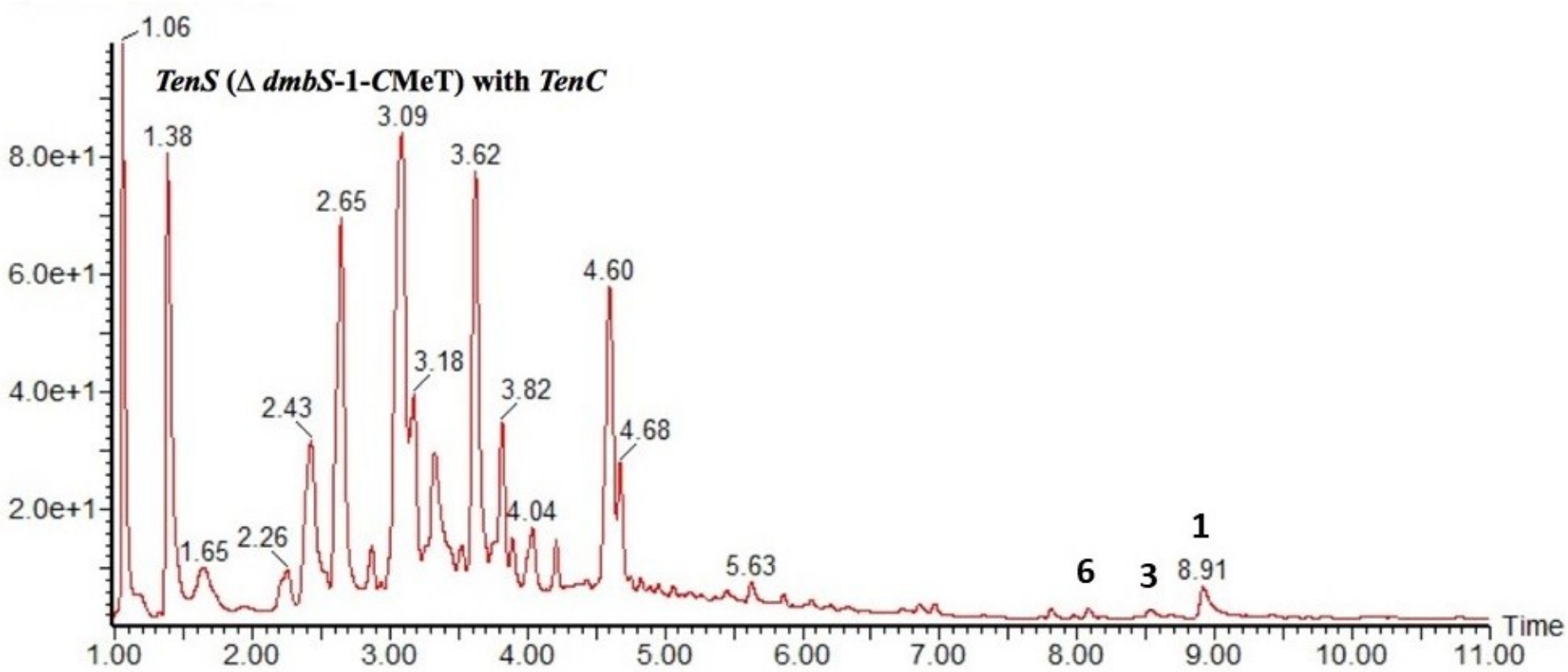
Amplified HPLC profile of Expt 3.



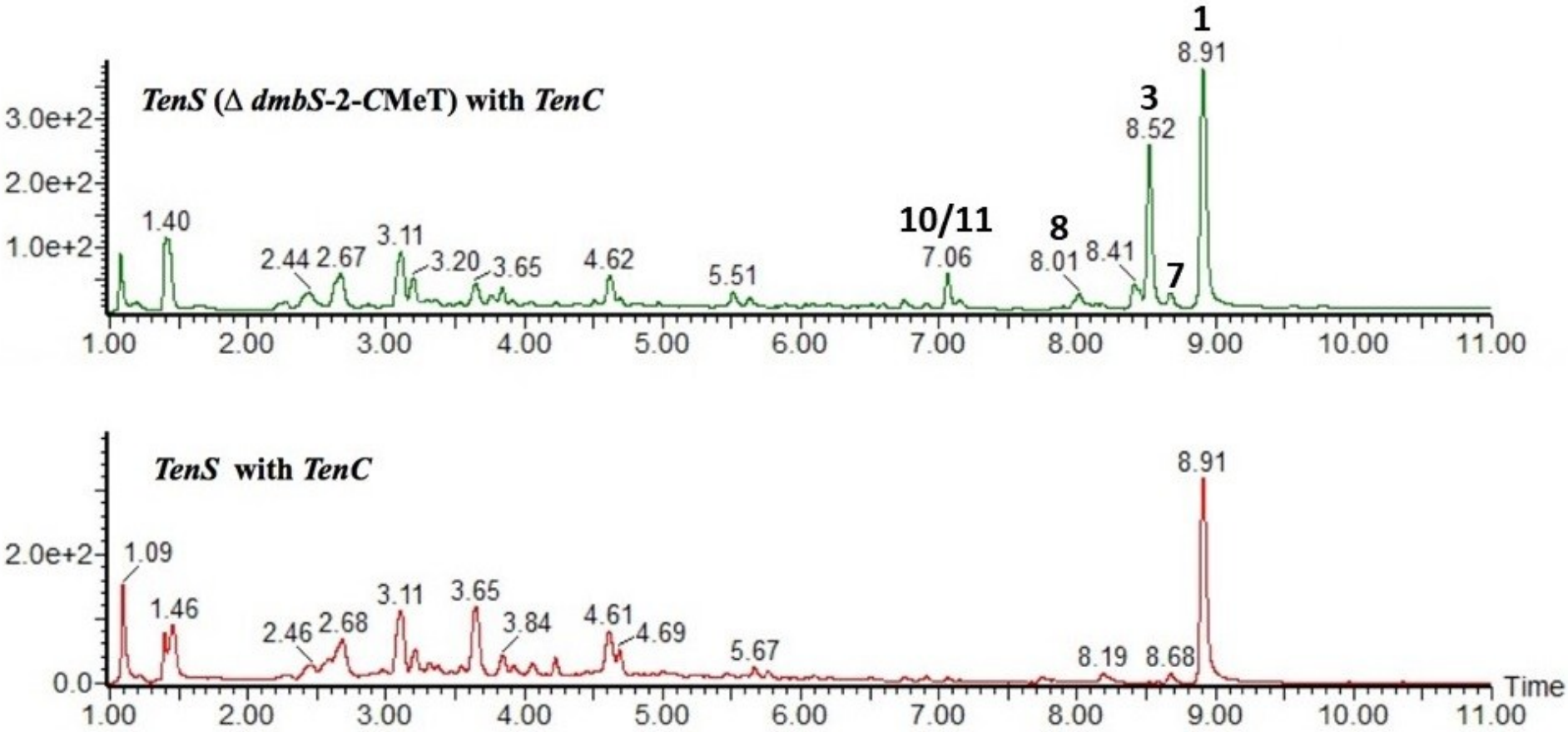
Expt 4. *Ten*S (Δ *dmb*S-1-*C*MeT) with *Ten*C



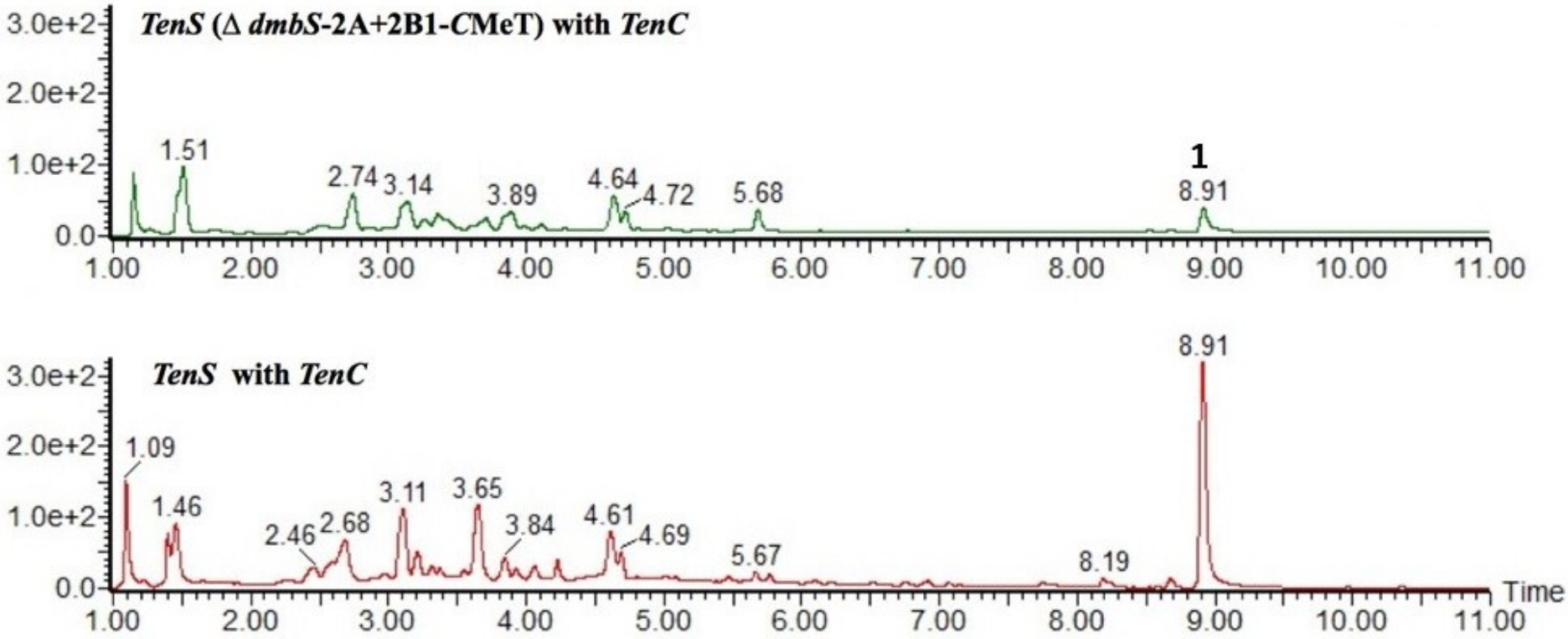
Amplified HPLC profile of Expt 4.



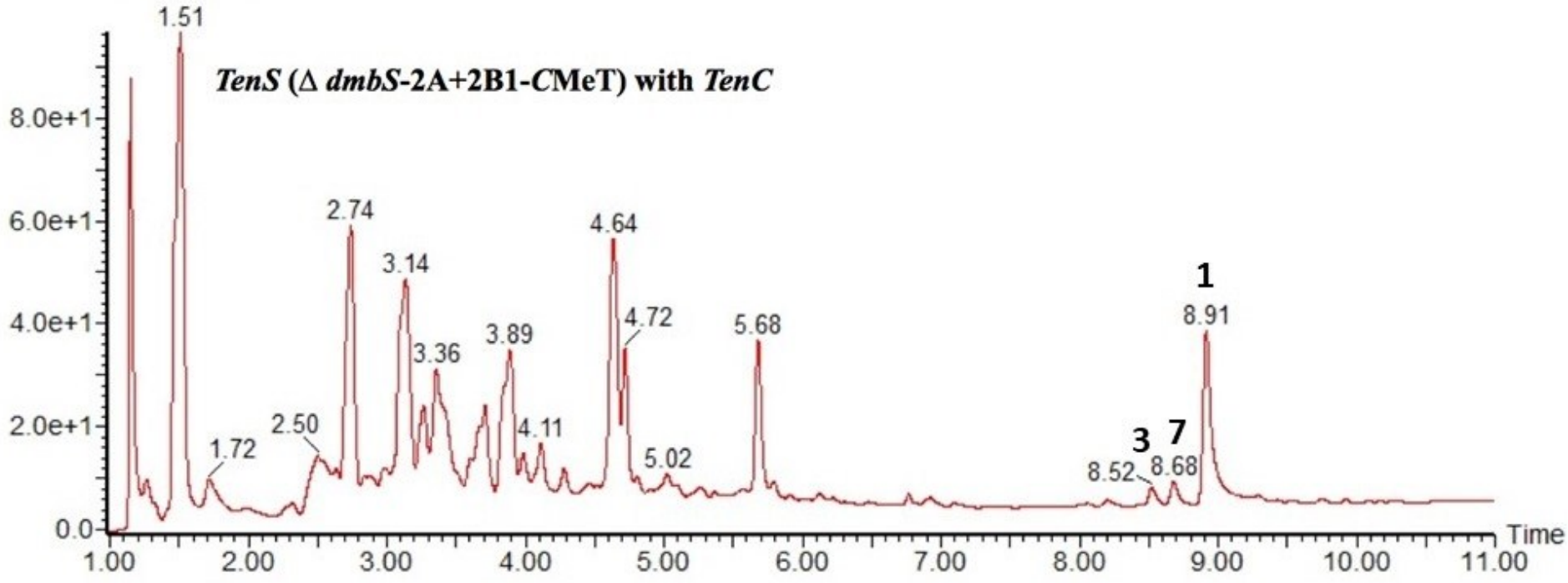
Expt 5. *Ten*S (Δ *dmb*S-2-*C*MeT) with *Ten*C



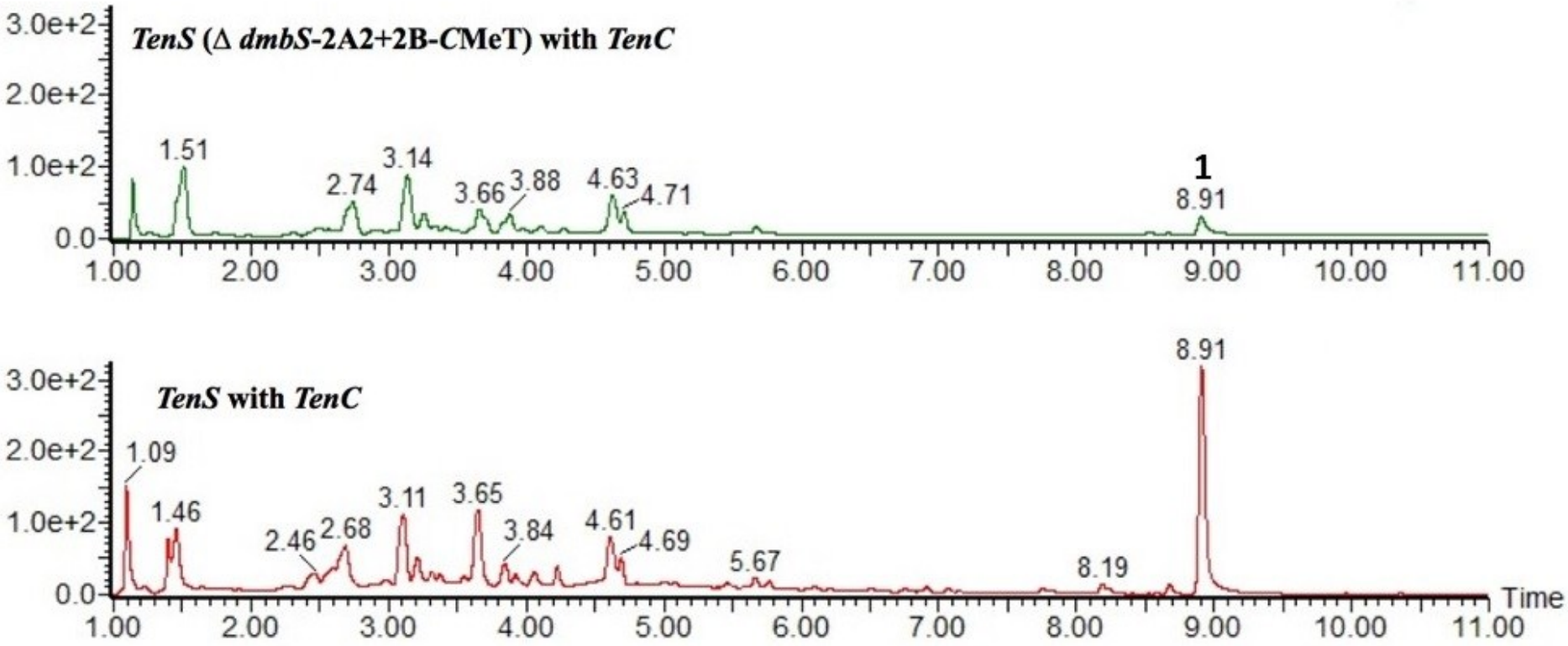
Expt 6. *Ten*S (Δ *dmb*S-2A+2B1-*C*MeT) with *Ten*C



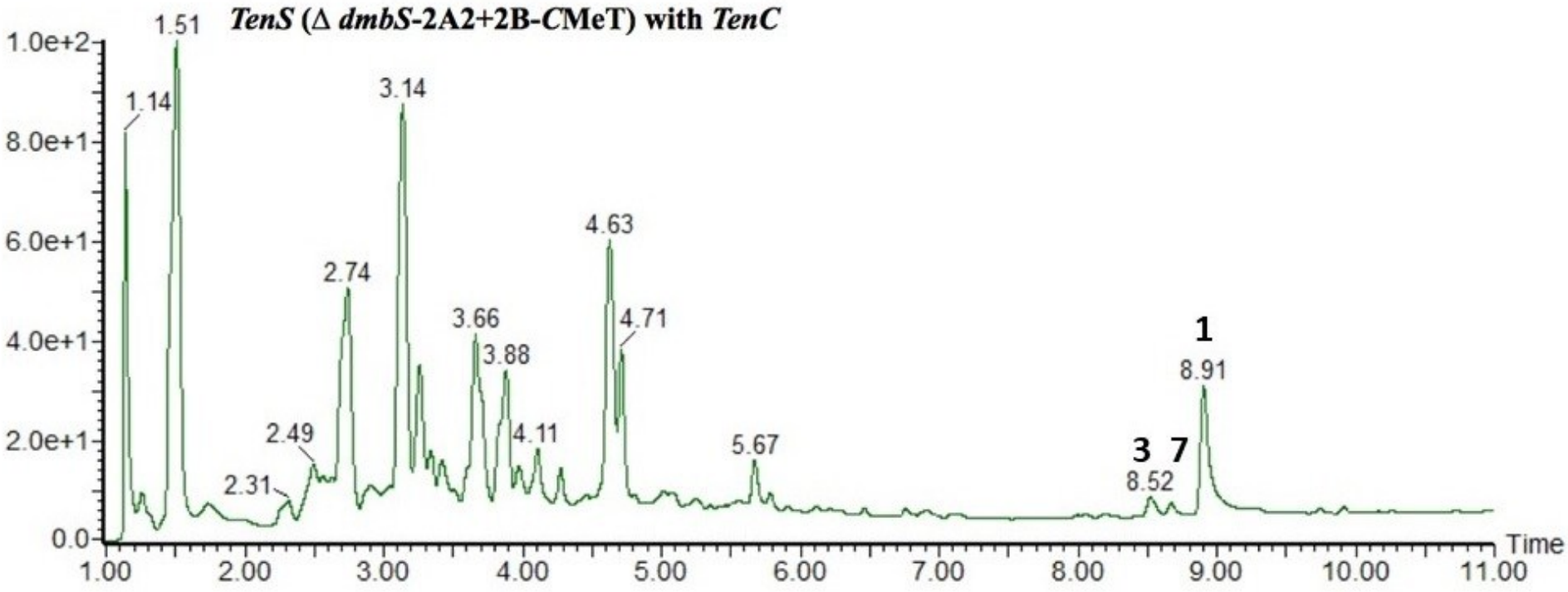
Amplified HPLC profile of Expt 6.



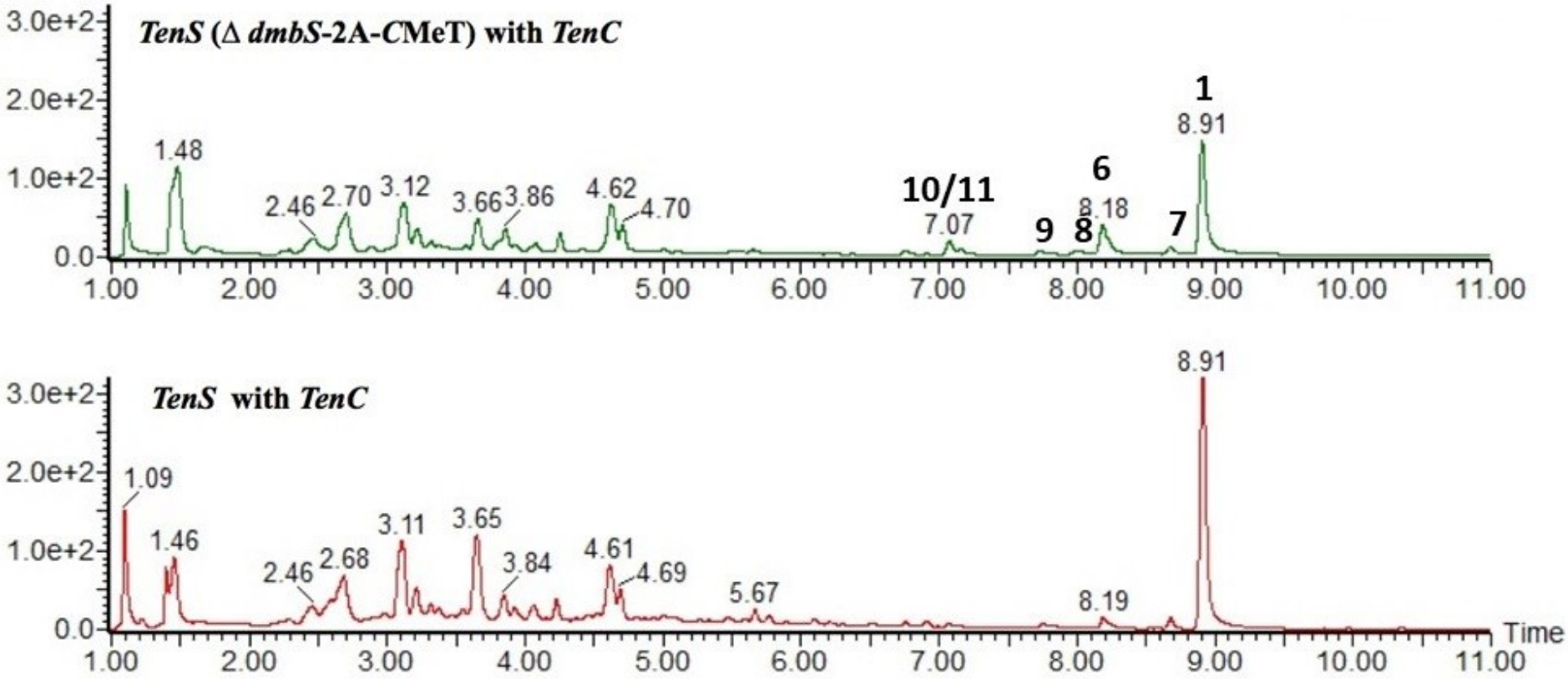
Expt 7. *Ten*S (Δ *dmb*S-2A2+2B-*C*MeT) with *Ten*C



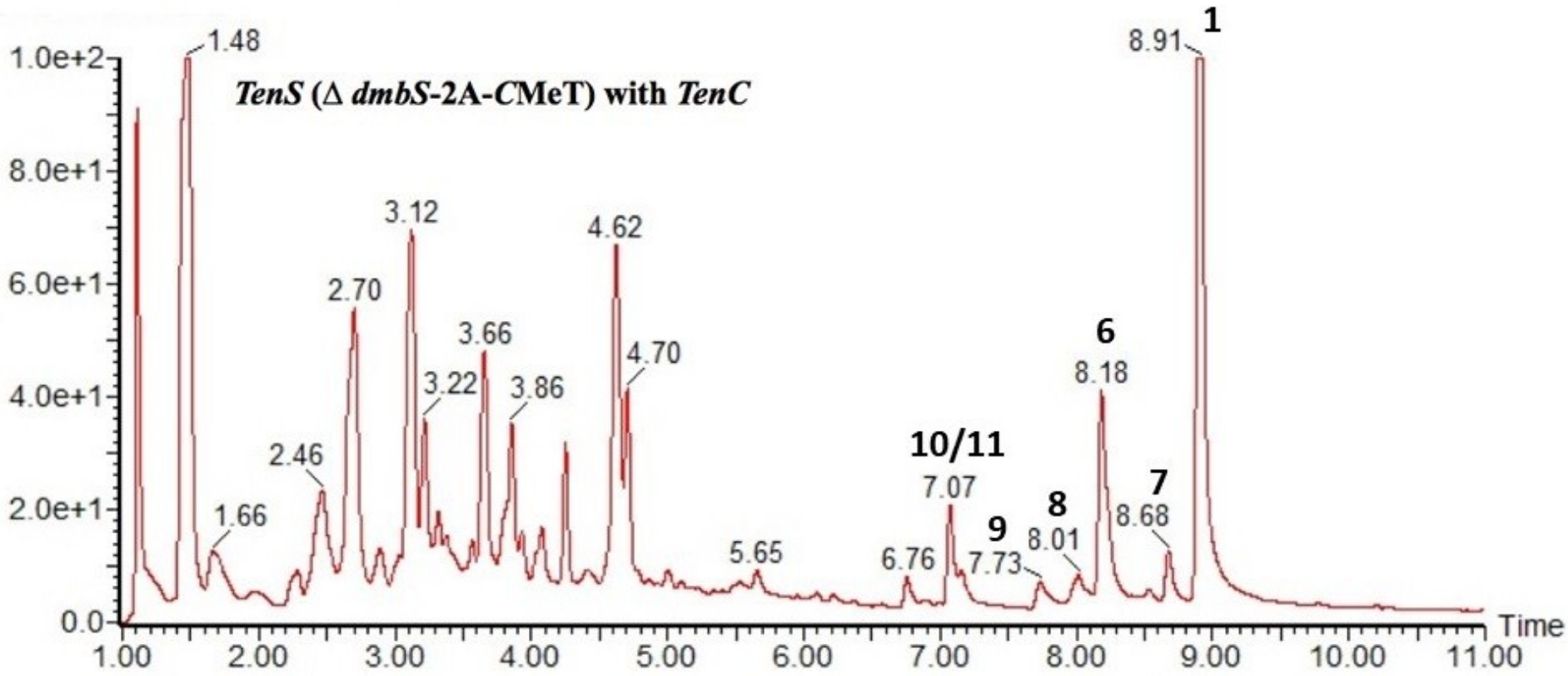
Amplified HPLC profile of Expt 7.



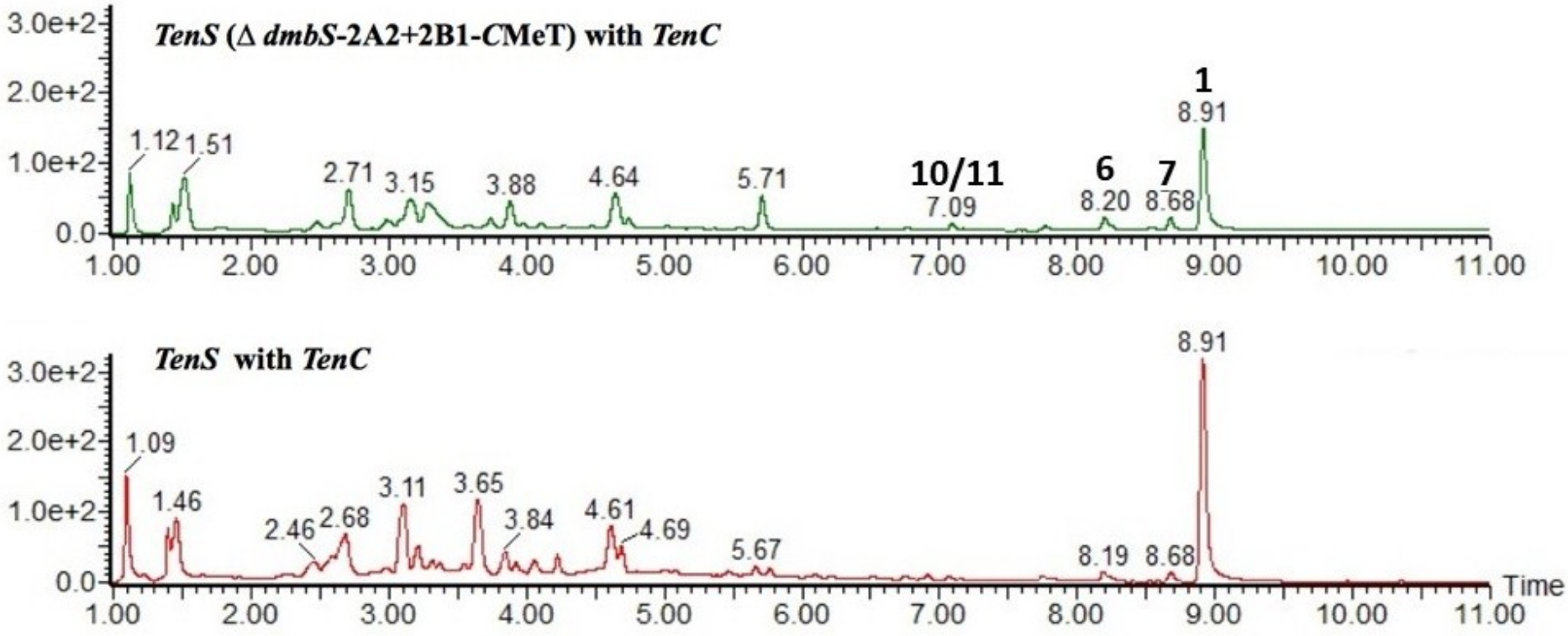
Expt 8. *Ten*S (Δ *dmb*S-2A-*C*MeT) with *Ten*C



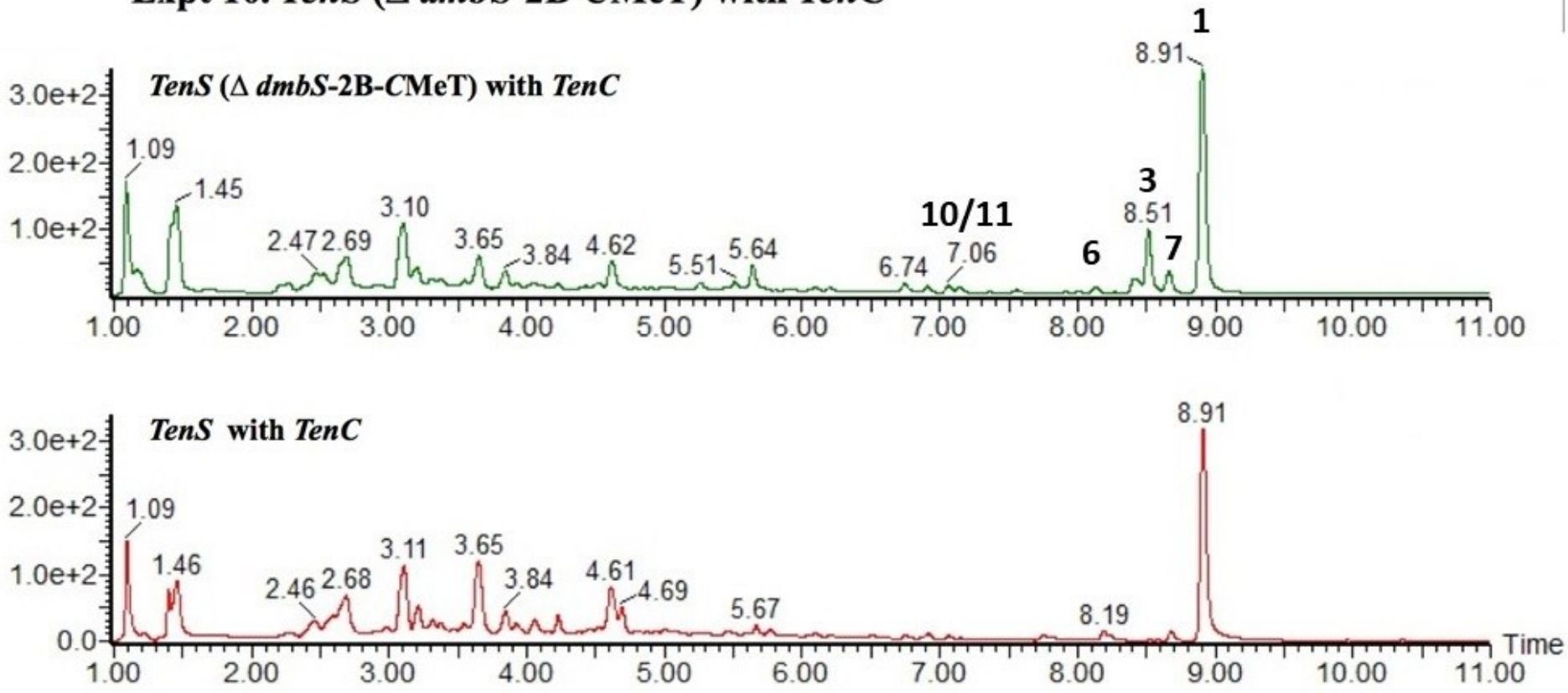
Expansion of Expt 8.



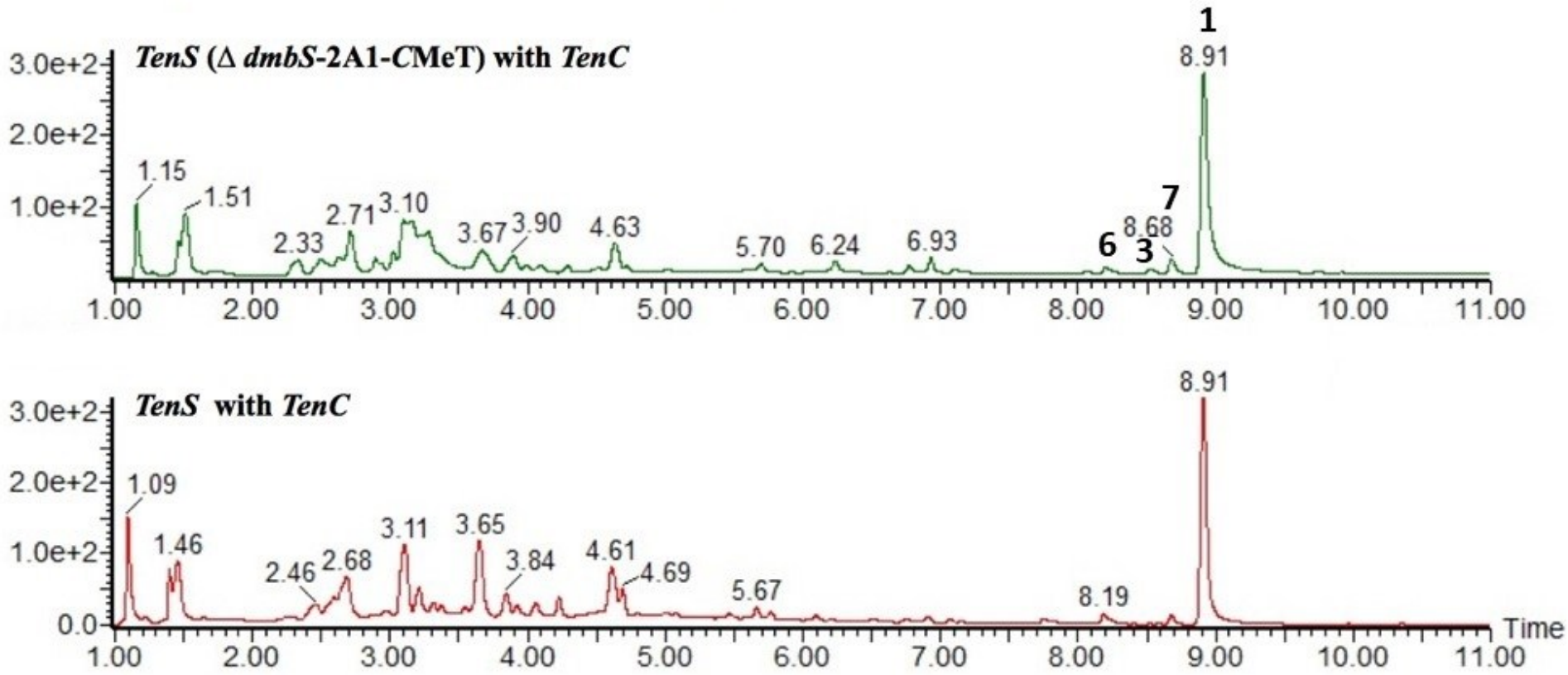
Expt 9. *Ten*S (Δ *dmb*S-2A2+2B1-*C*MeT) with *Ten*C



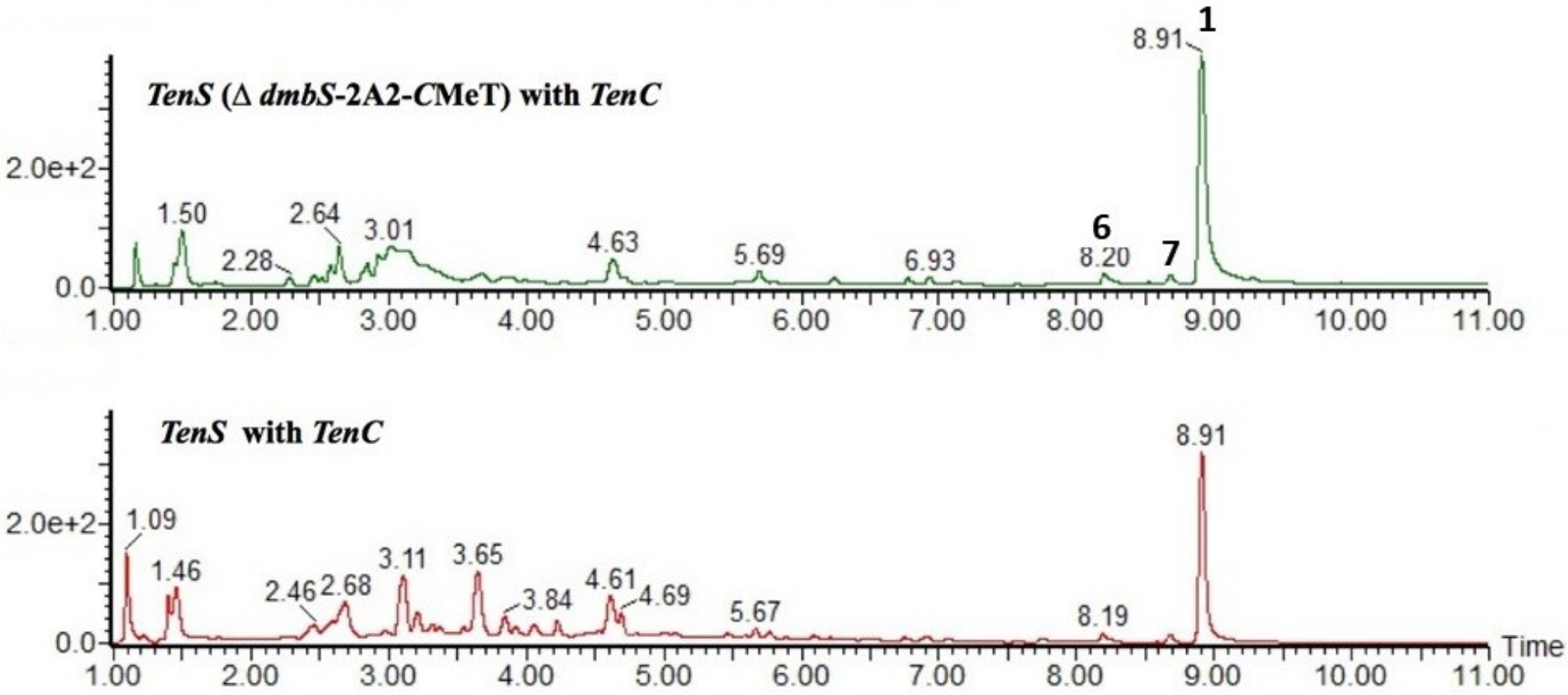
Expt 10. *Ten*S (Δ *dmb*S-2B-*C*MeT) with *Ten*C



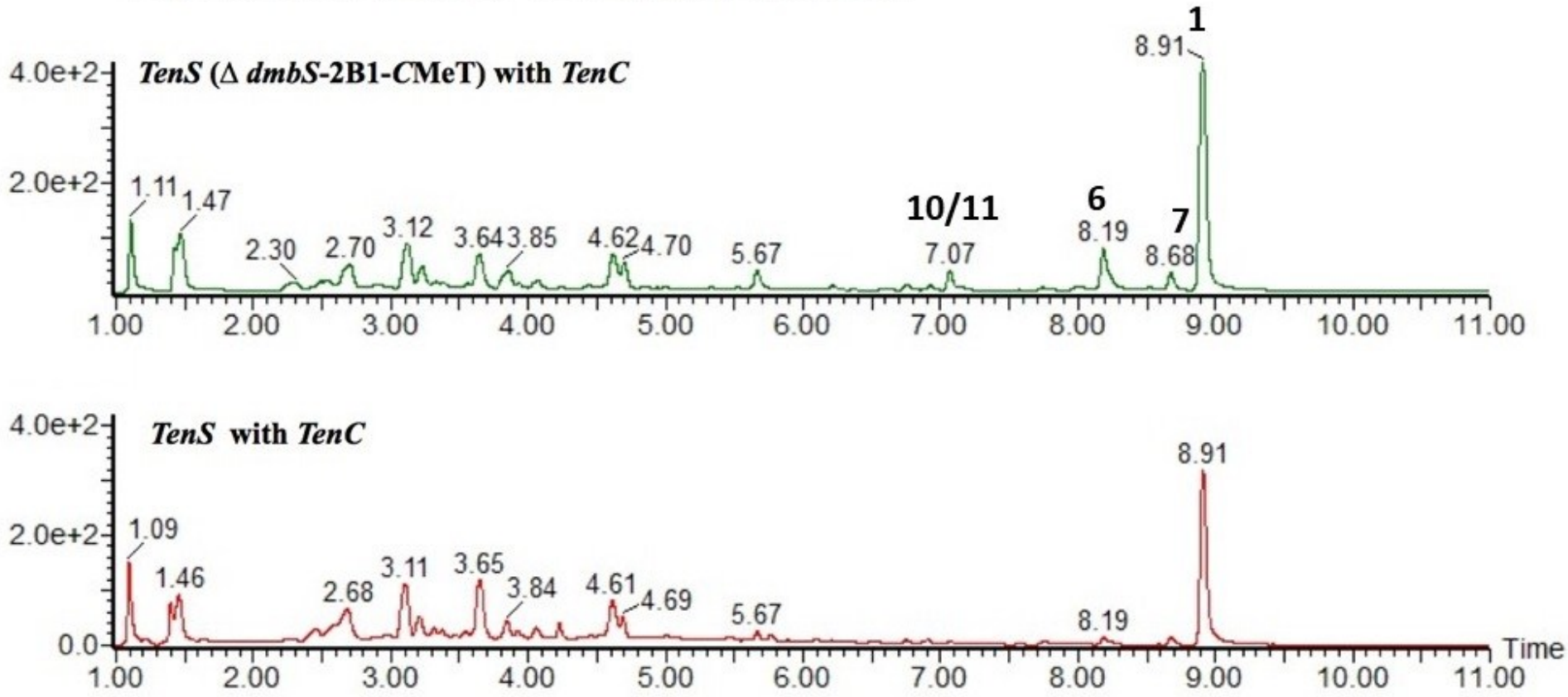
Expt 11. *Ten*S (Δ *dmb*S-2A1-*C*MeT) with *Ten*C



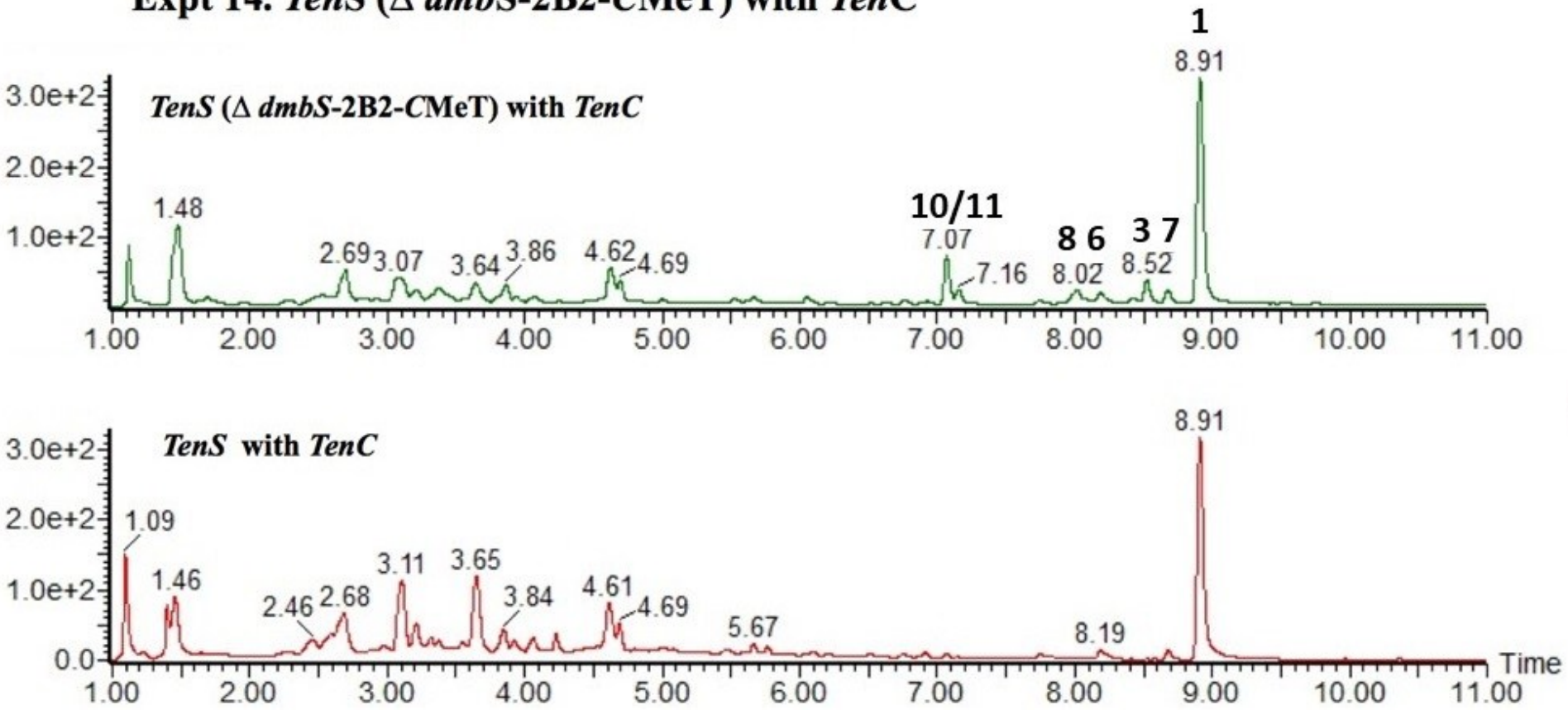
Expt 12. *Ten*S (Δ *dmb*S-2A2-*C*MeT) with *Ten*C



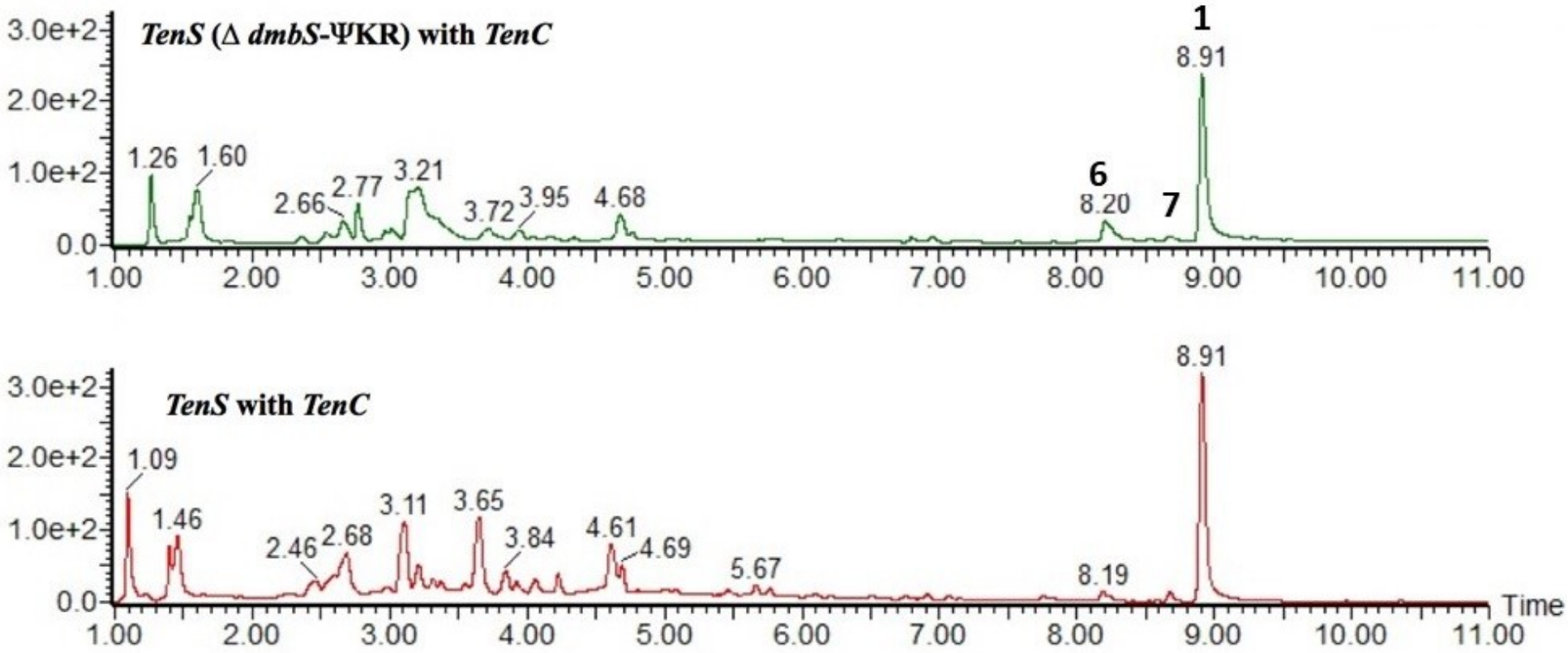
Expt 13. *Ten*S (Δ *dmb*S-2B1-*C*MeT) with *Ten*C



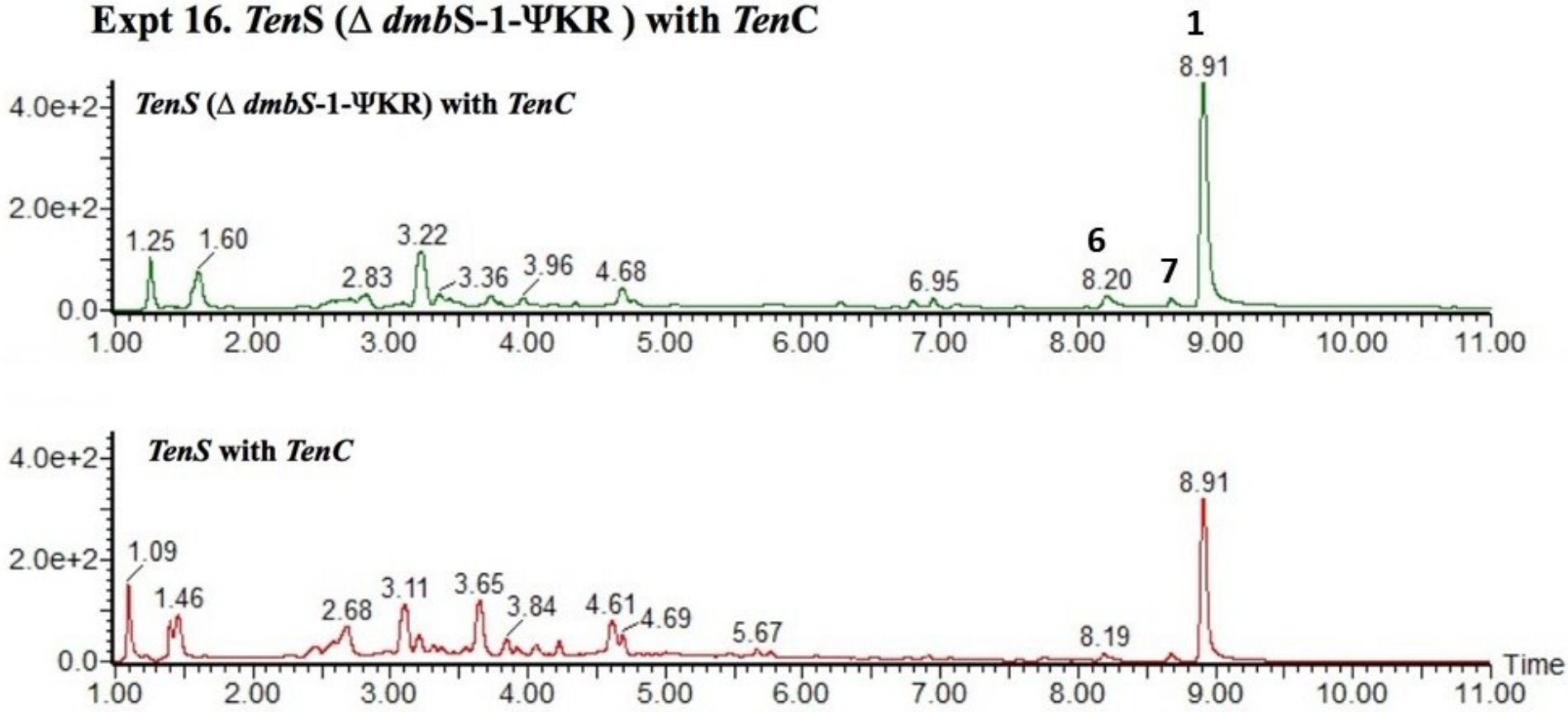
Expt 14. *Ten*S (Δ *dmb*S-2B2-*C*MeT) with *Ten*C



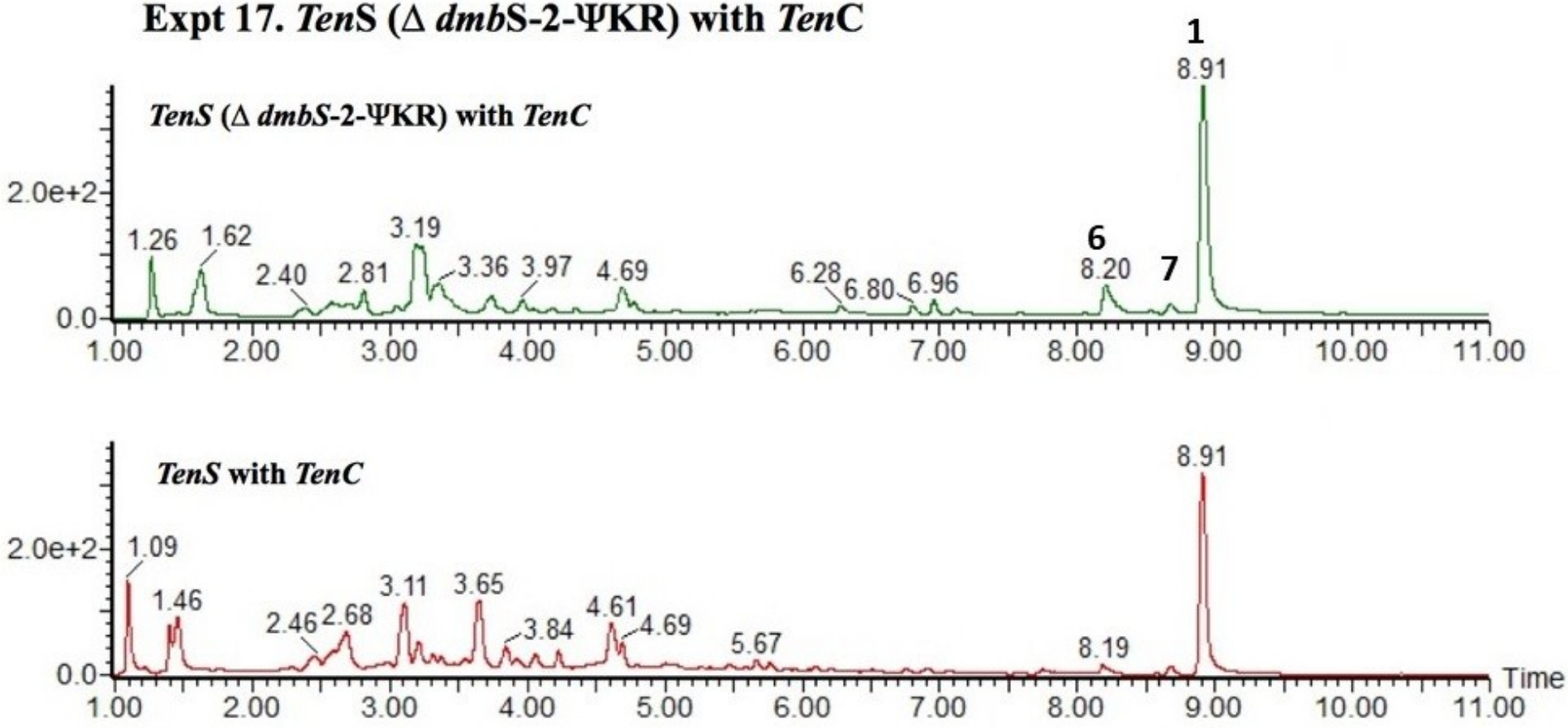
Expt 15. *Ten*S (Δ *dmb*S-ΨKR) with *Ten*C



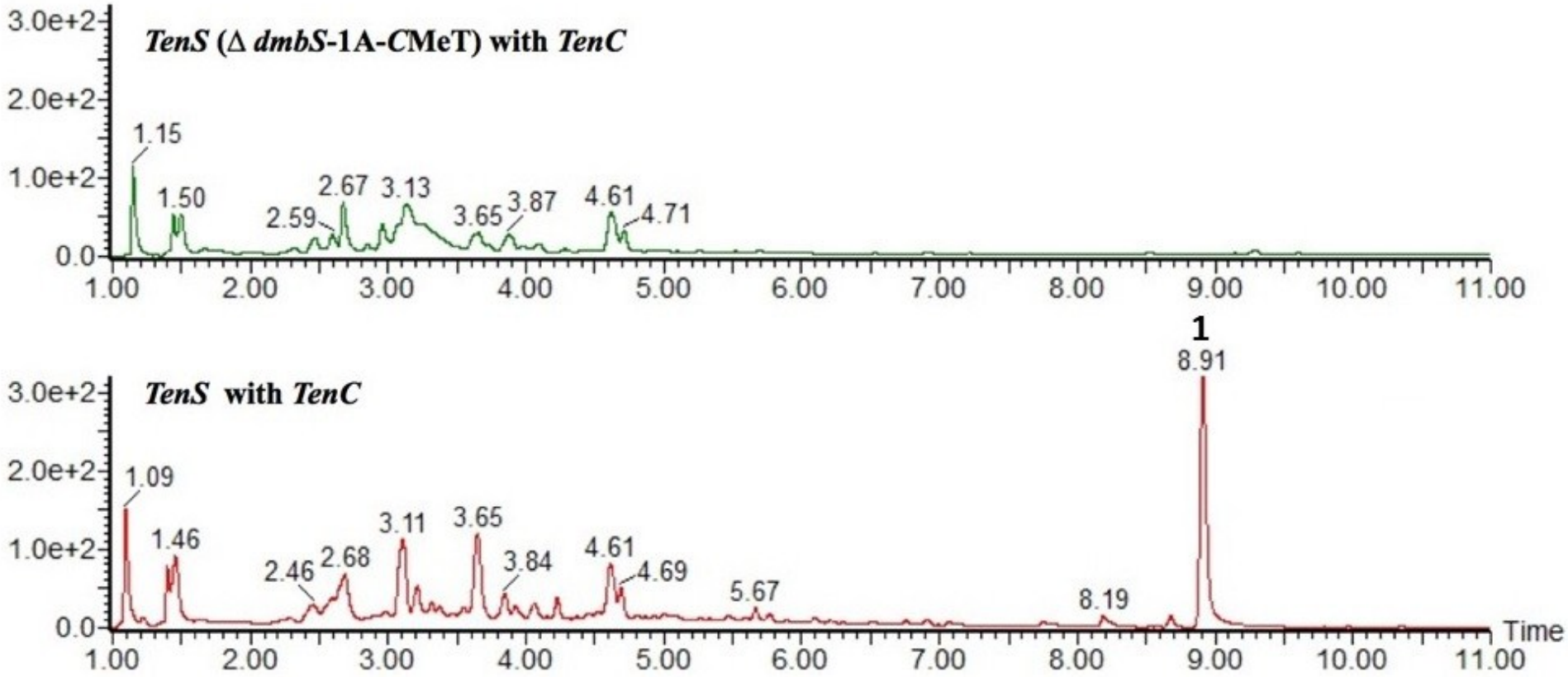
Expt 16. *Ten*S (Δ *dmb*S-1-ΨKR) with *Ten*C



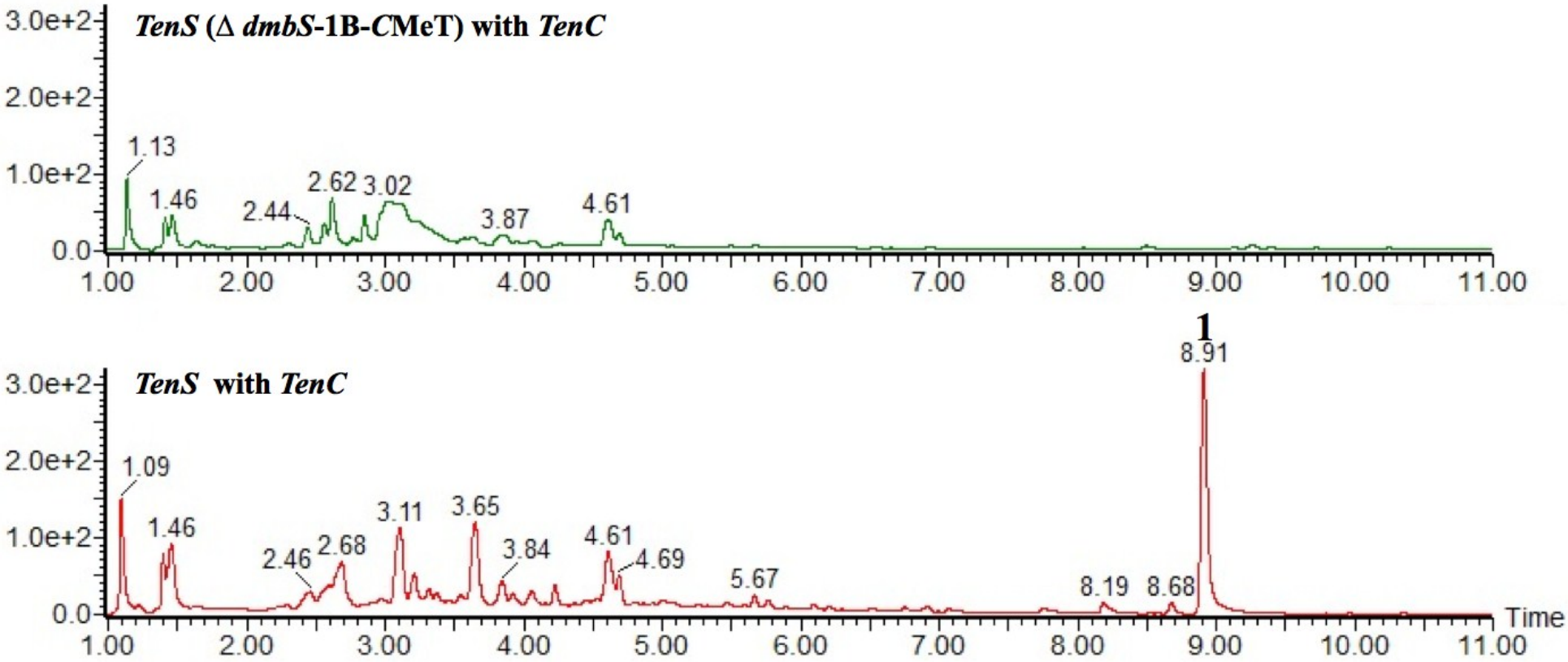
Expt 17. *Ten*S (Δ *dmb*S-2-ΨKR) with *Ten*C



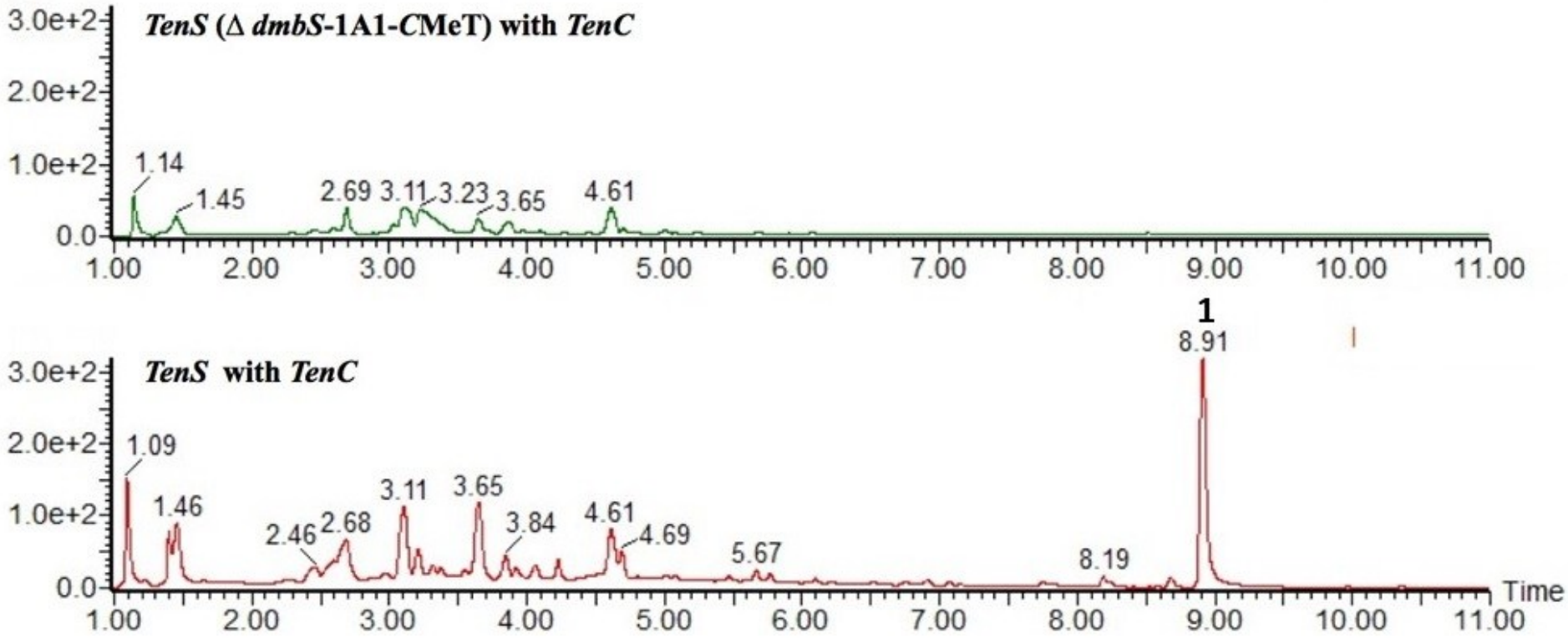
Expt 18. *Ten*S (Δ *dmb*S-1A-*C*MeT) with *Ten*C



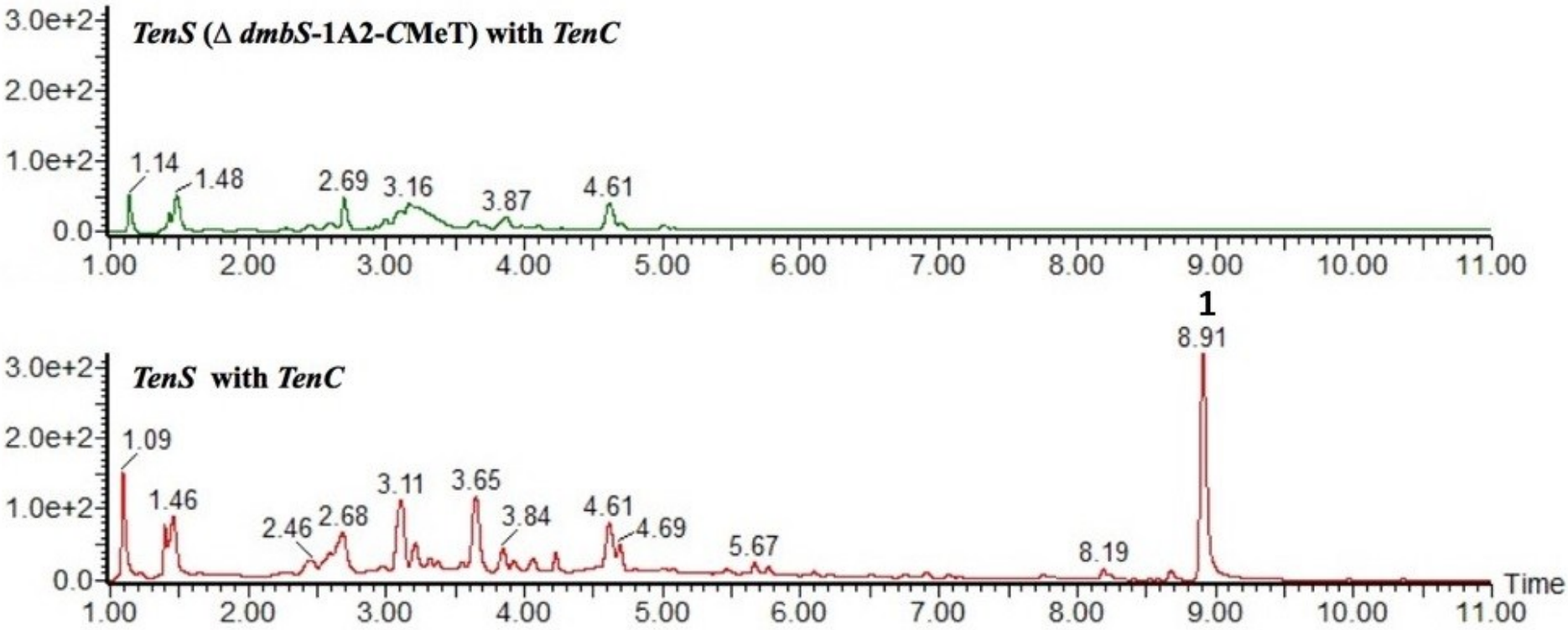
Expt 19. *Ten*S (Δ *dmb*S-1B-*C*MeT) with *Ten*C



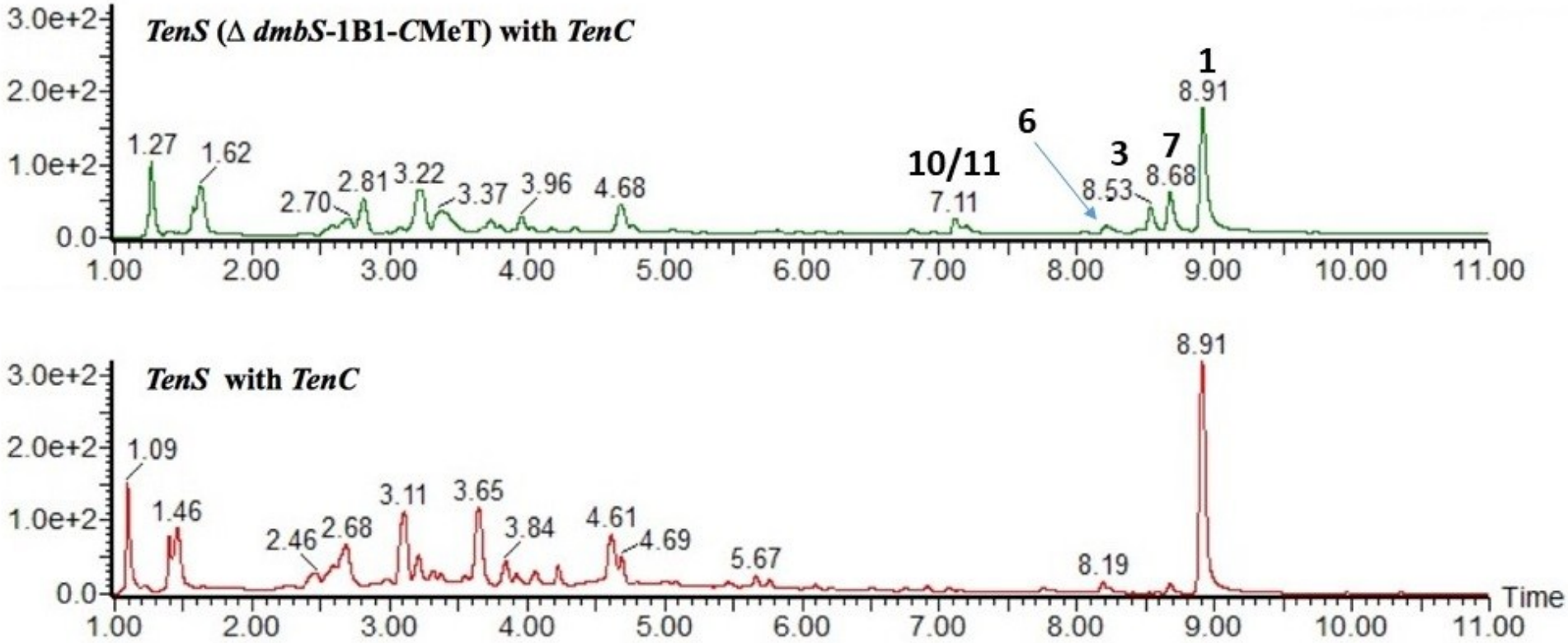
Expt 20. *Ten*S (Δ *dmb*S-1A1-*C*MeT) with *Ten*C



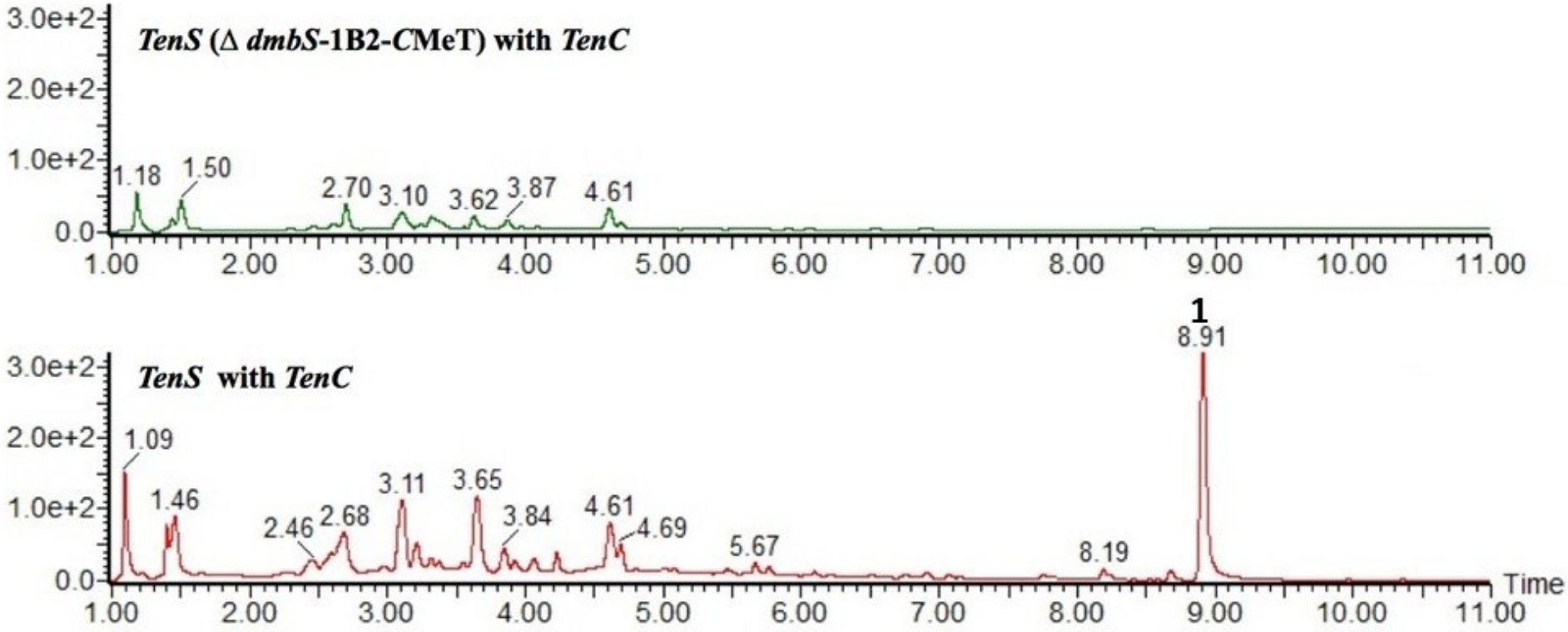
Expt 21. *Ten*S (Δ *dmb*S-1A2-*C*MeT) with *Ten*C



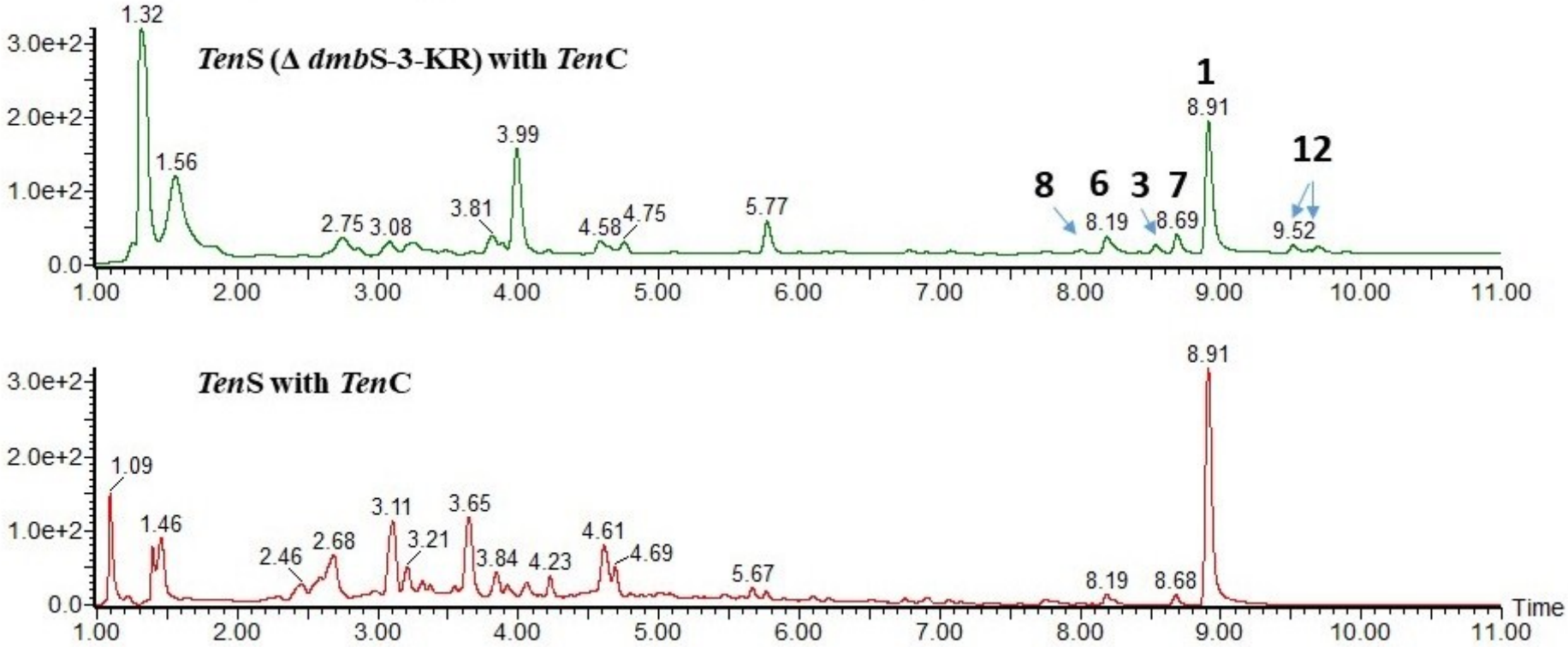
Expt 22. pTYGS-arg-*Ten*C+*Ten*S (Δ *dmb*S-1B1-*C*MeT)



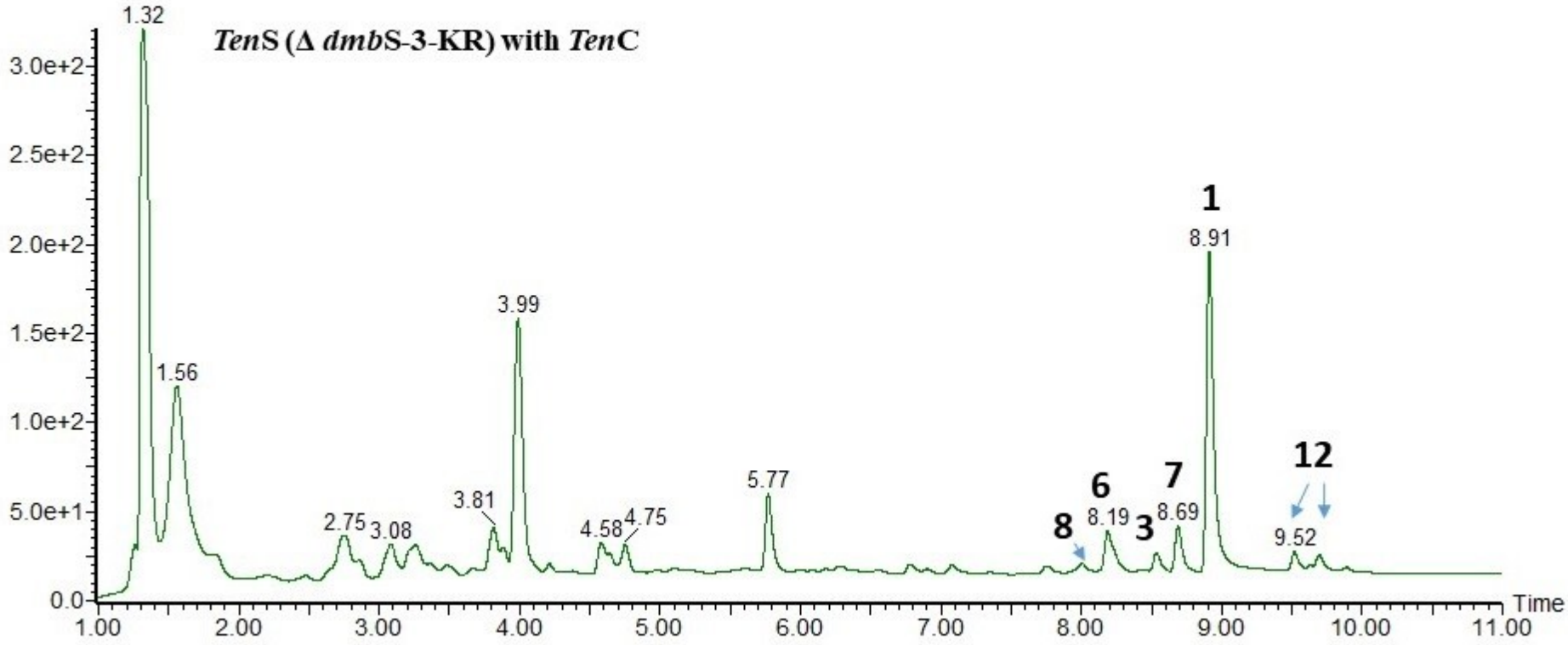
Expt 23. pTYGS-arg-*Ten*C+*Ten*S (Δ *dmb*S-1B2-*C*MeT)



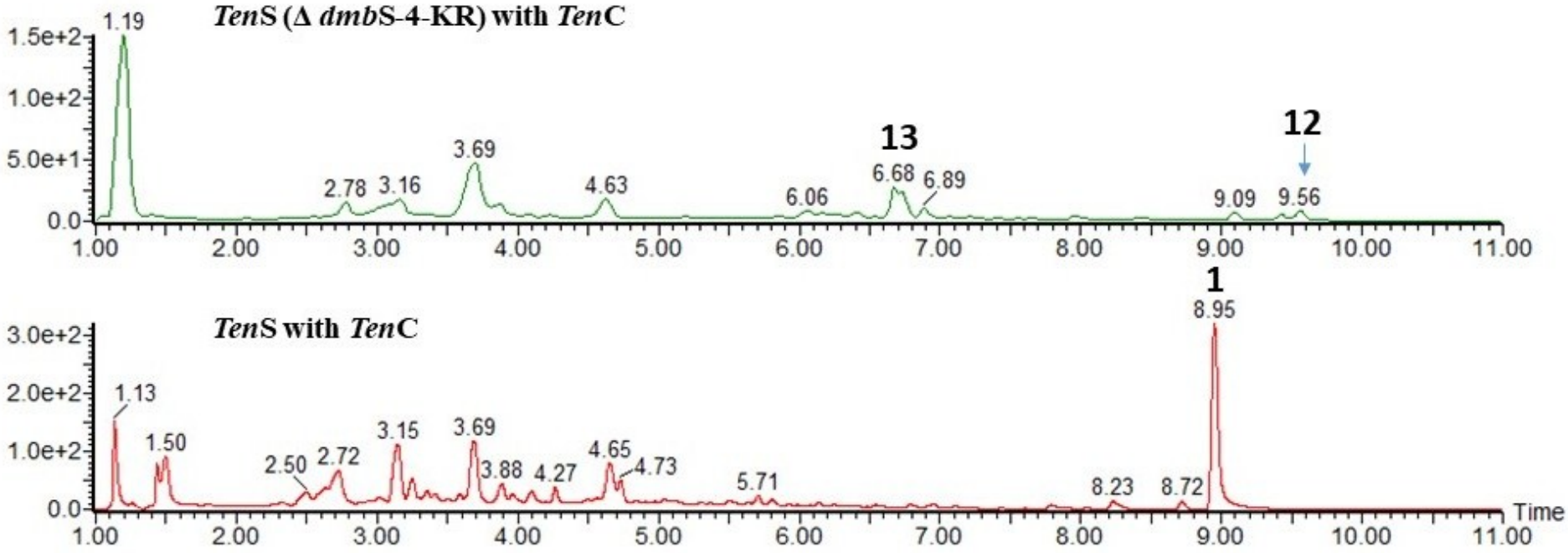
Expt 25. pTYGS-arg-*Ten*C+*Ten*S (Δ *dmb*S-3-KR)



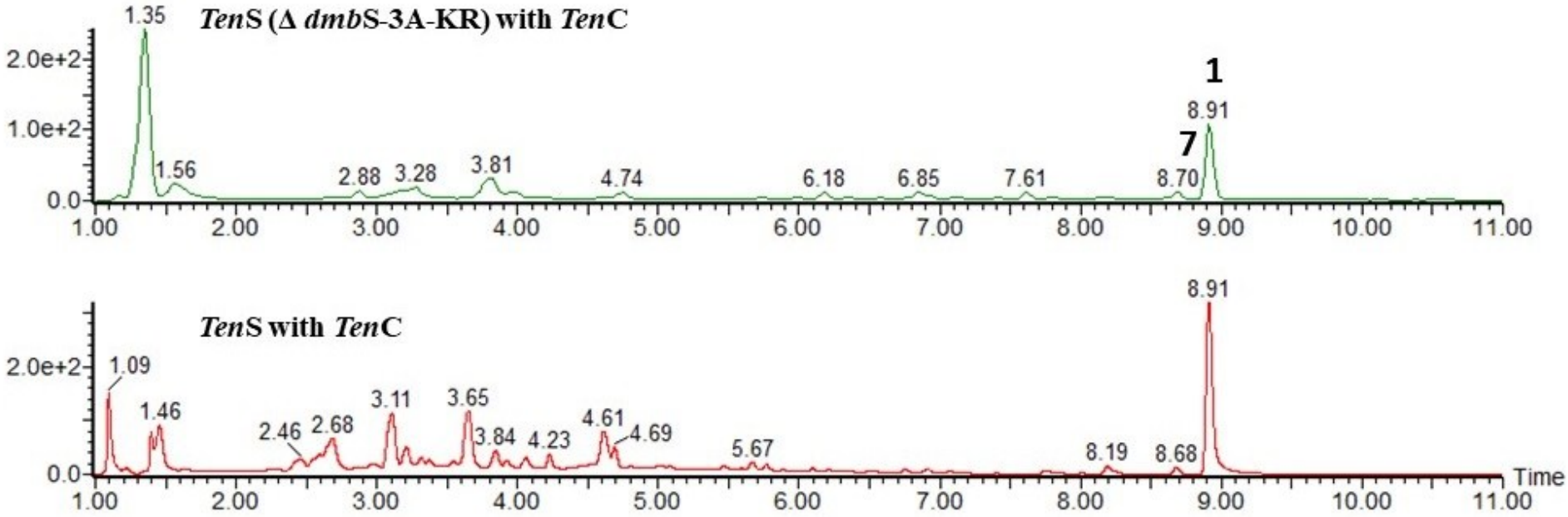
Ampflified HPLC profile of Expt 25.



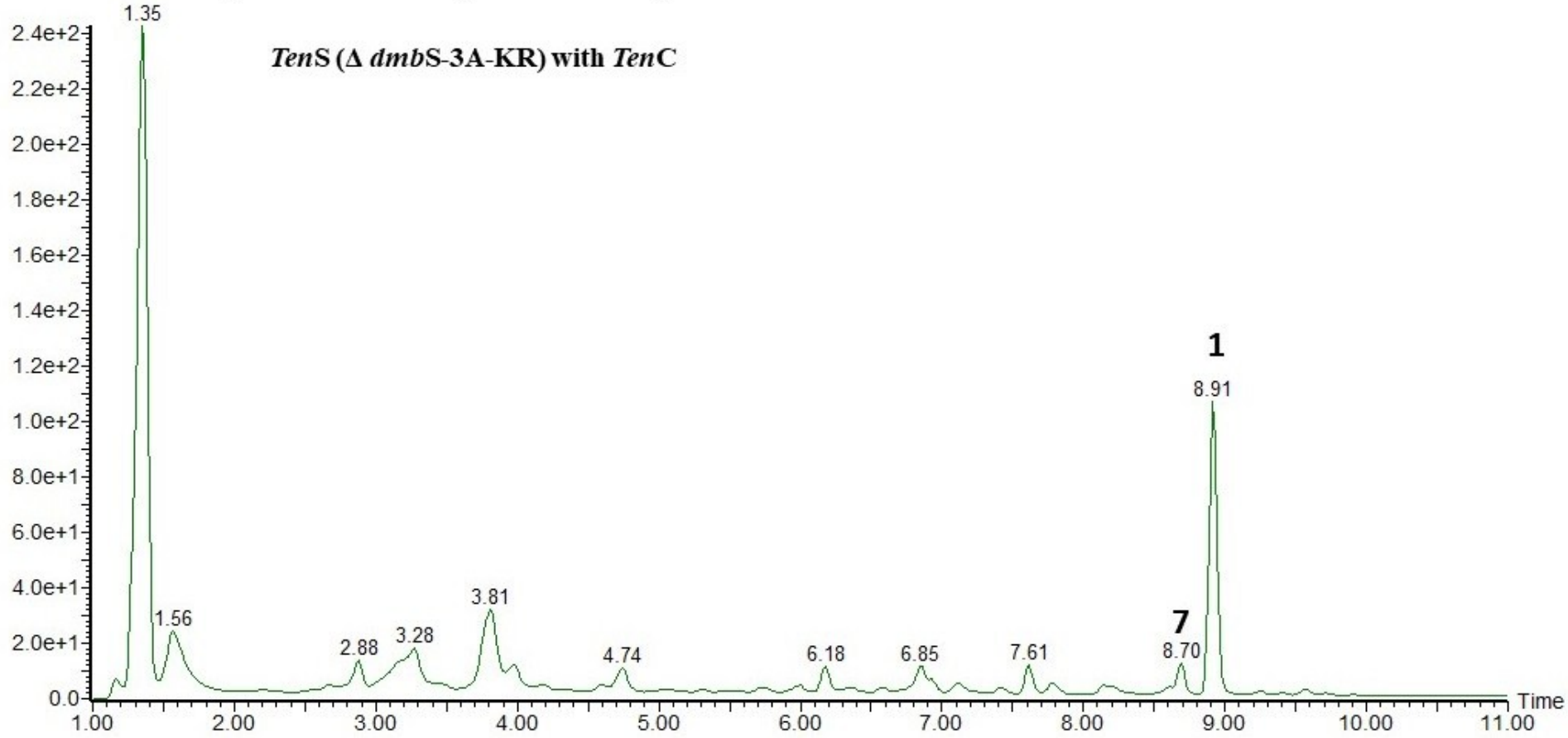
Expt 26. pTYGS-arg-*Ten*C+*Ten*S (Δ *dmb*S-4-KR)



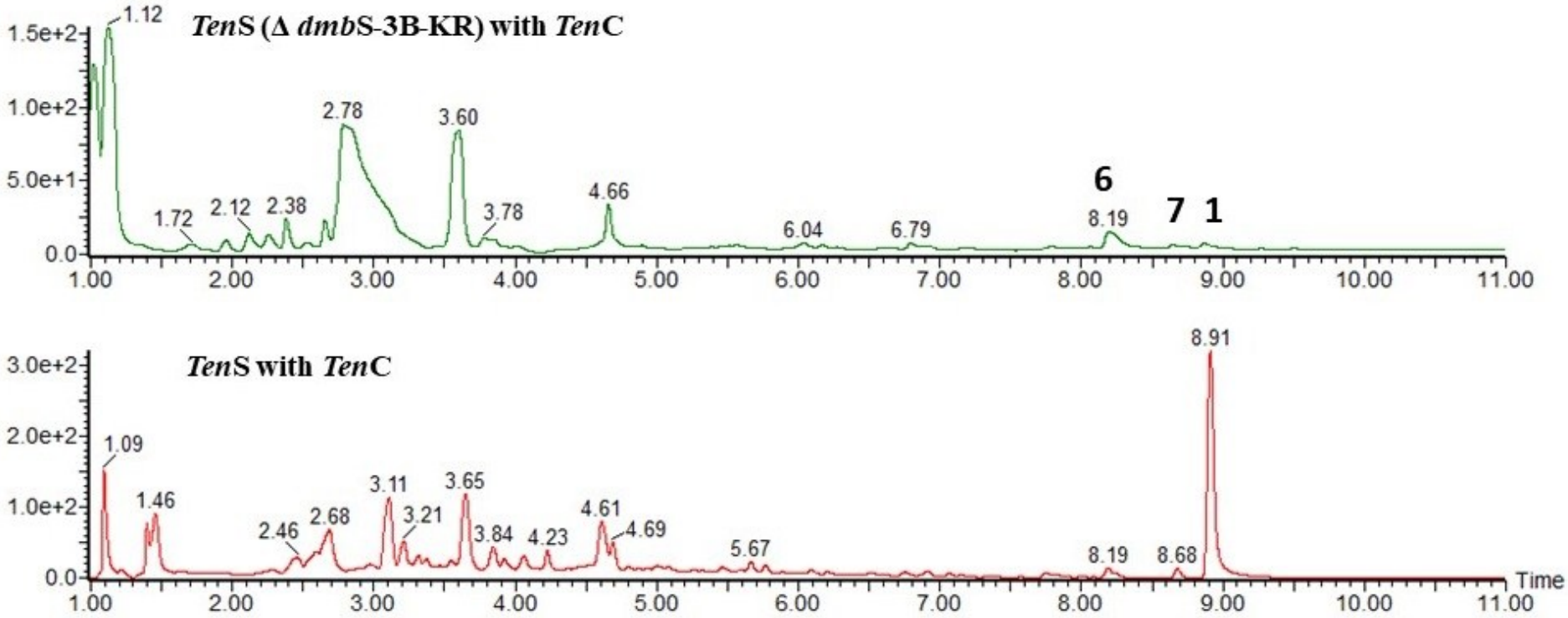
Expt 27. pTYGS-arg-*Ten*C+*Ten*S (Δ *dmb*S-3A-KR)



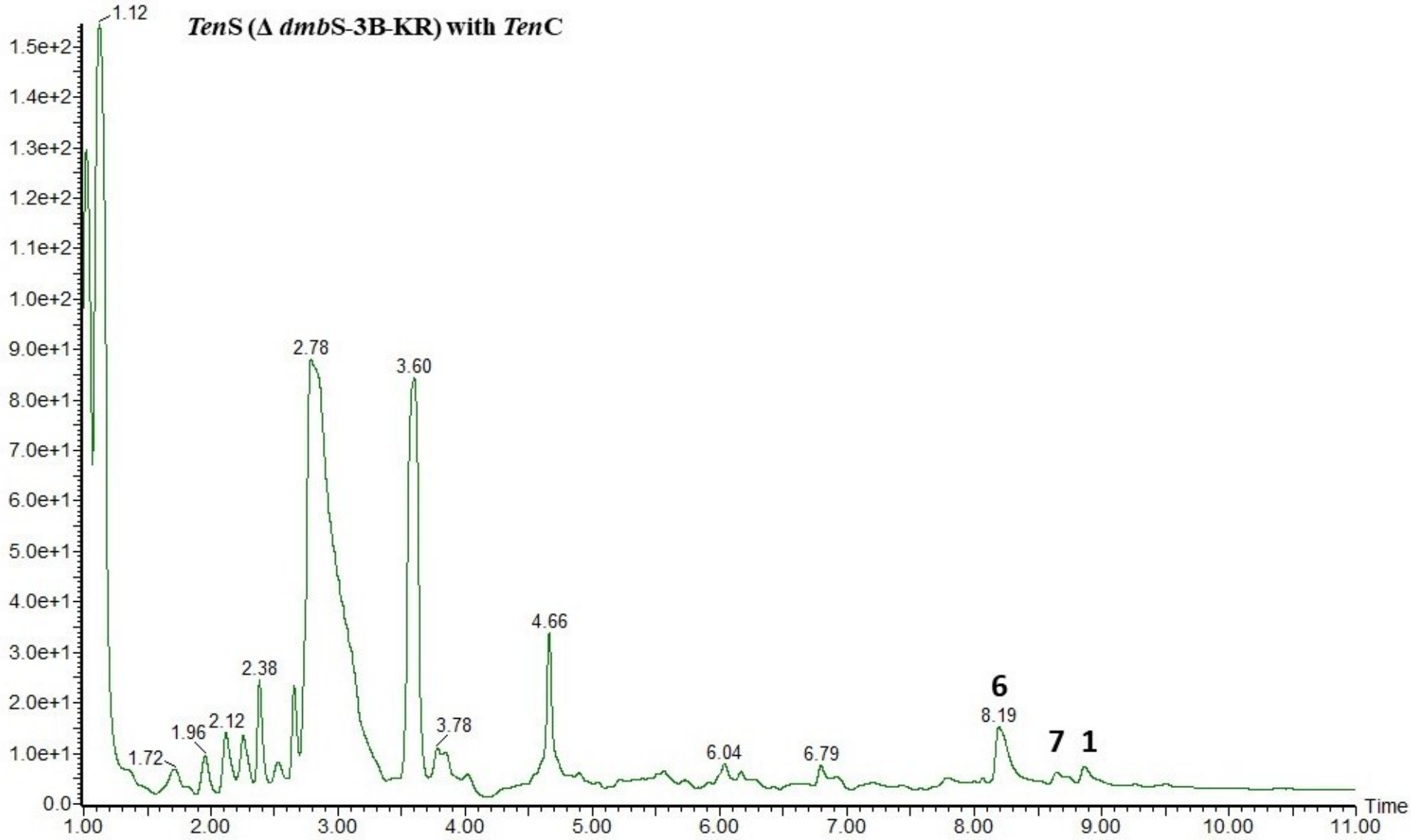
Ampflified HPLC profile of Expt 27.



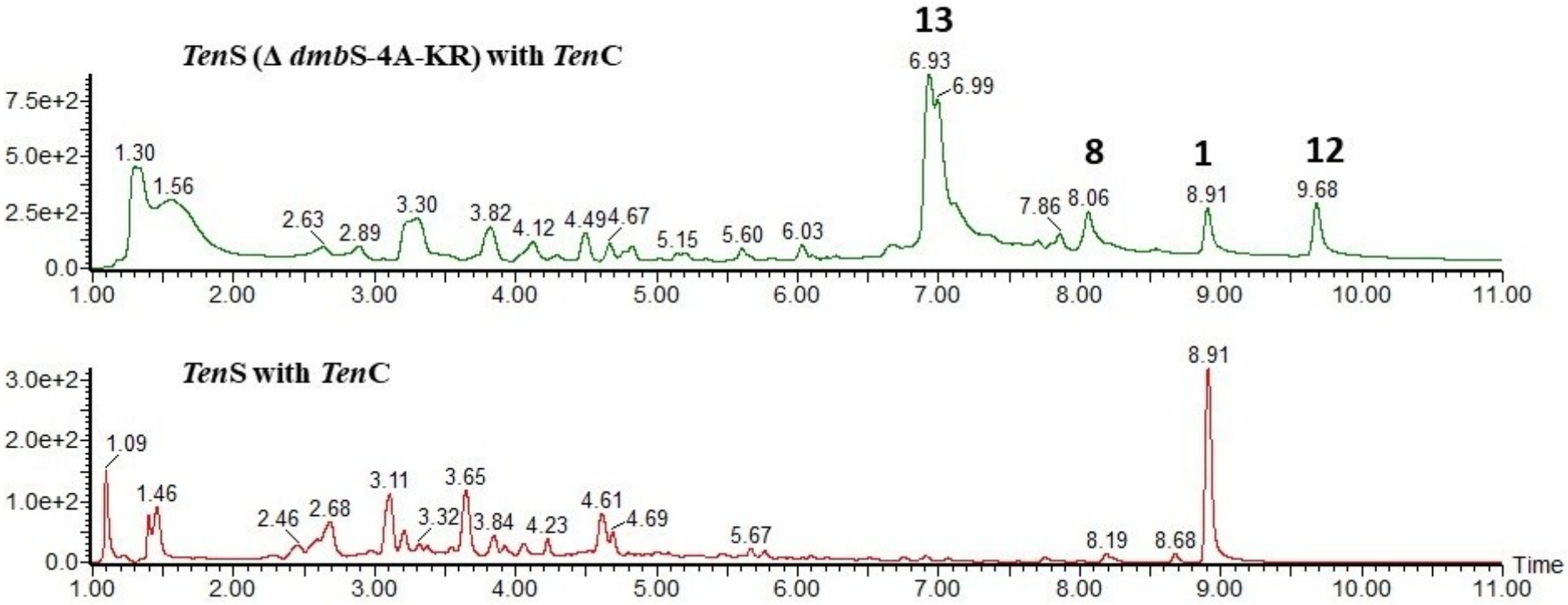
Expt 28. pTYGS-arg-*Ten*C+*Ten*S (Δ *dmb*S-3B-KR)



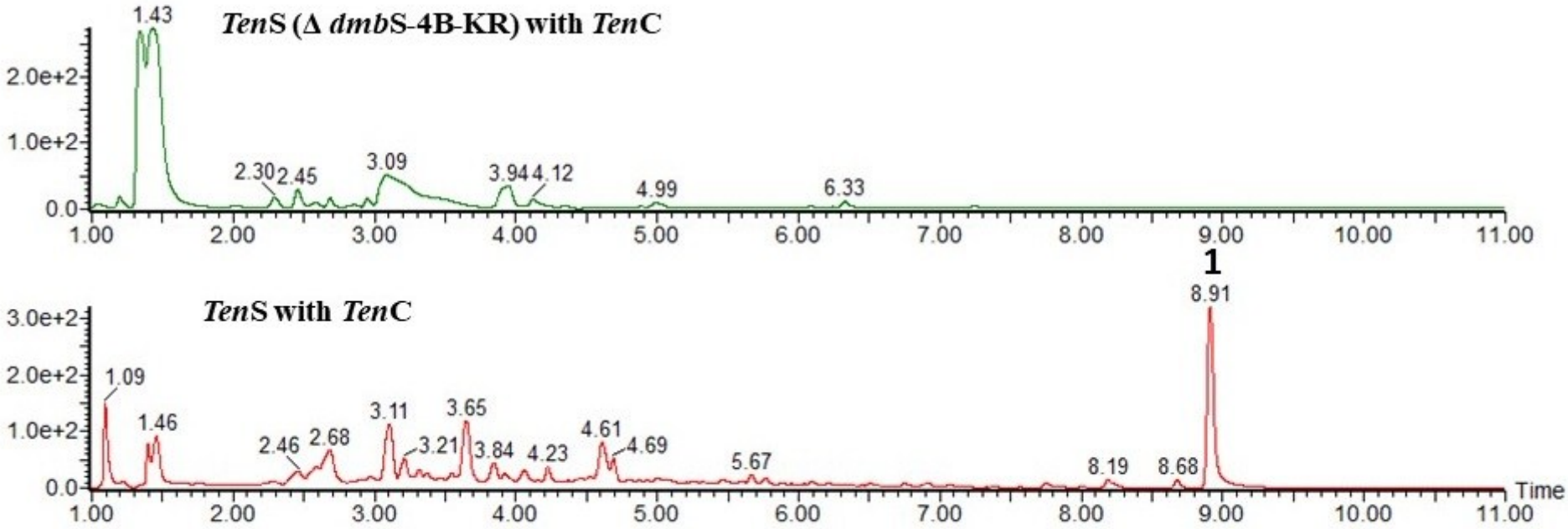
Ampflified HPLC profile of Expt 28.



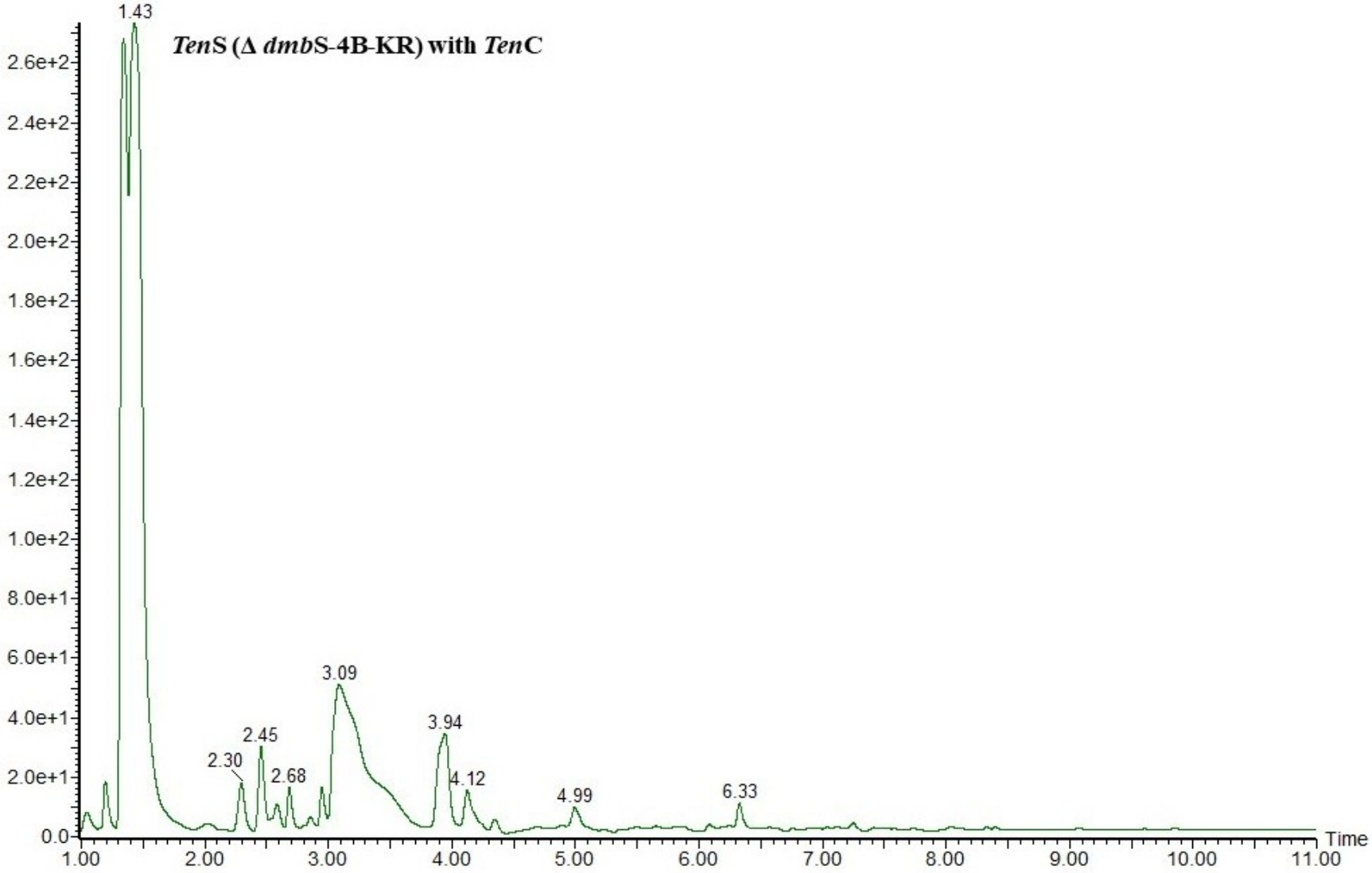
Expt 29. pTYGS-arg-*Ten*C+*Ten*S (Δ *dmb*S-4A-KR)



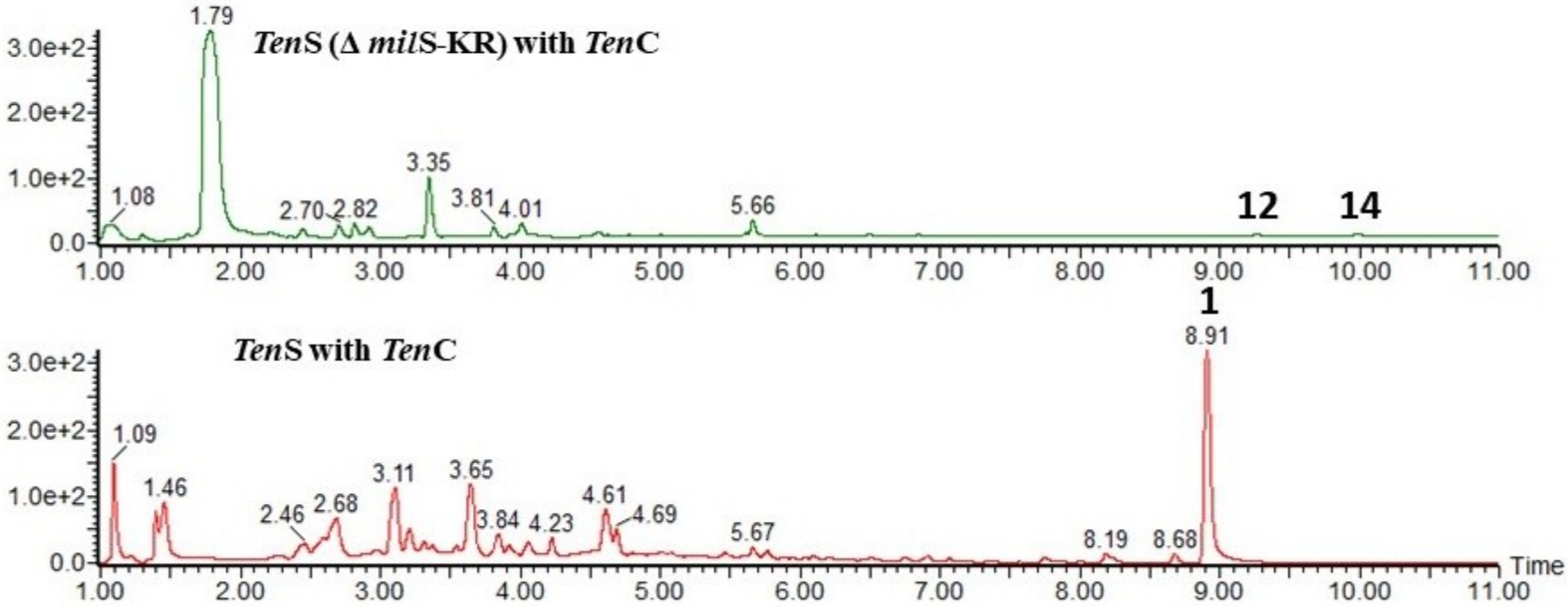
Expt 30. pTYGS-arg-*Ten*C+*Ten*S (Δ *dmb*S-4B-KR)



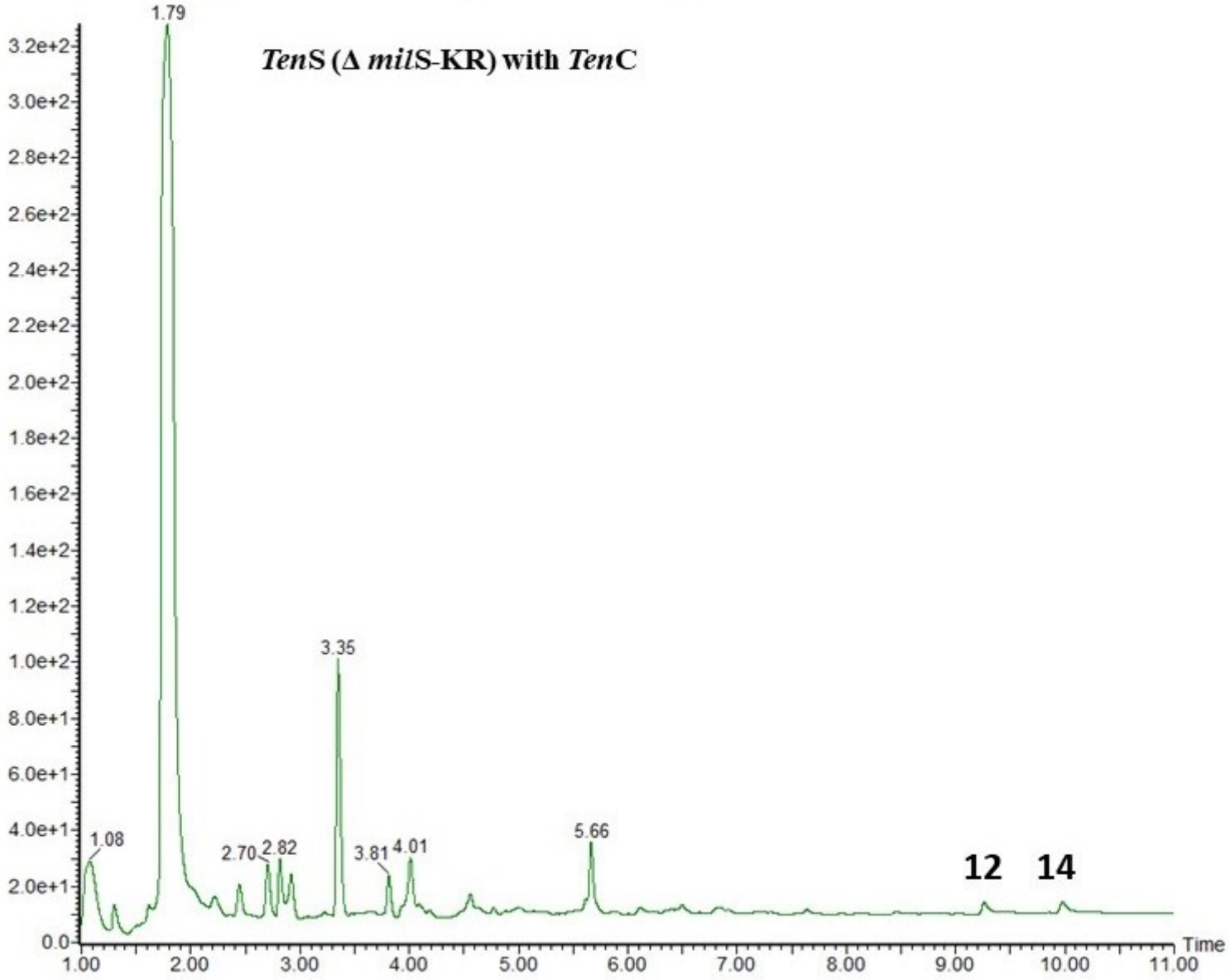
Ampflified HPLC profile of Expt 30.



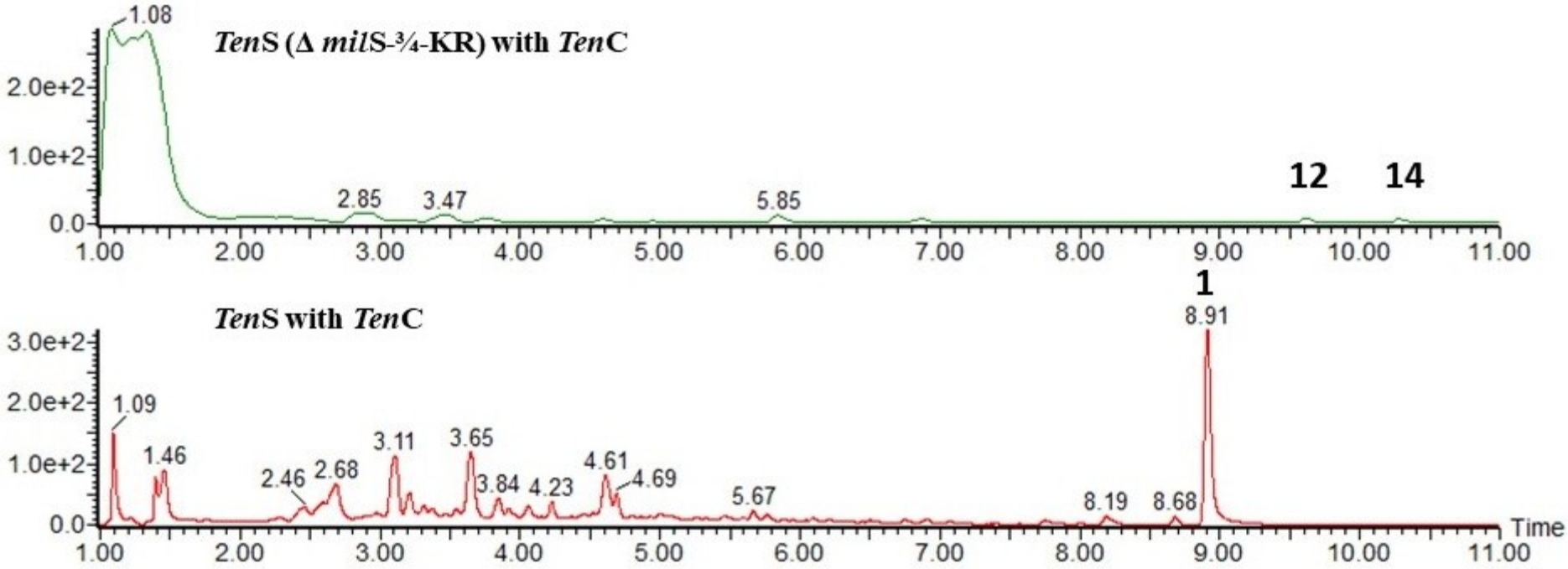
Expt 31. pTYGS-arg-*Ten*C+*Ten*S (Δ *mil*S-KR)



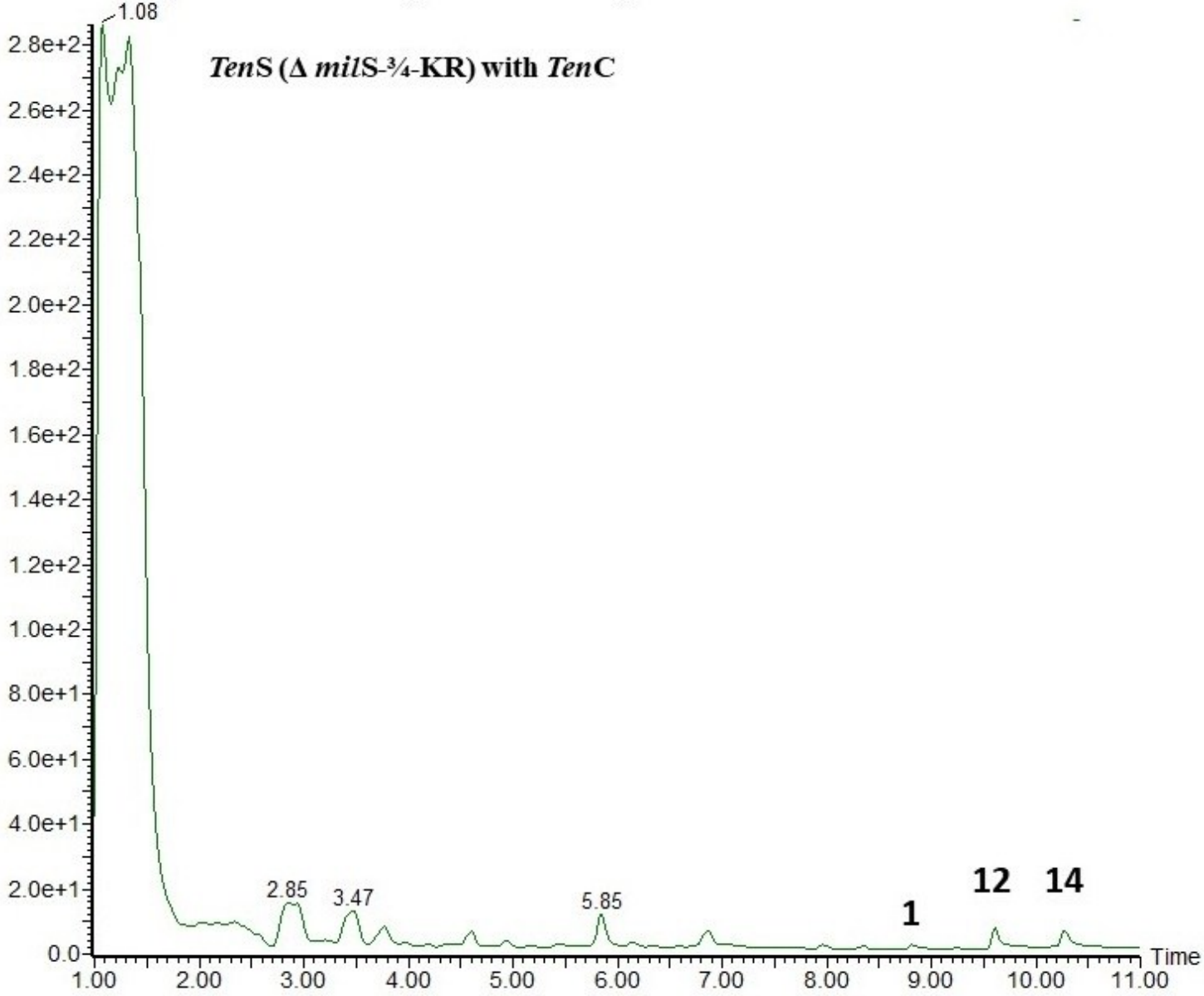
Ampflified HPLC profile of Expt 31.



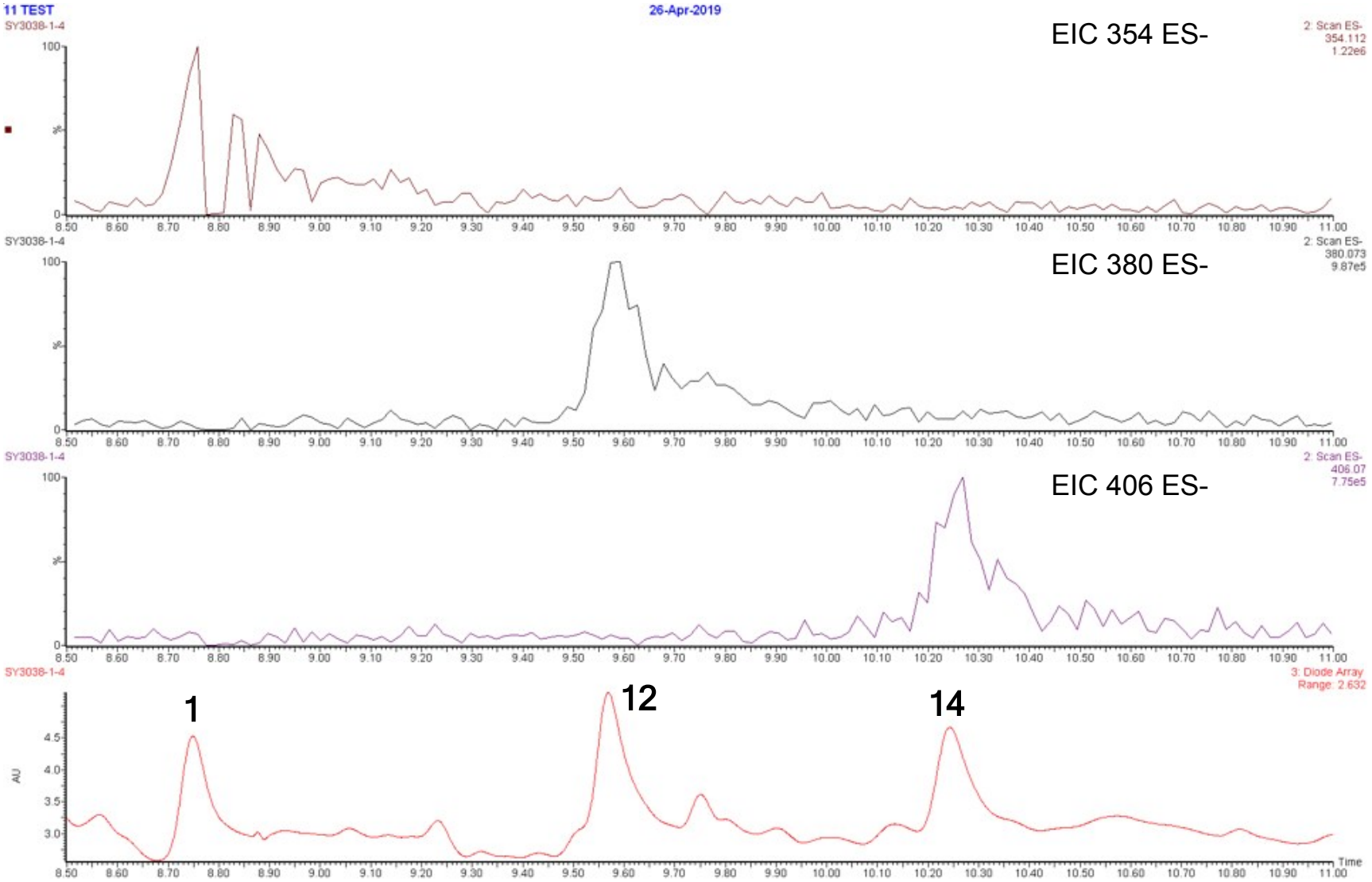
Expt 32. pTYGS-arg-*Ten*C+*Ten*S (Δ *mil*S-¾-KR)



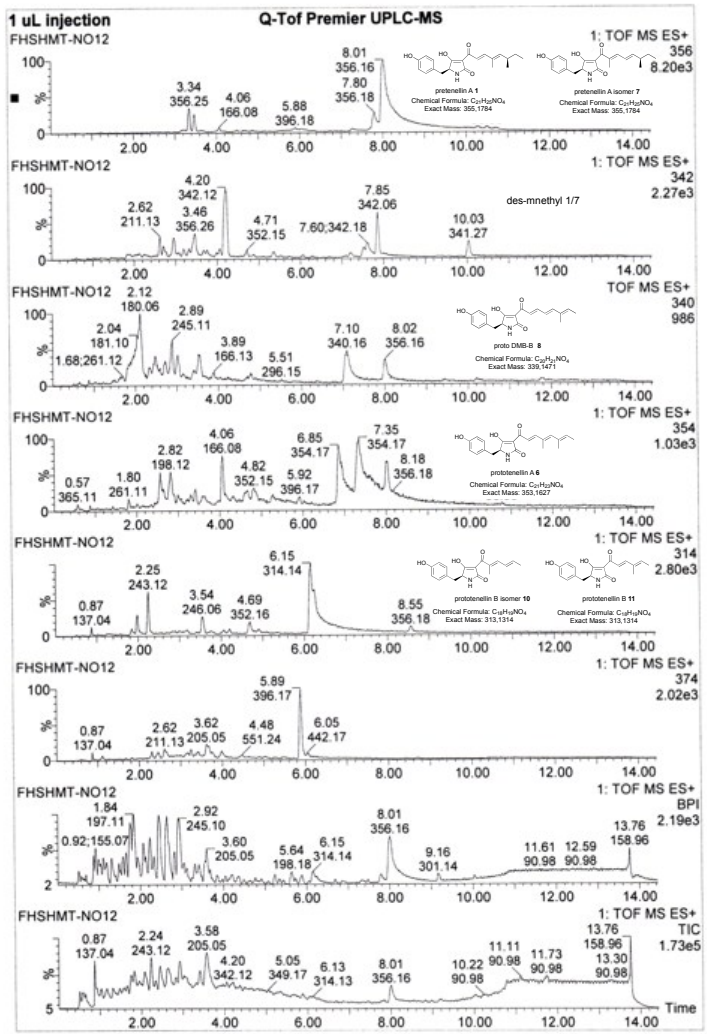
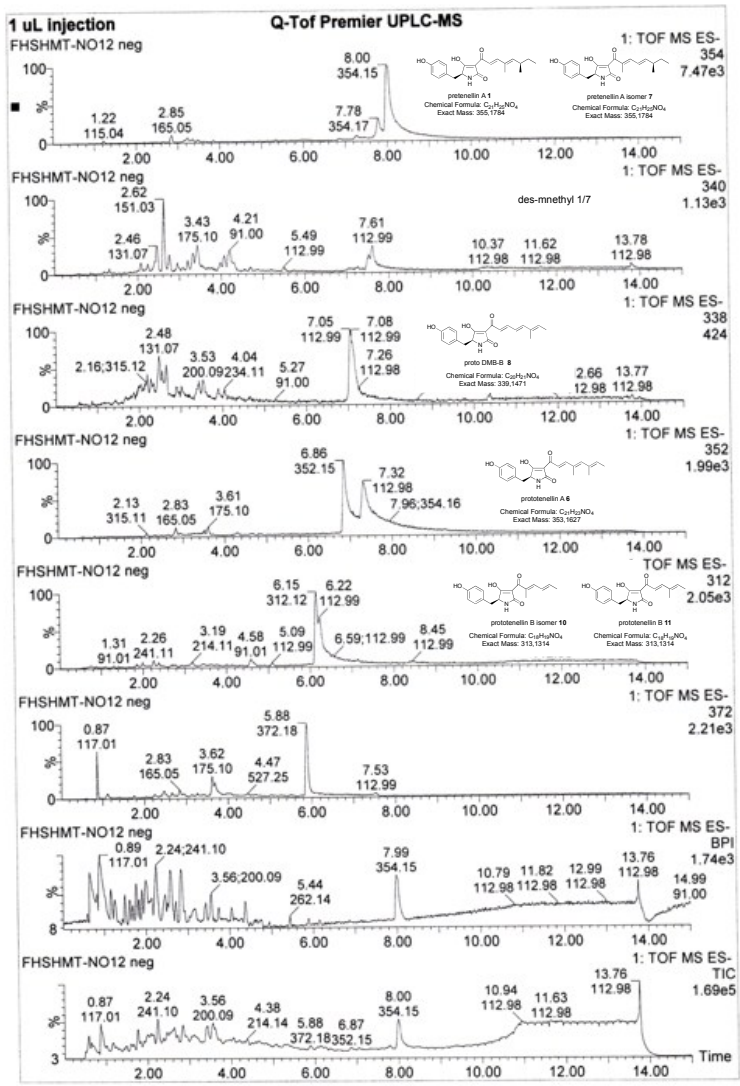
Ampflified HPLC profile of Expt 32.



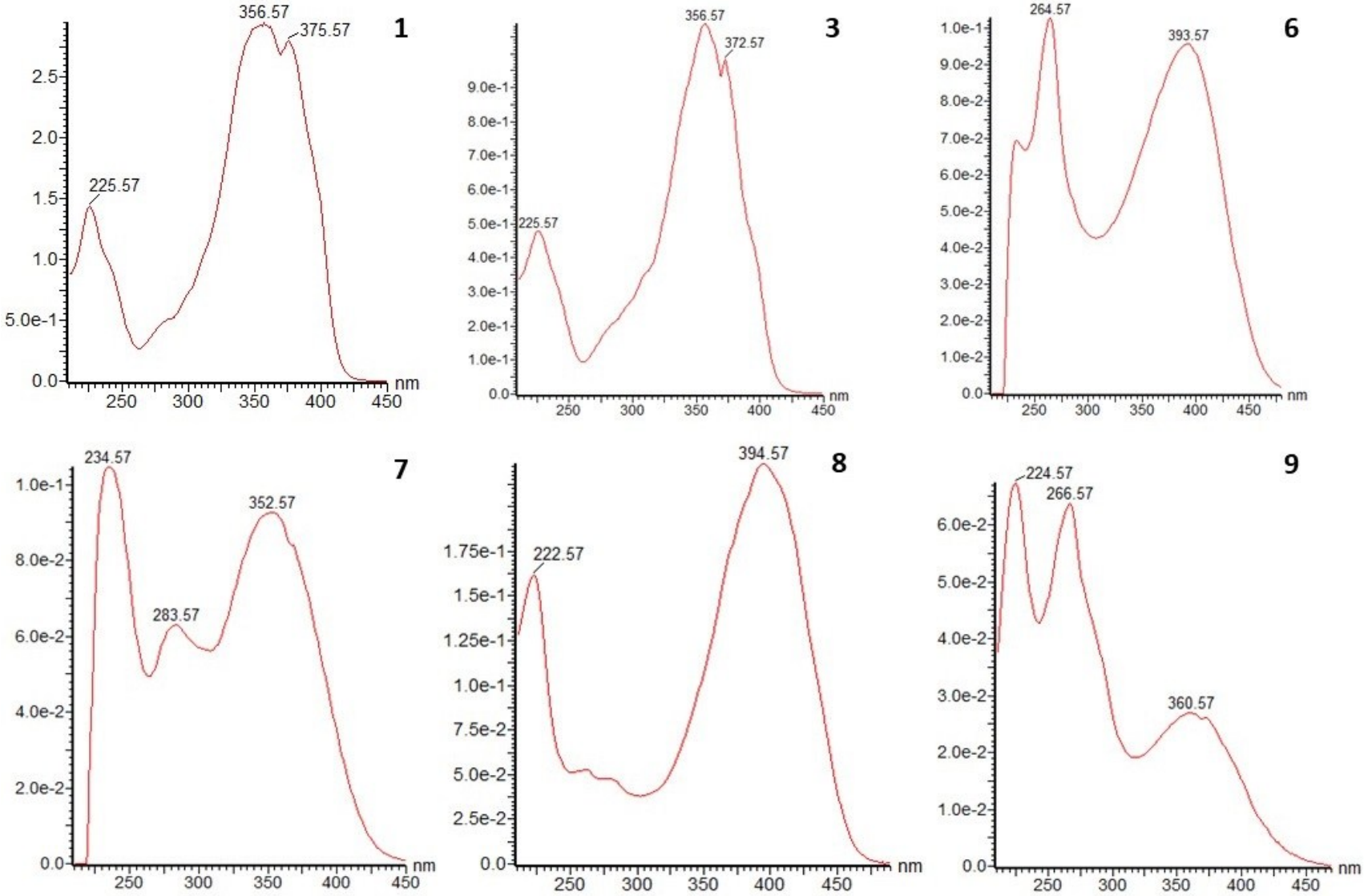
Expt. 33. pTYGS-arg-*TenC*+*TenS*(Δ*MilS* Q2398-V2409)

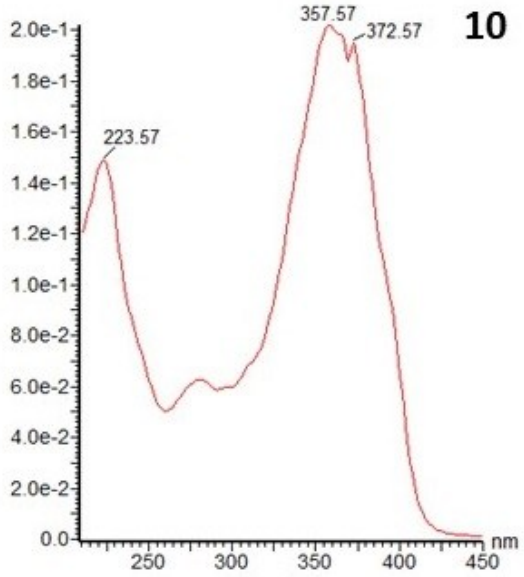


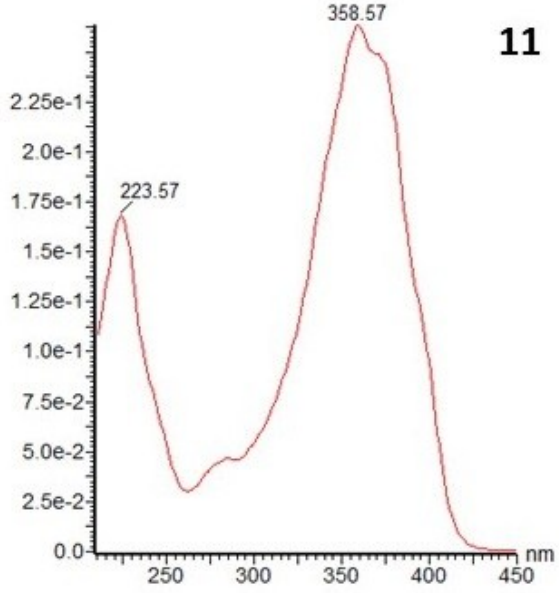
4. LC-HRMS data for extract from experiment 8.

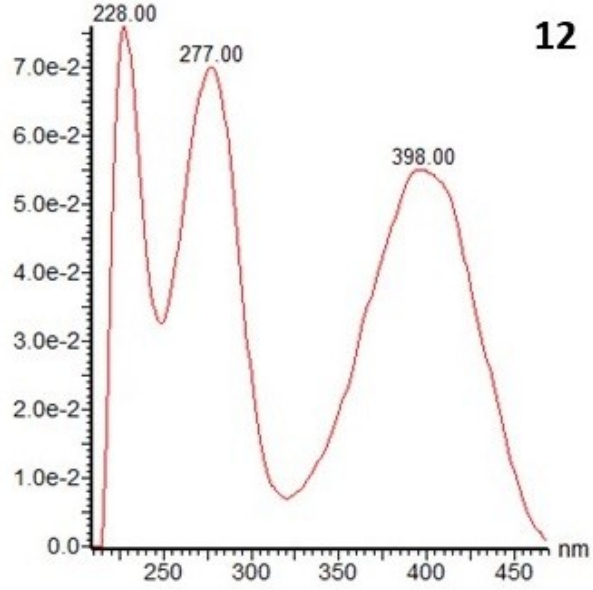
Extracted ions ES+	Extracted ions ES-
	

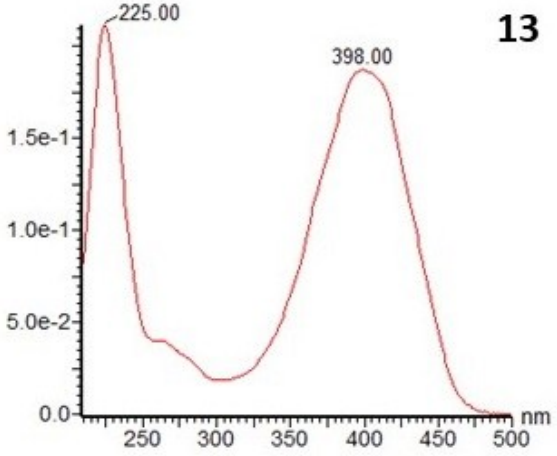
5. UV spectra of compounds

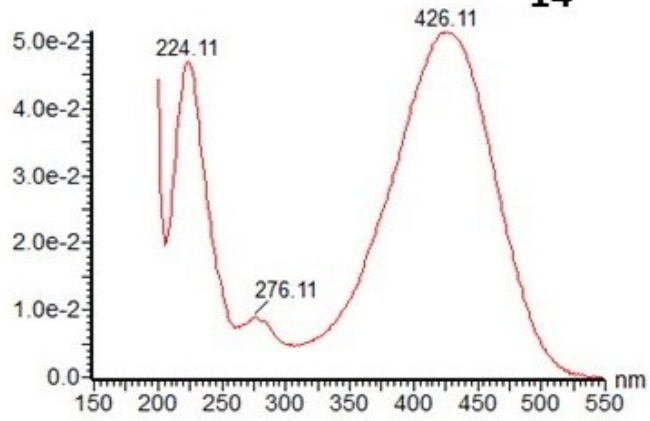



10
357.57
372.57
223.57
nm

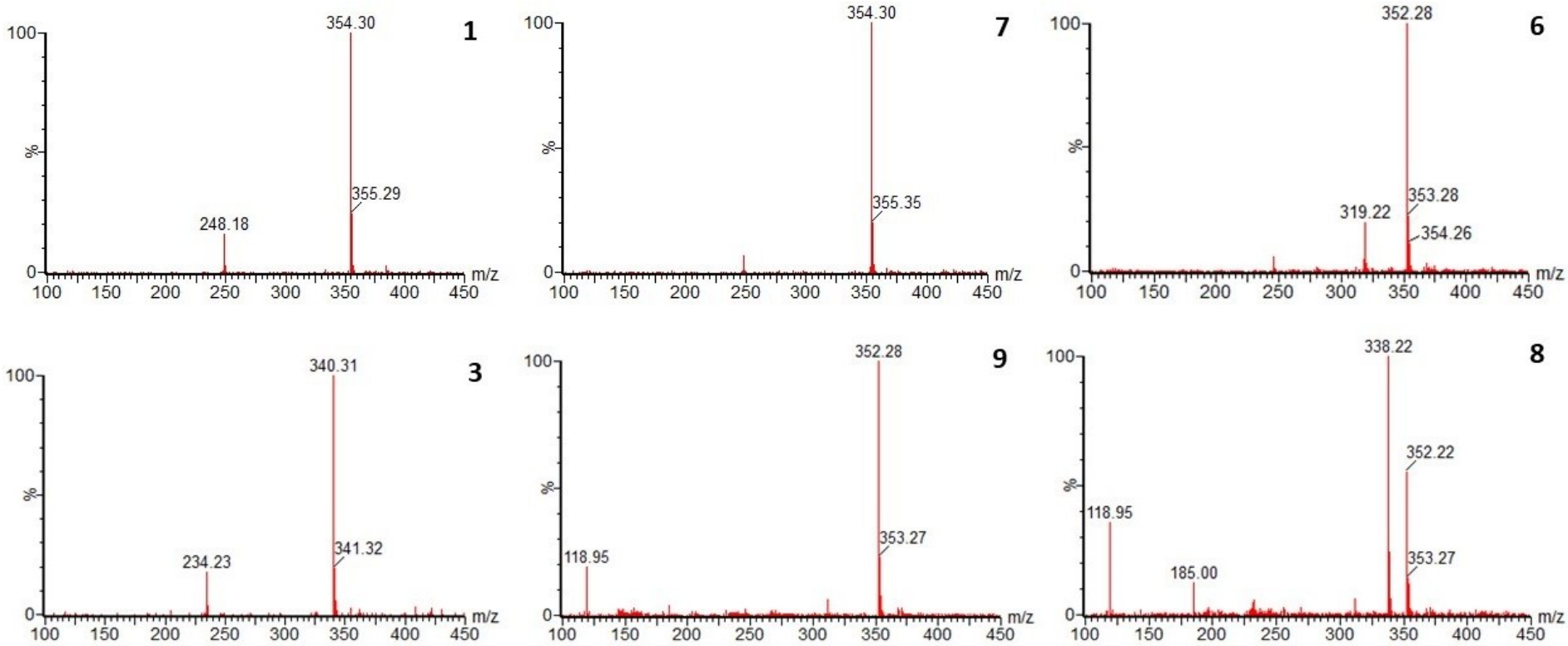

11
358.57
223.57
nm

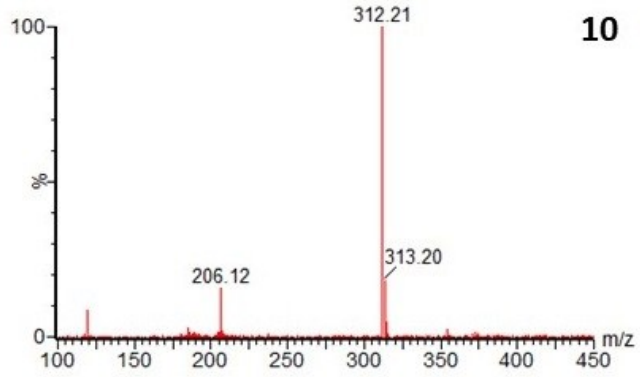

12
228.00
277.00
398.00
nm

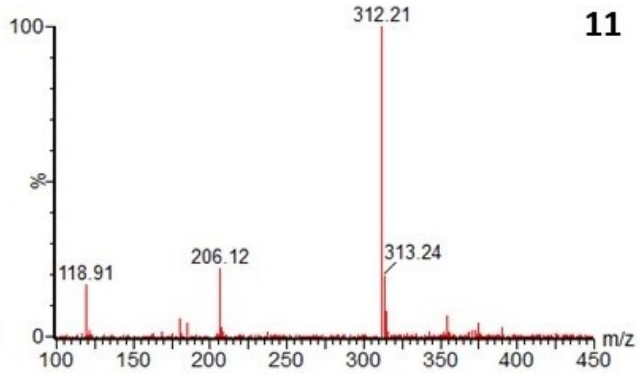

13
225.00
398.00
nm

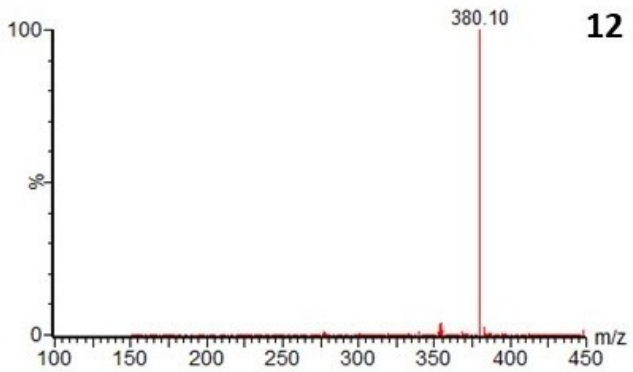

14
426.11
224.11
276.11
nm

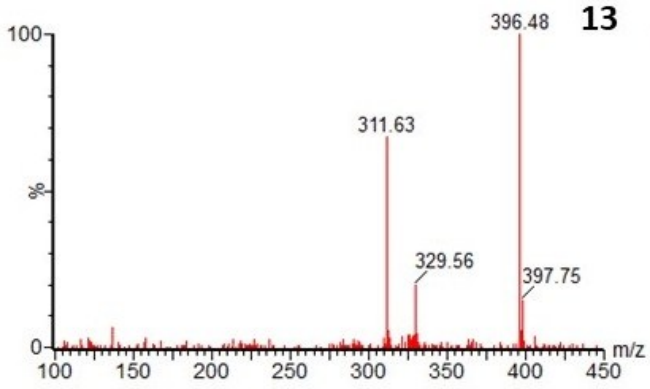
6. MS data for compounds (ESI, negative ion mode)

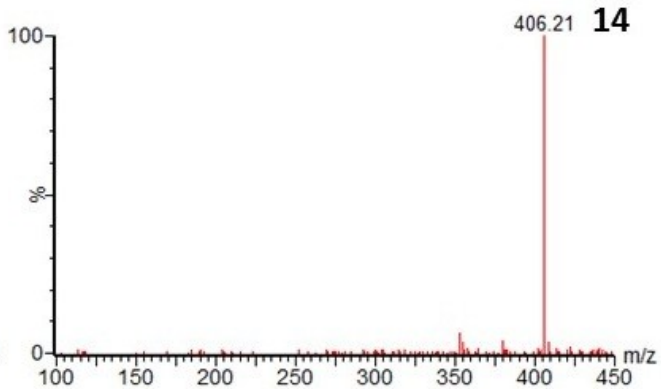



10
312.21
313.20
206.12
100
%
0
m/z
100 150 200 250 300 350 400 450


11
312.21
313.24
206.12
118.91
100
%
0
m/z
100 150 200 250 300 350 400 450


12
380.10
100
%
0
m/z
100 150 200 250 300 350 400 450


13
396.48
311.63
329.56
397.75
100
%
0
m/z
100 150 200 250 300 350 400 450


14
406.21
100
%
0
m/z
100 150 200 250 300 350 400 450

7. HR-MS data for compounds 1, 3, 6-14.

HR-MS of 1

Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

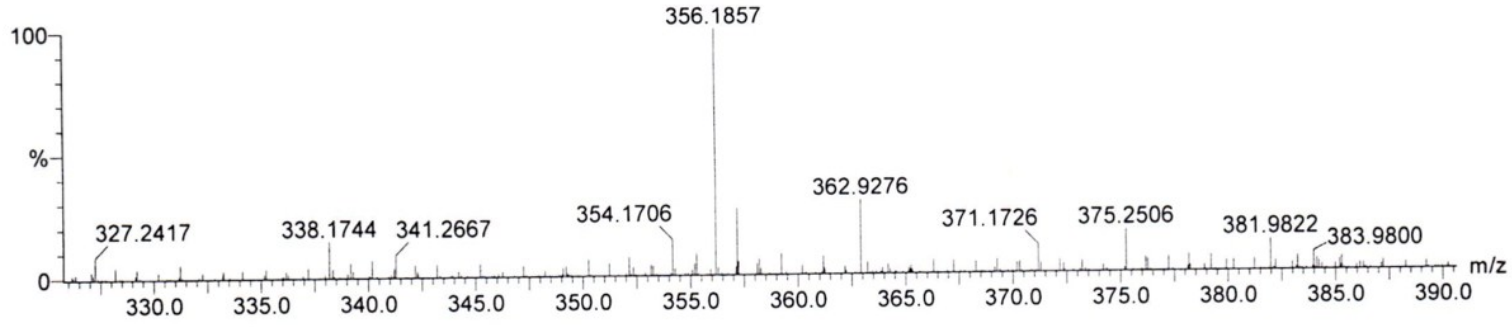
Monoisotopic Mass, Even Electron Ions
323 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-4 O: 0-20

1 uL injection

Q-Tof Premier UPLC-MS

13-Nov-2014
10:49:05
1: TOF MS ES+
1.43e+004

FHSHMT-NO12 951 (9.724) AM (Cen,5, 90.00, Ar,10000.0,556.28,0.70,LS 5); Cm (923:951)



Minimum:				-1.5		
Maximum:		10.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
356.1857	356.1862	-0.5	-1.4	9.5	73.6	C21 H26 N O4
	356.1822	3.5	9.8	5.5	215.3	C16 H26 N3 O6
	356.1921	-6.4	-18.0	0.5	333.8	C14 H30 N O9
	356.1763	9.4	26.4	14.5	99.2	C23 H22 N3 O

HR-MS of 3

Elemental Composition Report

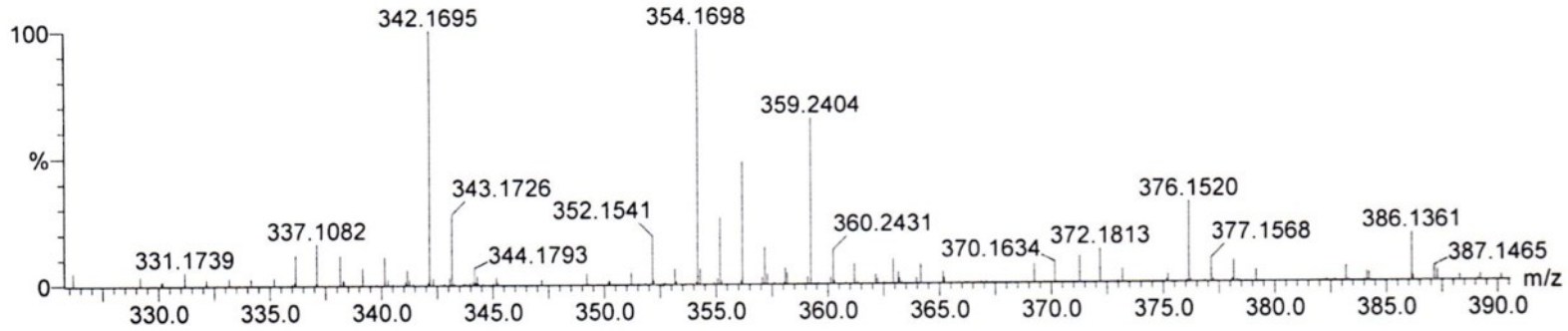
Single Mass Analysis

Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
299 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-4 O: 0-20

1 uL injection Q-Tof Premier UPLC-MS 13-Nov-2014 10:49:05

FHSHMT-NO12 744 (7.606) AM (Cen,5, 90.00, Ar,10000.0,556.28,0.70,LS 5); Cm (742:748) 1: TOF MS ES+ 3.00e+003



Minimum:				-1.5		
Maximum:		10.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
342.1695	342.1705	-1.0	-2.9	9.5	27.0	C20 H24 N O4
	342.1665	3.0	8.8	5.5	66.7	C15 H24 N3 O6
	342.1764	-6.9	-20.2	0.5	97.8	C13 H28 N O9
	342.1606	8.9	26.0	14.5	27.2	C22 H20 N3 O

HR-MS of 6

Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

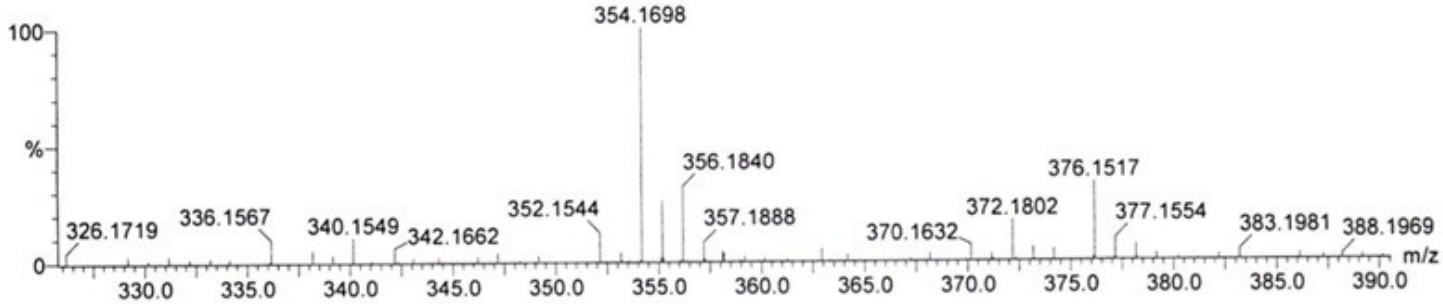
Monoisotopic Mass, Even Electron Ions
318 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-4 O: 0-20

1 uL injection

Q-Tof Premier UPLC-MS

13-Nov-2014
10:49:05
1: TOF MS ES+
3.31e+003

FHSHMT-NO12 728 (7.443) AM (Cen,5, 90.00, Ar,10000.0,556.28,0.70,LS 5); Cm (724:728)



Minimum:				-1.5		
Maximum:		10.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
354.1698	354.1705	-0.7	-2.0	10.5	412.7	C21 H24 N O4
	354.1665	3.3	9.3	6.5	451.9	C16 H24 N3 O6
	354.1764	-6.6	-18.6	1.5	471.7	C14 H28 N O9
	354.1606	9.2	26.0	15.5	426.0	C23 H20 N3 O

HR-MS of 7

Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

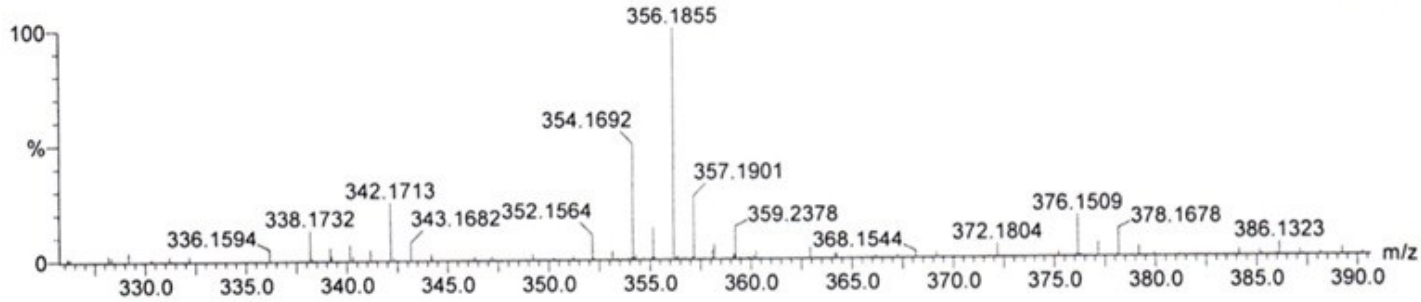
Monoisotopic Mass, Even Electron Ions
323 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-4 O: 0-20

1 uL injection

Q-Tof Premier UPLC-MS

13-Nov-2014
10:49:05
1: TOF MS ES+
3.57e+003

FHSHMT-NO12 757 (7.743) AM (Cen,5, 50.00, Ar,10000.0,556.28,0.70,LS 5); Cm (753:757)



Minimum:				-1.5		
Maximum:		10.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
356.1855	356.1862	-0.7	-2.0	9.5	18.7	C21 H26 N O4
	356.1822	3.3	9.3	5.5	55.8	C16 H26 N3 O6
	356.1921	-6.6	-18.5	0.5	86.8	C14 H30 N O9
	356.1763	9.2	25.8	14.5	23.6	C23 H22 N3 O

HR-MS of 8

Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

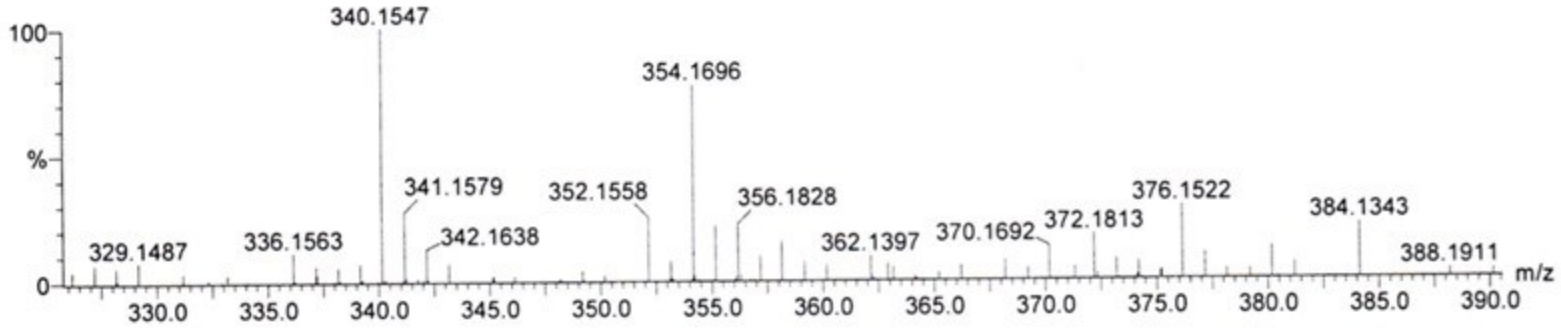
Monoisotopic Mass, Even Electron Ions
301 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-4 O: 0-20

1 uL injection

Q-Tof Premier UPLC-MS

13-Nov-2014
10:49:05
1: TOF MS ES+
2.92e+003

FHSHMT-NO12 694 (7.098) AM (Cen,5, 90.00, Ar,10000.0,556.28,0.70,LS 5); Cm (690:696)



Minimum:				-1.5		
Maximum:		10.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
340.1547	340.1549	-0.2	-0.6	10.5	101.5	C20 H22 N O4
	340.1509	3.8	11.2	6.5	144.0	C15 H22 N3 O6
	340.1608	-6.1	-17.9	1.5	170.7	C13 H26 N O9
	340.1450	9.7	28.5	15.5	104.4	C22 H18 N3 O

HR-MS of 9

Elemental Composition Report

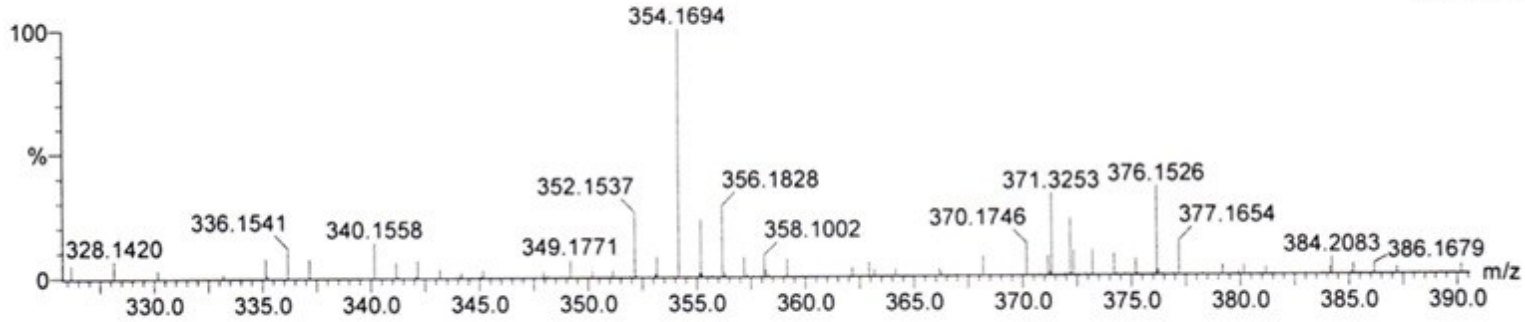
Single Mass Analysis

Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
318 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-4 O: 0-20

1 uL injection Q-Tof Premier UPLC-MS 13-Nov-2014 10:49:05

FHSHMT-NO12 685 (7.007) AM (Cen,5, 90.00, Ar,10000.0,556.28,0.70,LS 5); Cm (681:685) 1: TOF MS ES+ 2.10e+003



Minimum:				-1.5		
Maximum:		10.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
354.1694	354.1705	-1.1	-3.1	10.5	226.7	C21 H24 N O4
	354.1665	2.9	8.2	6.5	240.7	C16 H24 N3 O6
	354.1764	-7.0	-19.8	1.5	248.8	C14 H28 N O9
	354.1606	8.8	24.8	15.5	239.8	C23 H20 N3 O

HR-MS of 10

Elemental Composition Report

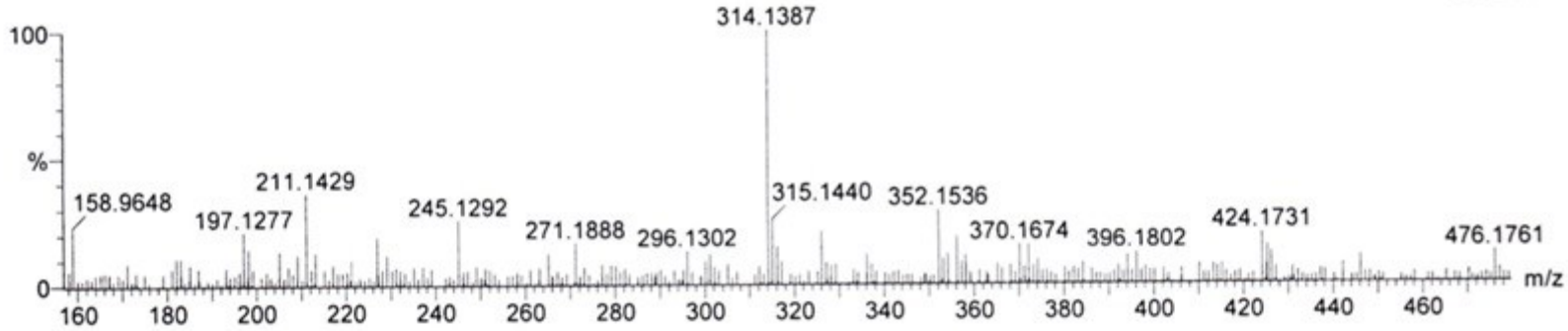
Single Mass Analysis

Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
257 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-4 O: 0-20

1 uL injection Q-Tof Premier UPLC-MS 13-Nov-2014 10:49:05

FHSHMT-NO12 648 (6.625) AM (Cen,5, 70.00, Ar,10000.0,556.28,0.70,LS 5); Cm (643:650) 1: TOF MS ES+ 3.52e+003



Minimum:				-1.5		
Maximum:		10.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
314.1387	314.1392	-0.5	-1.6	9.5	175.8	C18 H20 N O4
	314.1352	3.5	11.1	5.5	233.0	C13 H20 N3 O6
	314.1451	-6.4	-20.4	0.5	266.3	C11 H24 N O9
	314.1293	9.4	29.9	14.5	182.7	C20 H16 N3 O

HR-MS of 11

Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

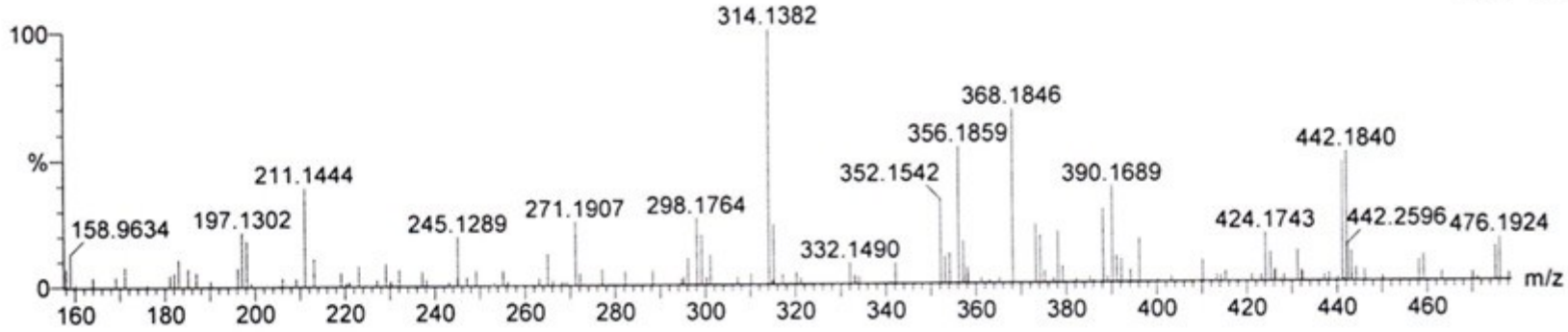
Monoisotopic Mass, Even Electron Ions
257 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-4 O: 0-20

1 uL injection

Q-Tof Premier UPLC-MS

13-Nov-2014
10:49:05
1: TOF MS ES+
5.91e+002

FHSHMT-NO12 596 (6.099) AM (Cen,5, 90.00, Ar,10000.0,556.28,0.70,LS 5)



Minimum:				-1.5		
Maximum:		10.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
314.1382	314.1392	-1.0	-3.2	9.5	n/a	C18 H20 N O4
	314.1352	3.0	9.5	5.5	n/a	C13 H20 N3 O6
	314.1451	-6.9	-22.0	0.5	n/a	C11 H24 N O9
	314.1293	8.9	28.3	14.5	n/a	C20 H16 N3 O

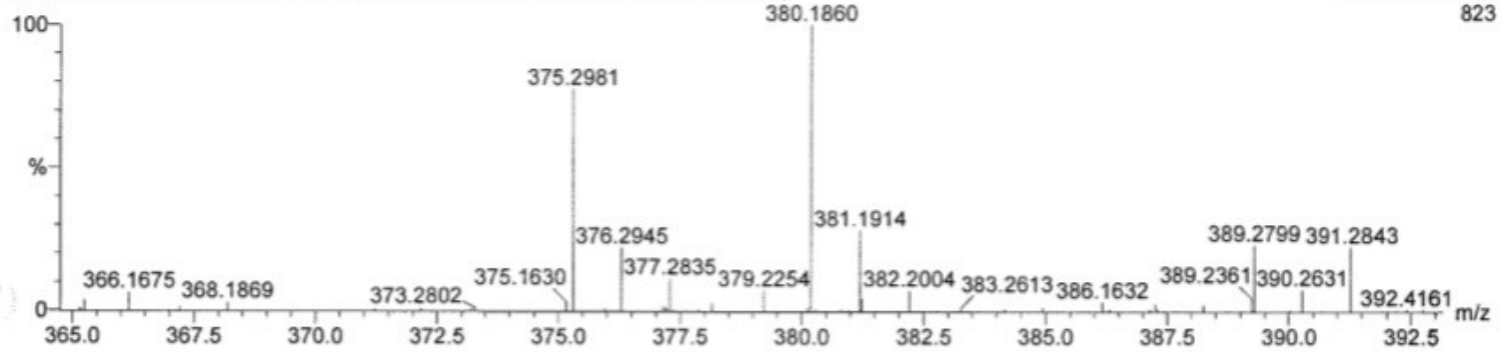
HR-MS of 12 (negative ion mode)

mental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
1096 formula(e) evaluated with 10 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-100 N: 0-12 O: 0-10 Na: 0-1

Friedrich Q-Tof Premier UPLC-MS 12-Jan-201711:49:03
SF 4-006-18-2, neg 973 (9.950) AM (Cen,4, 19.00, Ar,9500.0,554.26,0.70,LS 5) 1: TOF MS ES-823



Minimum:				-1.5		
Maximum:		5.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
380.1860	380.1862	-0.2	-0.5	11.5	4.6	C23 H26 N O4
	380.1853	0.7	1.8	-0.5	48.1	C7 H26 N9 O9
	380.1851	0.9	2.4	13.5	7.4	C22 H23 N5 Na
	380.1870	-1.0	-2.6	0.5	37.2	C10 H27 N7 O7 Na
	380.1875	-1.5	-3.9	16.5	5.1	C24 H22 N5
	380.1838	2.2	5.8	8.5	8.0	C21 H27 N O4 Na
	380.1883	-2.3	-6.0	5.5	31.4	C11 H23 N11 O3 Na
	380.1835	2.5	6.6	12.5	10.0	C19 H22 N7 O2
	380.1894	-3.4	-8.9	3.5	27.6	C12 H26 N7 O7
	380.1897	-3.7	-9.7	-0.5	25.0	C14 H31 N O9 Na

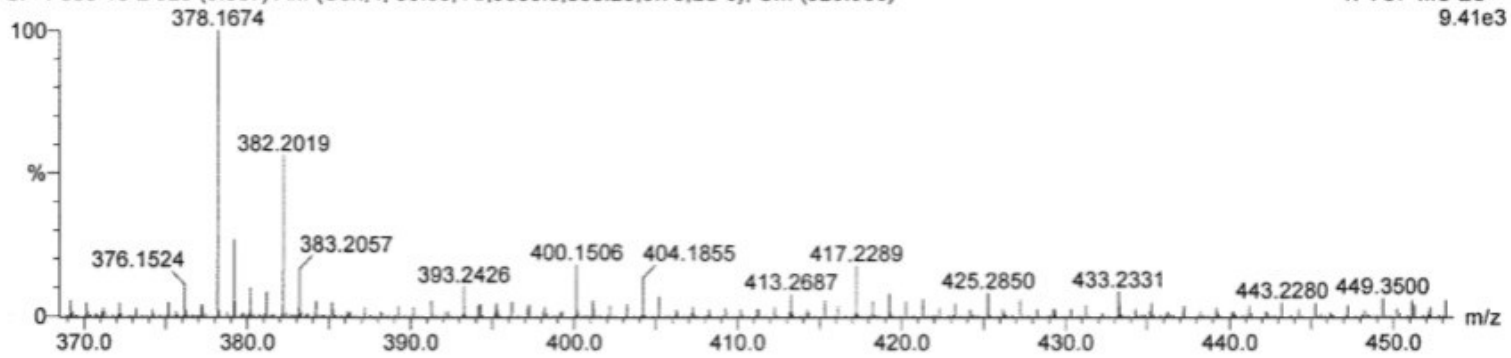
HR-MS of 12 (positive ion mode)

ɜmental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
1103 formula(e) evaluated with 10 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-100 N: 0-12 O: 0-10 Na: 0-1

Friedrich Q-Tof Premier UPLC-MS 12-Jan-201712:04:35
SF 4-006-18-2 929 (9.507) AM (Cen,4, 35.00, Ar,9500.0,556.28,0.70,LS 5); Cm (929:968) 1: TOF MS ES+
9.41e3



Minimum:				-1.5		
Maximum:		5.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
382.2019	382.2018	0.1	0.3	10.5	73.3	C23 H28 N O4
	382.2026	-0.7	-1.8	-0.5	314.8	C10 H29 N7 O7 Na
	382.2010	0.9	2.4	-1.5	386.7	C7 H28 N9 O9
	382.2008	1.1	2.9	12.5	96.5	C22 H25 N5 Na
	382.2032	-1.3	-3.4	15.5	74.0	C24 H24 N5
	382.2040	-2.1	-5.5	4.5	276.0	C11 H25 N11 O3 Na
	382.1994	2.5	6.5	7.5	102.8	C21 H29 N O4 Na
	382.1991	2.8	7.3	11.5	119.0	C19 H24 N7 O2
	382.2050	-3.1	-8.1	2.5	248.3	C12 H28 N7 O7
	382.2053	-3.4	-8.9	-1.5	229.3	C14 H33 N O9 Na

HR-MS of 13 (negative ion mode)

.lemental Composition Report **Page 1**

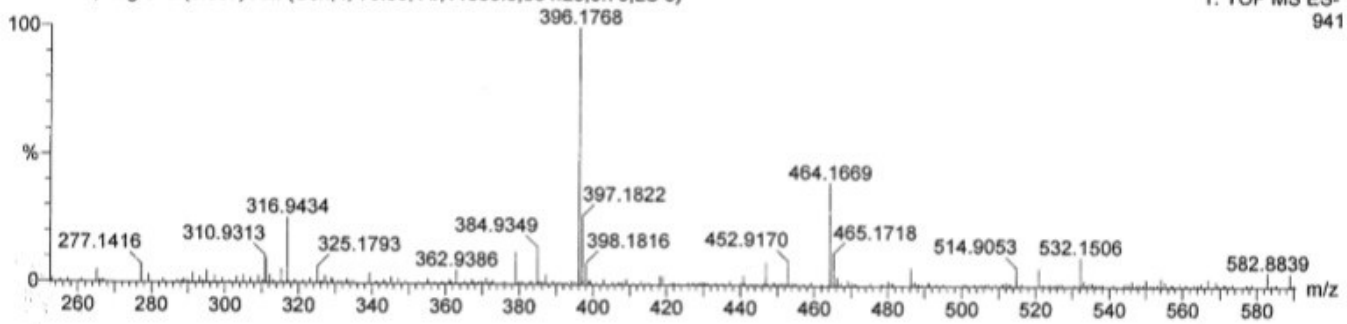
Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
736 formula(e) evaluated with 14 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-40 H: 0-100 N: 0-11 O: 0-16

Friedrich
SY 2011A, neg 349 (3.307) AM (Cen,4, 70.00, Ar,11000.0,554.26,0.70,LS 5)

Q-Tof Premier UPLC-MS

05-Oct-201710:30:24
1: TOF MS ES-
941



Minimum:				-1.5		
Maximum:		5.0	20.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
396.1768	396.1771	-0.3	-0.8	7.5	14.4	C18 H26 N3 O7
	396.1752	1.6	4.0	20.5	12.0	C30 H22 N
	396.1784	-1.6	-4.0	12.5	13.6	C19 H22 N7 O3
	396.1744	2.4	6.1	8.5	23.9	C14 H22 N9 O5
	396.1803	-3.5	-8.8	-0.5	52.8	C7 H26 N9 O10
	396.1731	3.7	9.3	3.5	28.1	C13 H26 N5 O9
	396.1811	-4.3	-10.9	11.5	8.5	C23 H26 N O5
	396.1717	5.1	12.9	-1.5	34.5	C12 H30 N O13
	396.1824	-5.6	-14.1	16.5	10.6	C24 H22 N5 O
	396.1712	5.6	14.1	16.5	9.8	C25 H22 N3 O2
	396.1829	-6.1	-15.4	-1.5	37.7	C11 H30 N3 O12
	396.1704	6.4	16.2	4.5	42.4	C9 H22 N11 O7
	396.1843	-7.5	-18.9	3.5	31.7	C12 H26 N7 O8
	396.1690	7.8	19.7	-0.5	50.4	C8 H26 N7 O11

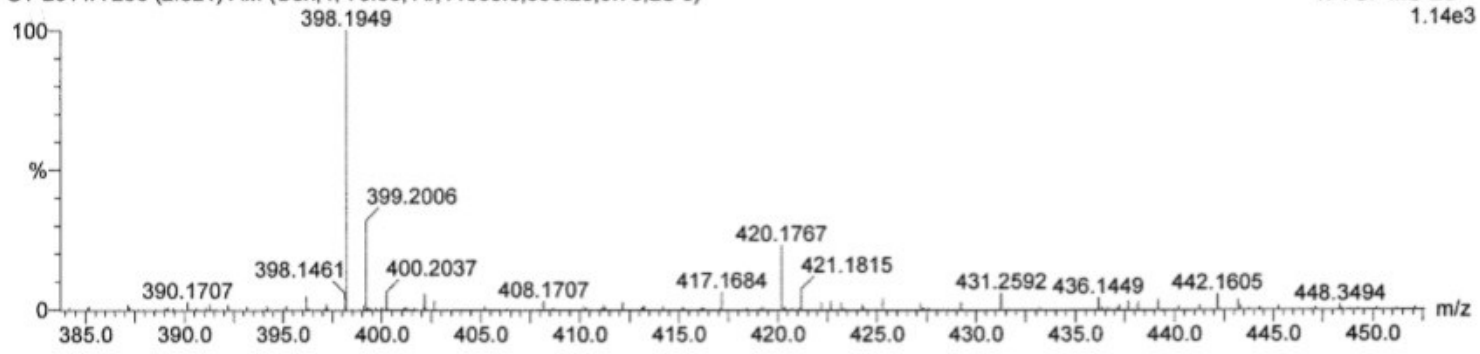
HR-MS of 13 (positive ion mode)

ɜmental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
740 formula(e) evaluated with 11 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-40 H: 0-100 N: 0-11 O: 0-16

Friedrich Q-Tof Premier UPLC-MS 05-Oct-201708:40:25
SY 2011A 256 (2.621) AM (Cen,4, 70.00, Ar,11000.0,556.28,0.70,LS 5) 1: TOF MS ES+
1.14e3



Minimum:				-1.5		
Maximum:		5.0	20.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
398.1949	398.1941	0.8	2.0	11.5	15.2	C19 H24 N7 O3
	398.1959	-1.0	-2.5	-1.5	78.6	C7 H28 N9 O10
	398.1967	-1.8	-4.5	10.5	6.6	C23 H28 N O5
	398.1927	2.2	5.5	6.5	20.8	C18 H28 N3 O7
	398.1981	-3.2	-8.0	15.5	4.4	C24 H24 N5 O
	398.1909	4.0	10.0	19.5	1.6	C30 H24 N
	398.1900	4.9	12.3	7.5	35.0	C14 H24 N9 O5
	398.1999	-5.0	-12.6	2.5	47.2	C12 H28 N7 O8
	398.1887	6.2	15.6	2.5	44.4	C13 H28 N5 O9
	398.2013	-6.4	-16.1	7.5	38.5	C13 H24 N11 O4
	398.2026	-7.7	-19.3	1.5	32.4	C16 H32 N O10

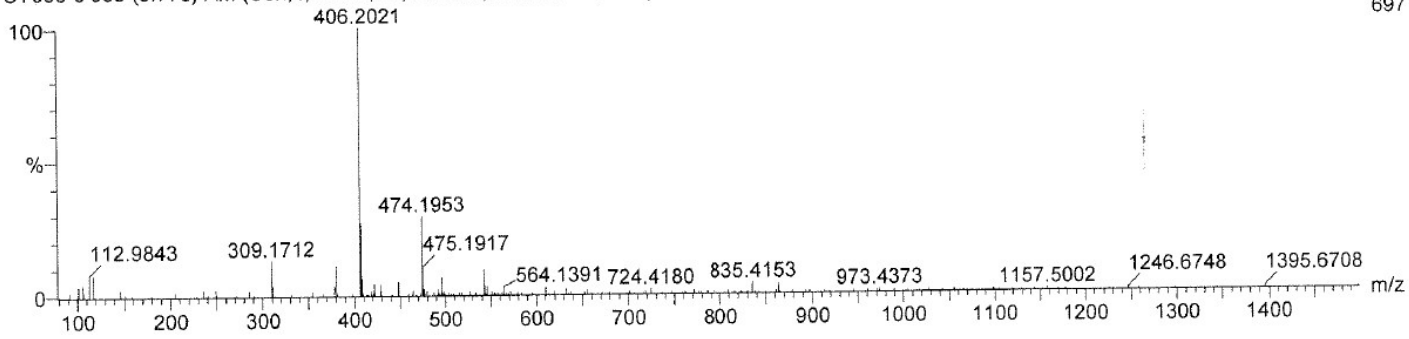
HRMS of 14

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -0.5, max = 60.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
765 formula(e) evaluated with 8 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-75 H: 0-120 B: 0-2 N: 0-1 O: 0-15 Na: 0-1

new sample, Sen Yin, BEH Phenyl up to 100% ACN Q-Tof Premier UPLC-MS
SY066-c 663 (6.778) AM (Cen,4, 70.00, Ar,11000.0,554.26,0.55,LS 5)

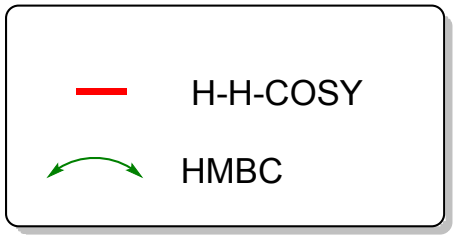
24-Jun-201910:20:16
1: TOF MS ES-
697

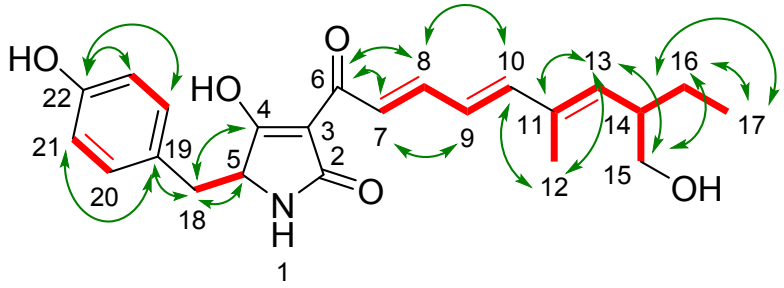


Minimum:				-0.5		
Maximum:		5.0	10.0	60.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
406.2021	406.2018	0.3	0.7	12.5	1.5	C25 H28 N O4
	406.2013	0.8	2.0	4.5	7.6	C18 H30 B N O7 Na
	406.2032	-1.1	-2.7	-0.5	18.5	C13 H31 B2 N O10 Na
	406.2037	-1.6	-3.9	7.5	4.1	C20 H29 B N O7
	406.1997	2.4	5.9	11.5	3.4	C22 H26 B2 N O5
	406.1994	2.7	6.6	9.5	2.2	C23 H29 N O4 Na
	406.2053	-3.2	-7.9	0.5	10.5	C16 H33 N O9 Na
	406.2056	-3.5	-8.6	2.5	12.8	C15 H30 B2 N O10

8. NMR Characterisation

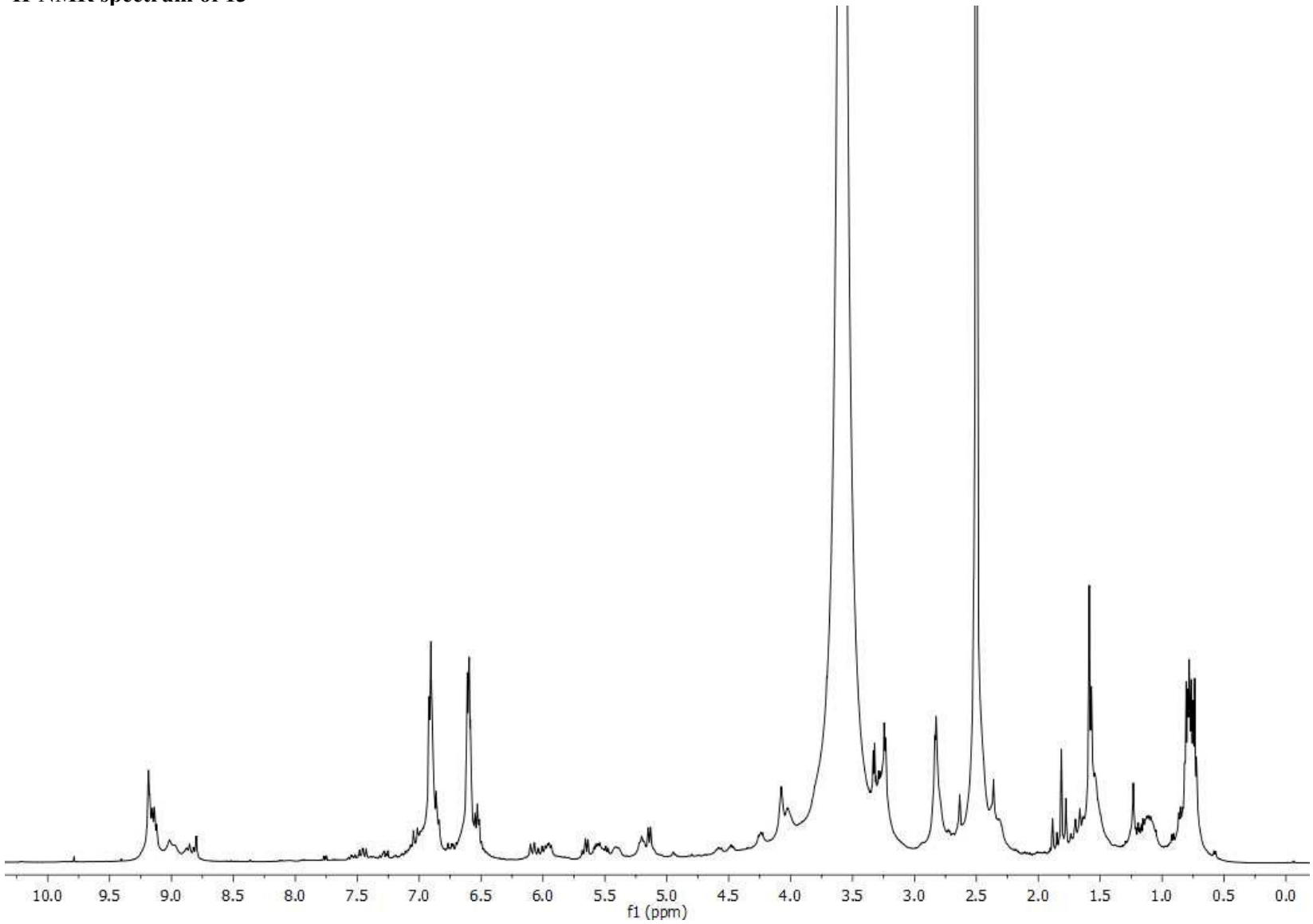
8.1 Compound 13



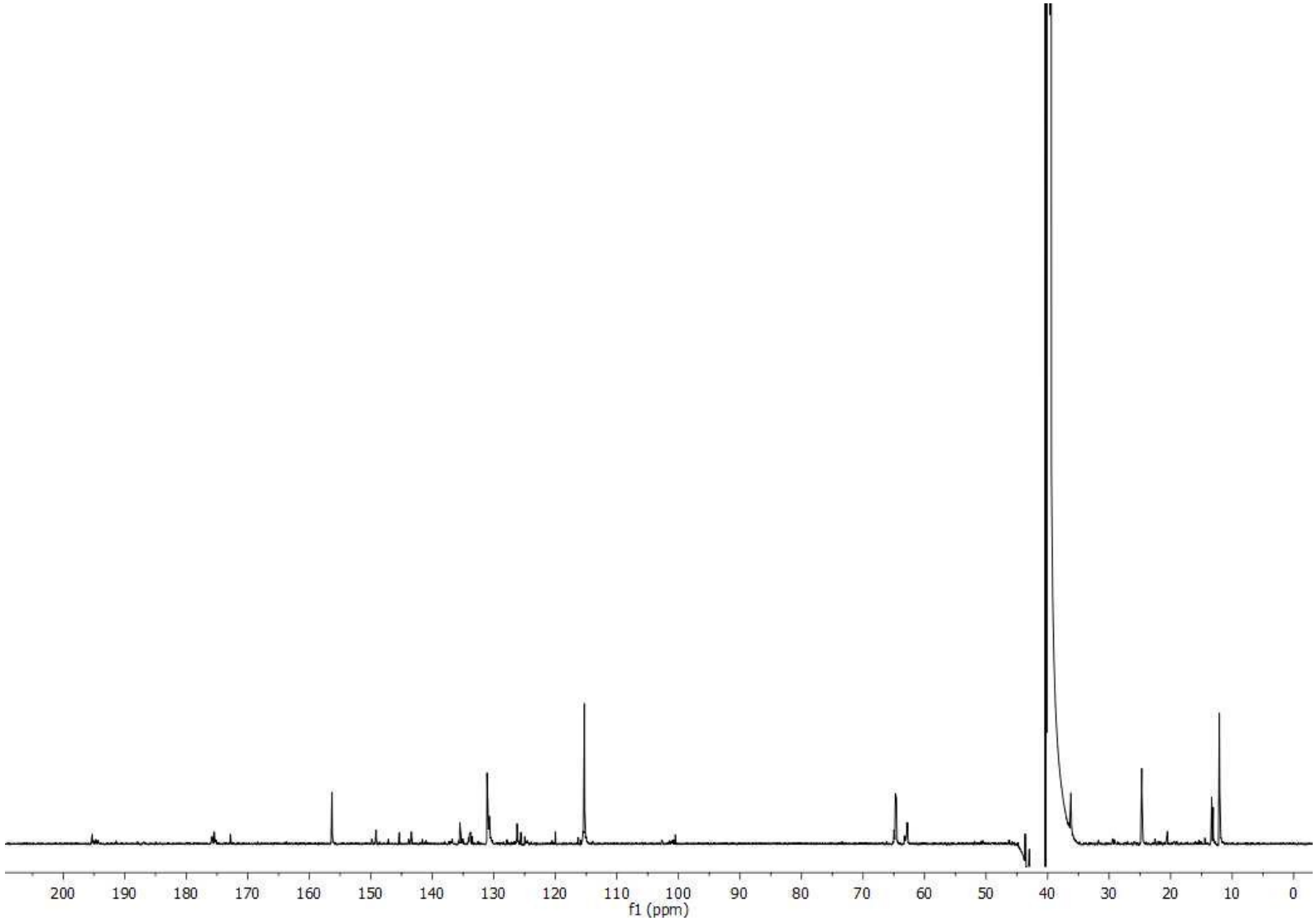


(DMSO-d_6, 600 MHz)					
position	δ_H /ppm	Mult.//	δ_C / ppm	COSY	HMBC (H to C)
2	-	-	163.6 (C)	-	-
3	-	-	100.4 (C)	-	-
4	-	-	194.9 (C)	-	-
5	4.06 (1H)	m	62.4 (CH)	18	4
6	-	-	172.4 (C)	-	-
7	7.04 (1H)	d (15.1 Hz)	119.6 (CH)	8	6, 9
8	7.46 (1H)	dd (15.0, 11.4 Hz)	144.9 (CH)	7, 9	6, 10
9	6.53 (1H)	m	125.7 (CH)	8, 10	7
10	6.88 (1H)	m	149.0 (CH)	9	8, 12
11	-	-	137.1 (C)	-	-
12	1.59 (3H)	s	12.9 (CH3)	13	13, 10
13	5.18 (1H)	m	135.1 (CH)	12	12, 15
14	2.35 (1H)	m	42.5 (CH)	13, 15, 16	
15	3.29 (2H)	m	64.3 (CH2)	14	13, 16
16	1.15 (2H)	m	24.3 (CH2)	14, 17	15, 17
17	0.78 (3H)	m	11.6 (CH3)	16	14, 16
18	2.83 (2H)	broad s	35.8 (CH2)	5	
19	-	-	126.0 (C)	-	-
20	6.92 (2H)	m	130.7 (CH)	21	
21	6.61 (2H)	m	114.9 (CH)	20	
22	-	-	155.9 (C)	-	-

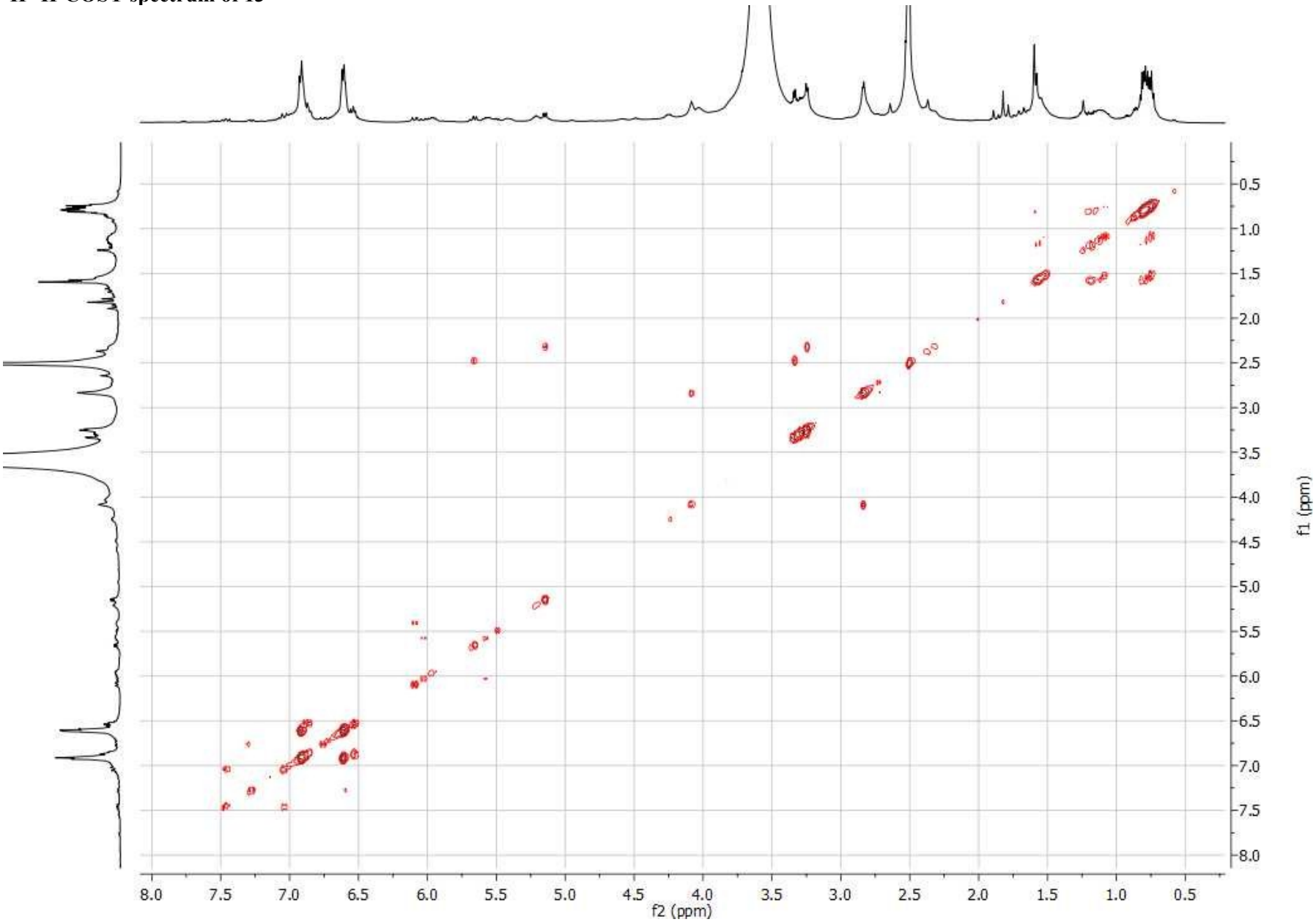
^{1}H-NMR spectrum of 13



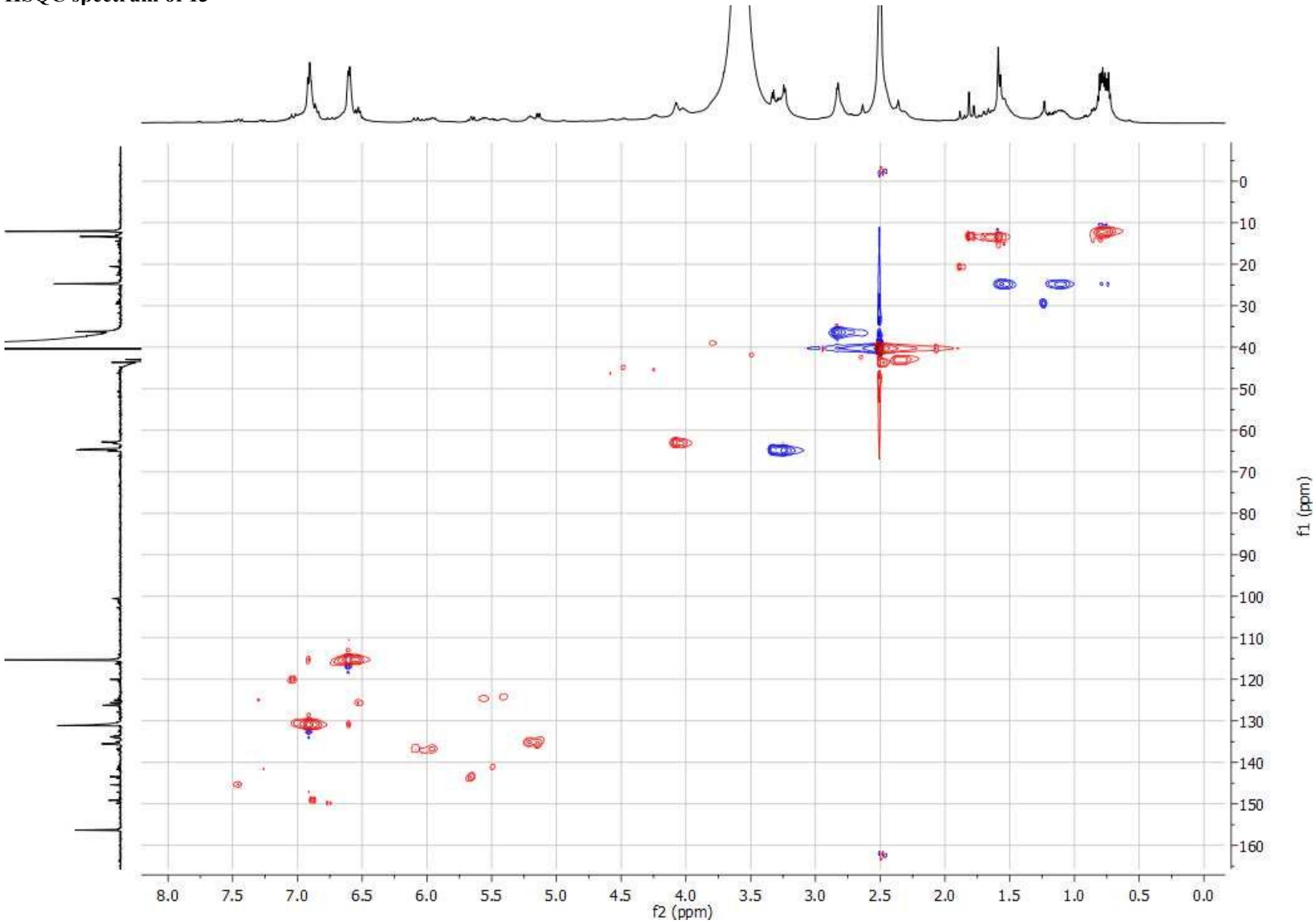
^{13}C-NMR spectrum of 13



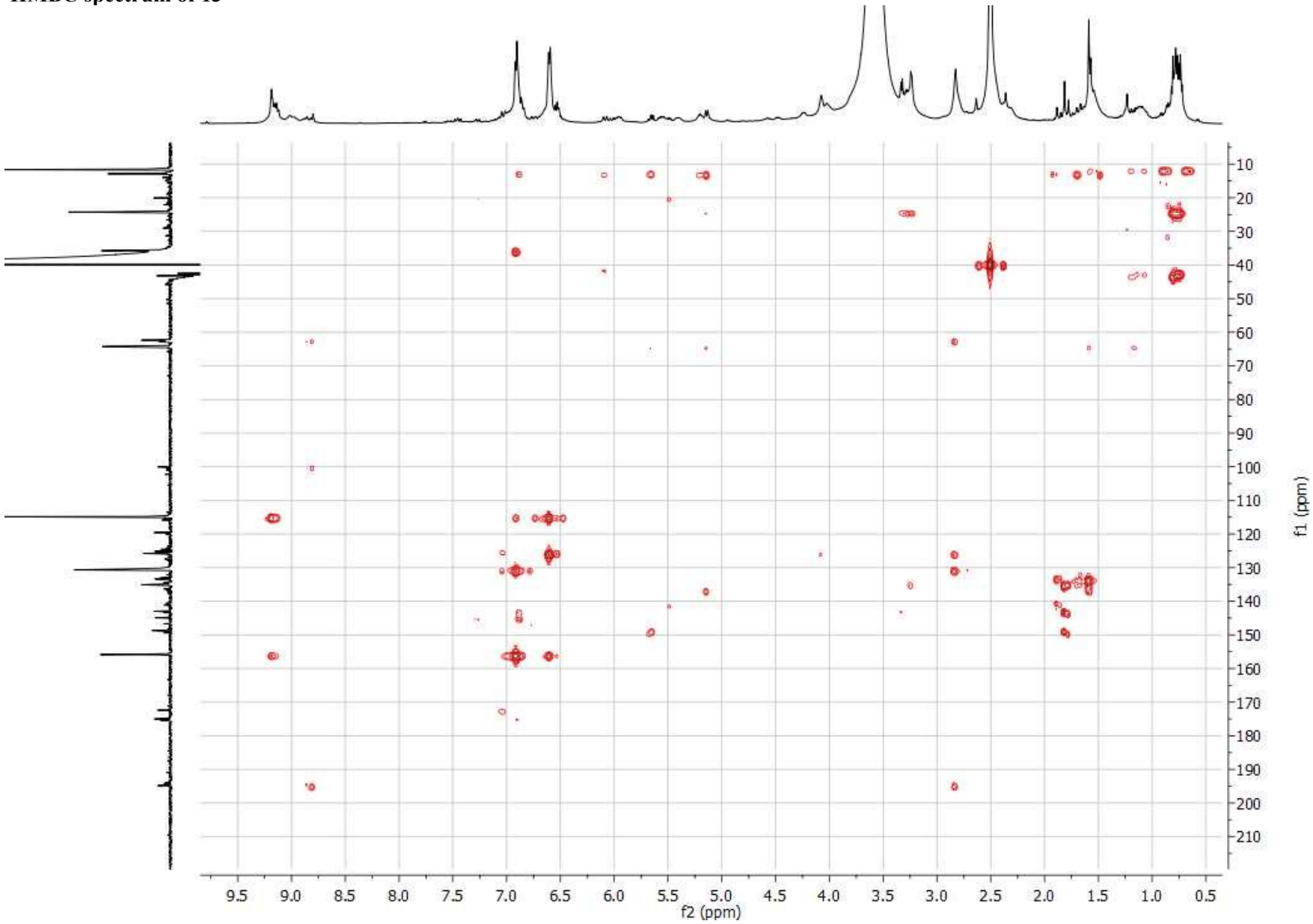
^{1}H-^{1}H-COSY spectrum of 13



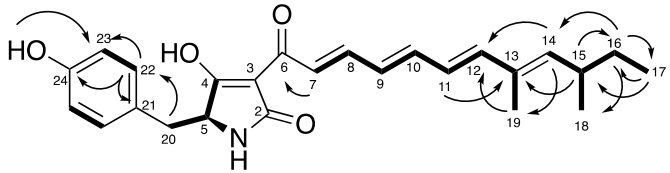
HSQC spectrum of 13



HMBC spectrum of 13

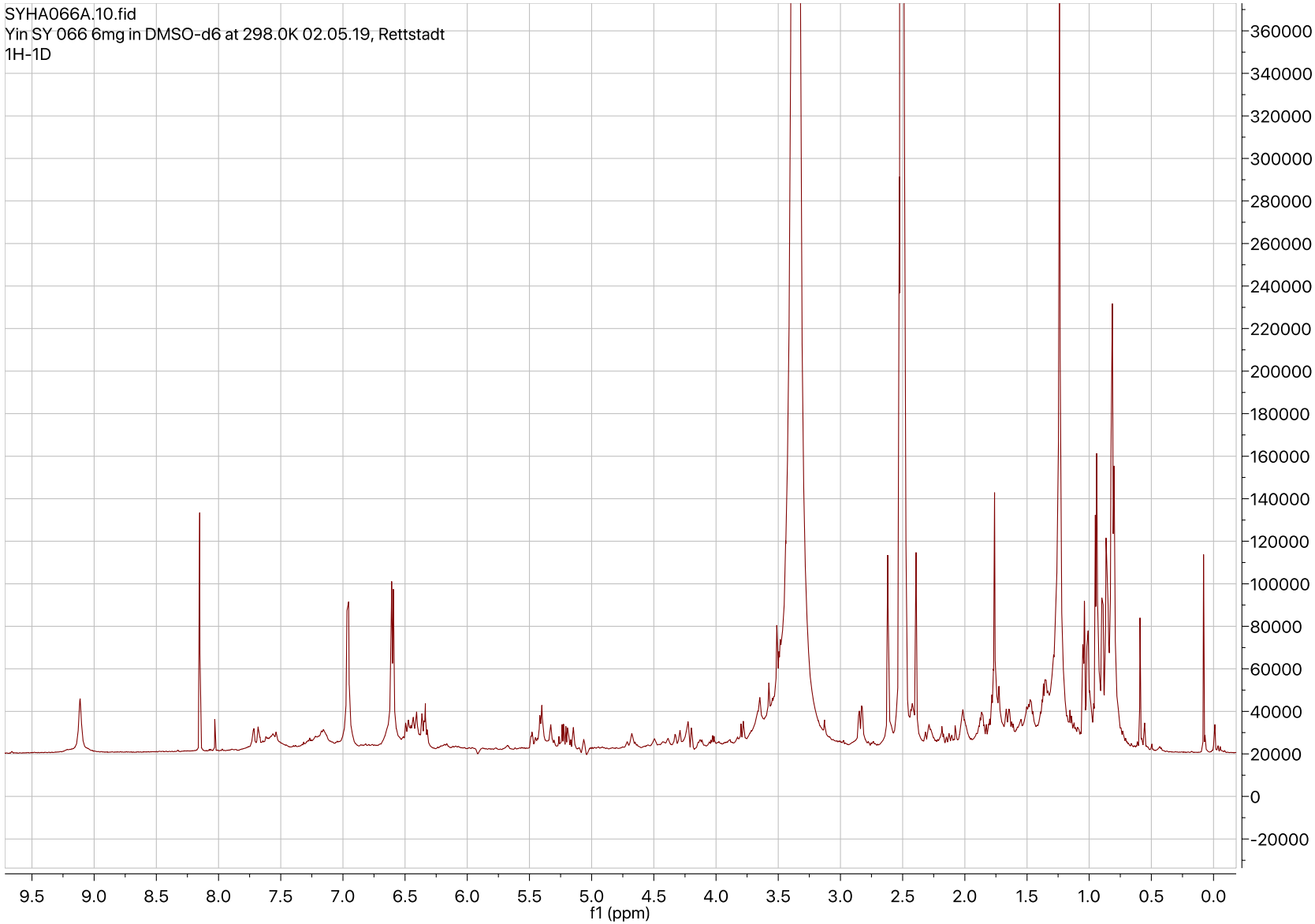


8.2 NMR Characterisation of 14

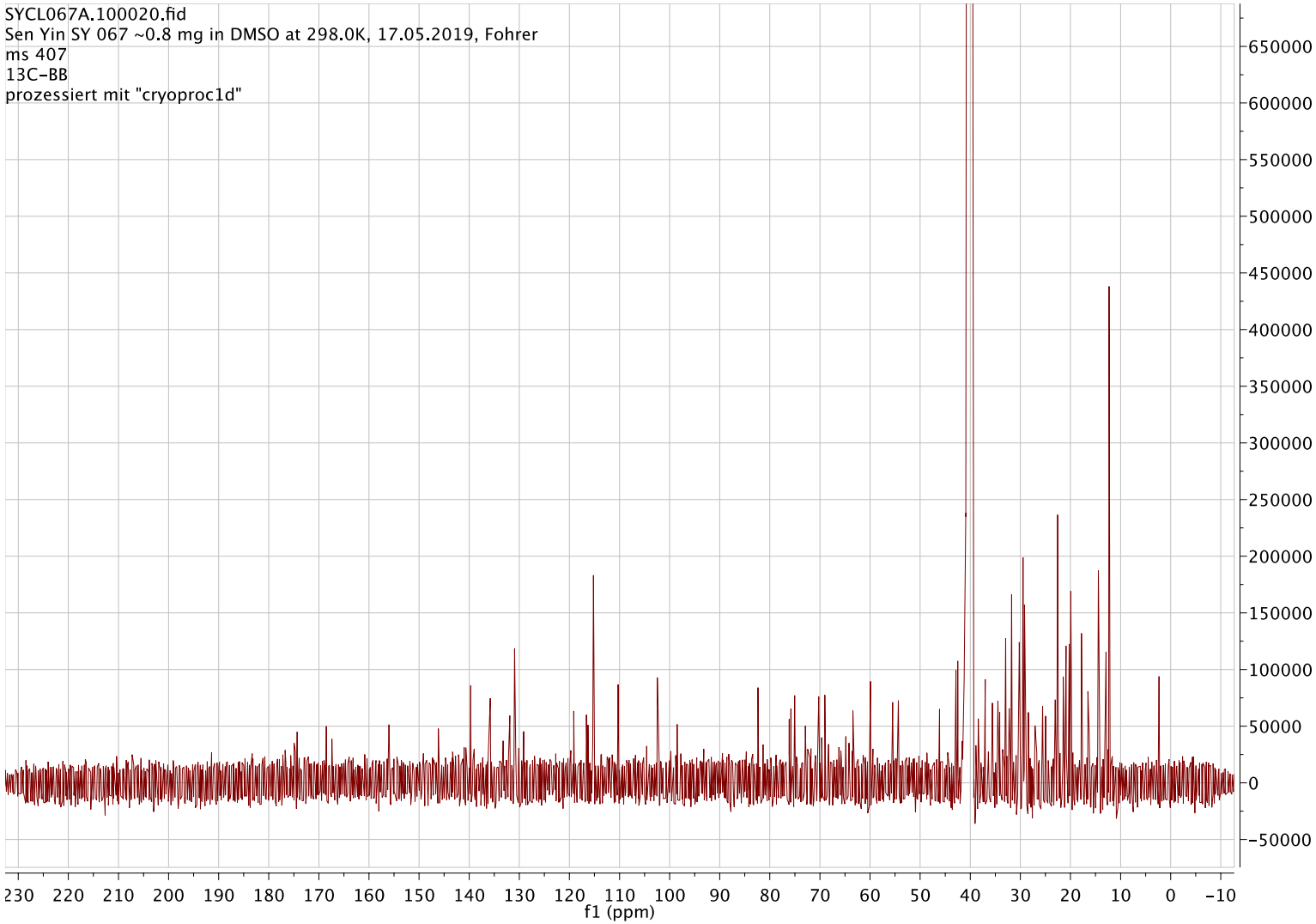


500 / 125 MHz, dmso-d_6					
Pos	**δ_H / ppm**	**mult. / Hz**	**δ_c / ppm**	**COSY**	**HMBC (H to C)**
2	-	-	175.1	-	-
3	-	-	102.5	-	-
4	-	-	191.2	-	-
5	3.68	m	69.9		
6	-	-	174.1	-	-
7	7.55	d, *J* = 15.8	129.1	8	6
8	7.18	m	139.5	7, 9	
9	6.47	m	131.3	8, 10	
10	6.63	m	140.2	9, 11	
11	6.34	m	127.0	10, 12	13
12	6.42	m	141.3	11	
13	-	-	133.1	-	-
14	5.41	d, *J* = 9.6	142.2	15, 19	12, 19
15	2.42	m	34.5	14, 18	13, 16
16	1.28, 1.36	m	29.8	15, 17	14, 15, 17, 18
17	0.81	t, *J* = 6.9	12.3	16	15, 16
18	0.95	d, *J* = 6.6	21.1	15	14, 15, 16
19	1.74	s	12.9	14	12, 13, 14
20	2.62, 2.84	m dd, *J* = 13.9, 3.9	37.5	5	22
21	-	-	128.5	-	-
22	6.96	d, *J* = 8.2	130.8	23	21, 23, 24
23	6.60	d, *J* = 8.2	115.2	22	21, 22, 24
24	-	-	156.1	-	-
24-OH	9.11	brs	-	-	23

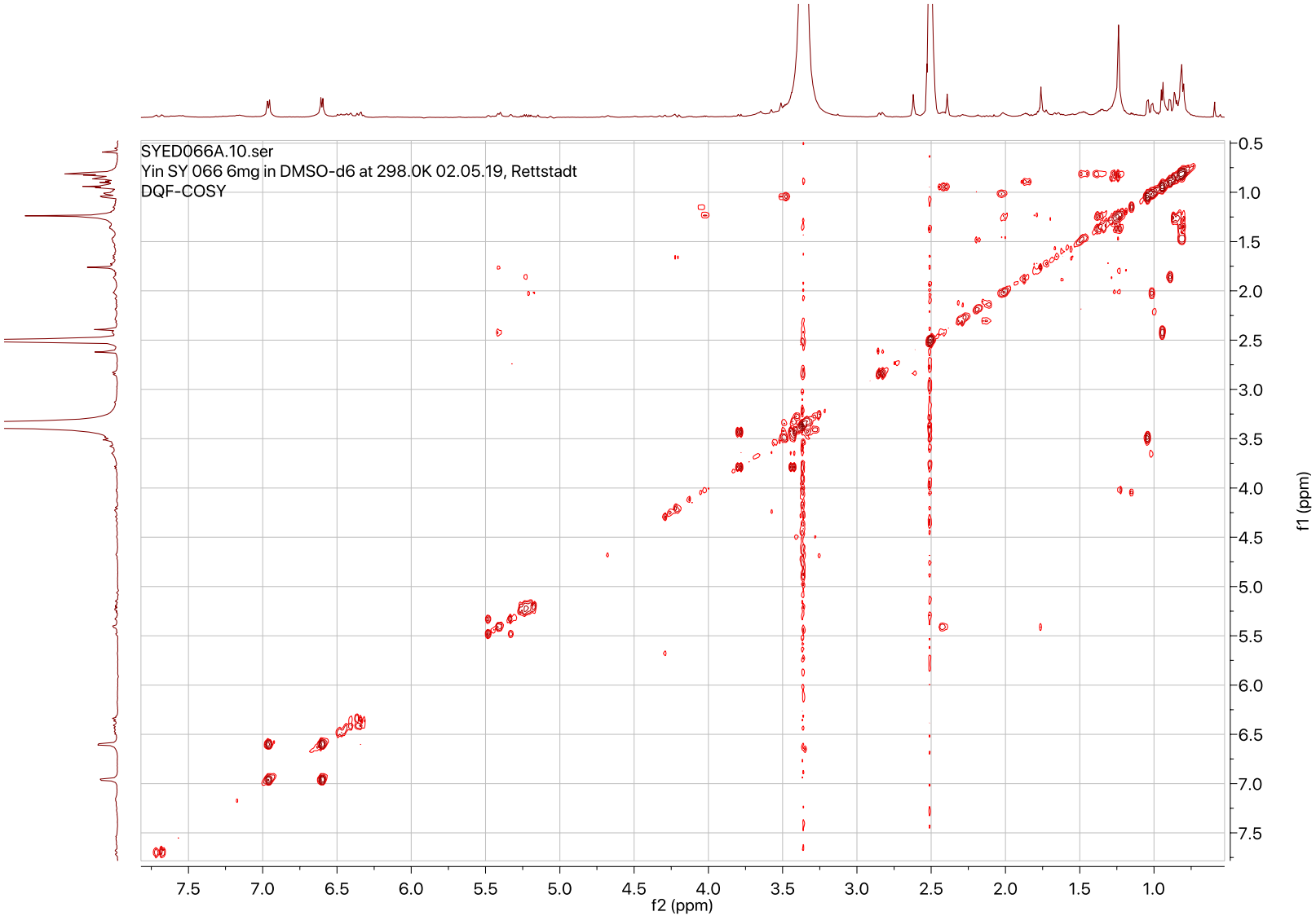
1H spectrum of 14



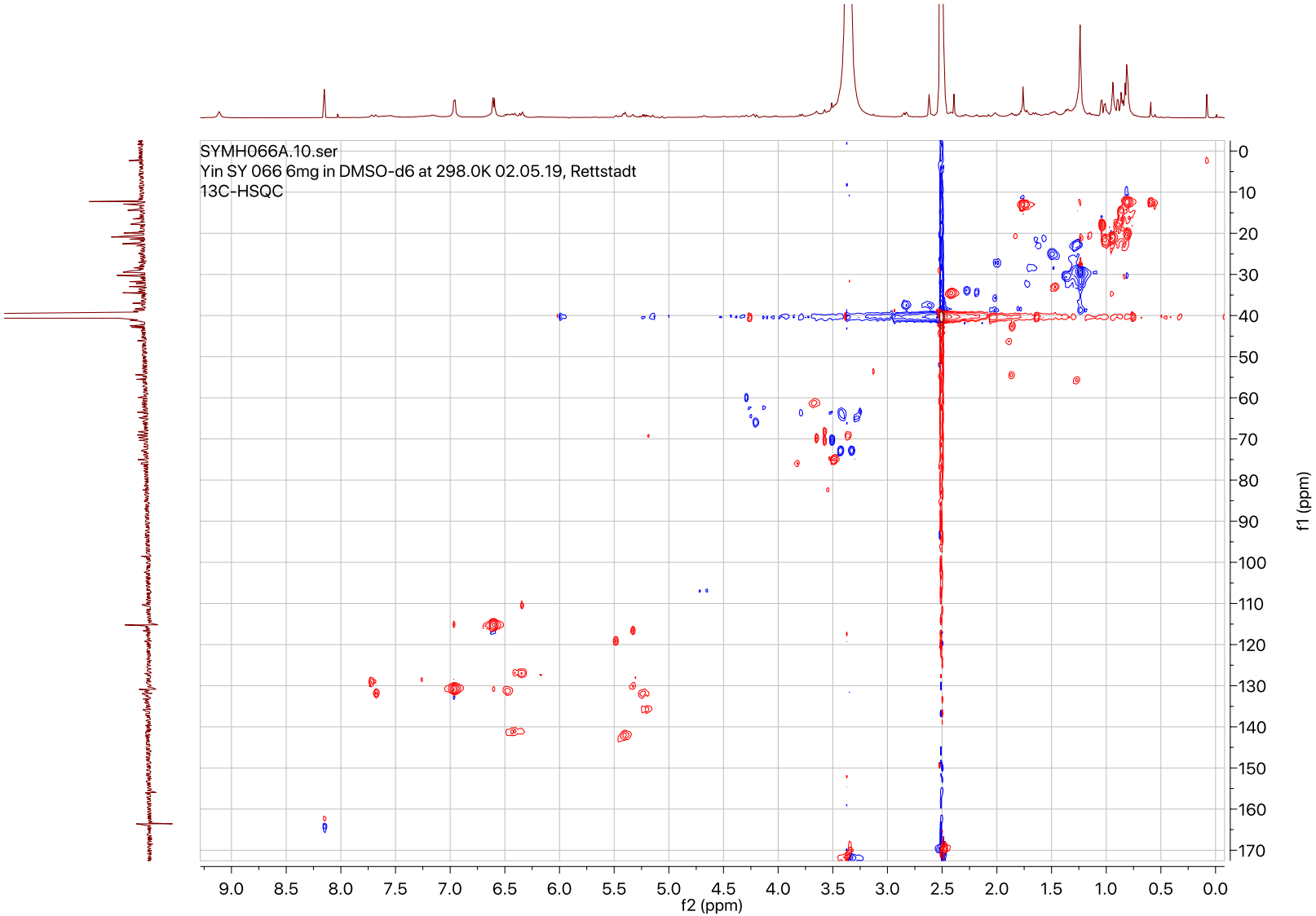
13C Spectrum of 14



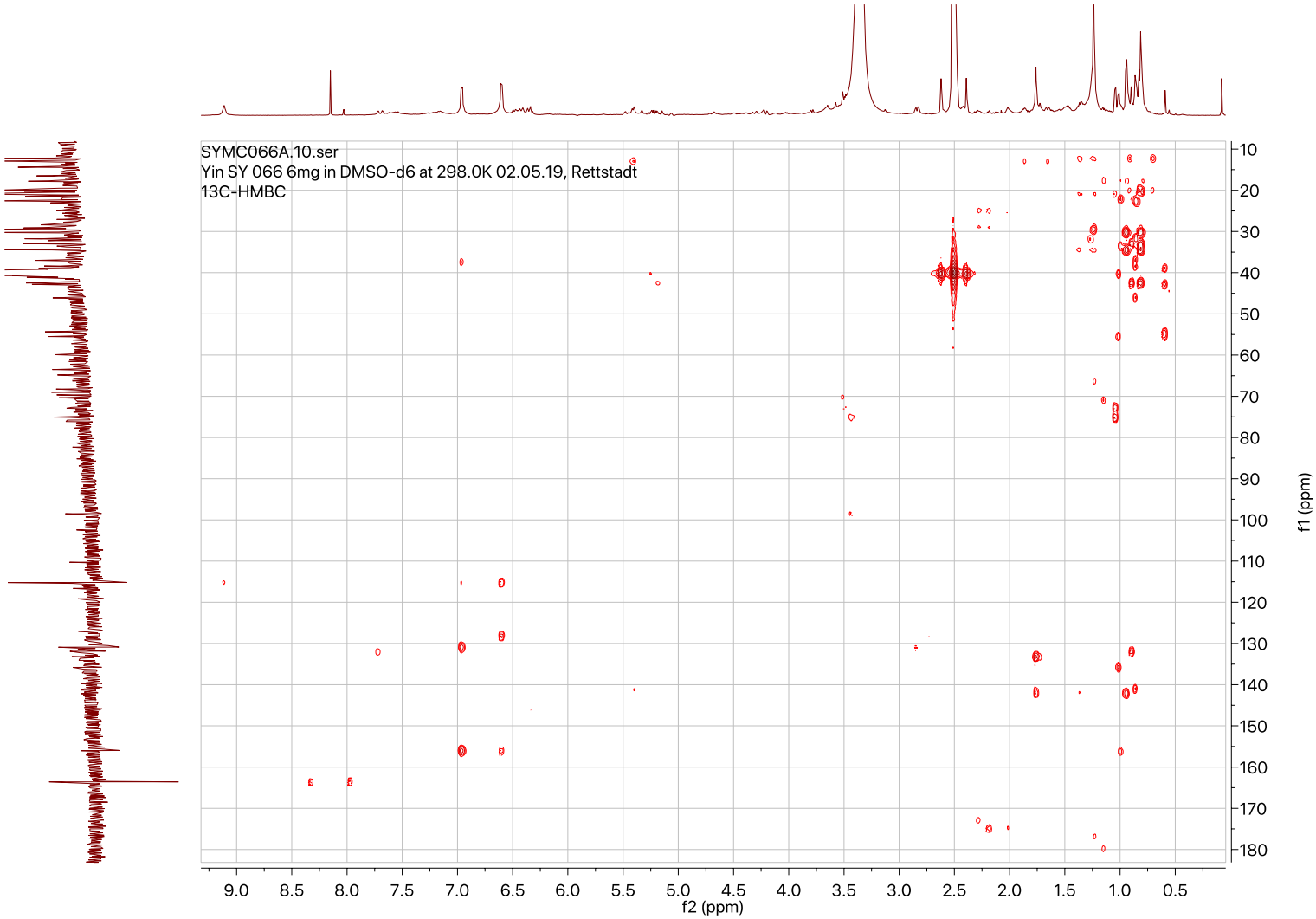
COSY spectrum of 14



HSQC Spectrum of 14



HMBC Spectrum of 14



9. Experimental Section

9.1 General

LC-MS data were obtained using a Waters LCMS system comprising of a Waters 2767 autosampler, Waters 2545 pump system, a Phenomenex Kinetex column (2.6 µ, C_{18}, 100 Å, 4.6 × 100 mm) equipped with a Phenomenex Security Guard precolumn (Luna C_5 300 Å) eluted at 1 mL/min. Detection was by Waters 2998 Diode Array detector between 200 and 400 nm; Waters 2424 ELSD and Waters SQD-2 mass detector operating simultaneously in ES+ and ES- modes between 100 *m/z* and 650 *m/z*. Solvents were: **A**, HPLC grade H_2O containing 0.05% formic acid; **B**, HPLC grade MeOH containing 0.045% formic acid; and **C**, HPLC grade CH_3CN containing 0.045% formic acid). Gradients were as follows.

Method 1. Kinetex/CH_3CN: 0 min, 10% **C**; 10 min, 90% **C**; 12 min, 90% **C**; 13 min, 10% **C**; 15 min, 10% **C**.

Semi-Preparative LCMS and compound purification.

Purification of compounds was generally achieved using a Waters mass-directed autopurification system comprising of a Waters 2767 autosampler, Waters 2545 pump system, a Phenomenex Kinetex Axia column (5µ, C_{18}, 100 Å, 21.2 × 250 mm) equipped with a Phenomenex Security Guard precolumn (Luna C_5 300 Å) eluted at 20 mL/min at ambient temperature. Solvent **A**, HPLC grade H_2O + 0.05% formic acid; Solvent **B**, HPLC grade CH_3CN + 0.045% formic acid. The post-column flow was split (100:1) and the minority flow was made up with HPLC grade MeOH + 0.045% formic acid to 1 $mL \cdot min^{-1}$ for simultaneous analysis by diode array (Waters 2998), evaporative light scattering (Waters 2424) and ESI mass spectrometry in positive and negative modes (Waters SQD-2). Detected peaks were collected into glass test tubes. Combined tubes were evaporated under a flow of dry N_2 gas, weighed, and residues dissolved directly in NMR solvent for NMR analysis.

9.2 Strains and culturing

Escherichia coli TOP10 (Invitrogen) was used as the host for plasmids that did not contain a Gateway destination cassette. Gateway destination vectors were propagated in *ccdB* survival cells (Invitrogen). *Saccharomyces cerevisiae* strain YPH499 (Stratagene) was used as the host for plasmid assembly by homologous recombination. *Aspergillus oryzae* strain M-2-3, an arginine auxotroph, was obtained from Professor Teruo Fujii, the University of Tokyo and mycelium was routinely maintained at 28 °C on MEA (3.36% malt extract agar). *Aspergillus oryzae* strain NSAR1 was obtained as a gift from the Kitamoto group.[1]

9.3 General techniques for DNA manipulation

Polymerase chain reactions were performed with PrimeSTAR® HS DNA Polymerase (TaKaRa Bio Inc.). PCR products were cloned into the pENTRY-YA vector (Invitrogen) through yeast homologous recombination and confirmed by DNA sequencing, and then transferred to the expression vector pTYGS-arg using Gateway LR *in vitro* recombination (Invitrogen) leading to constructs. Restriction digests were carried out according to the manufacturer's protocols (NEB, Fermentas, Promega). The primers used to amplify each fragment were synthesized by Sigma, and are listed in Table S1.

9.4 Rebuilding tenS with tenC expression system

The pTYGS-arg vector was digested with *Asc*I to produce the vector fragment. PCR fragment *tenC* was amplified using primers *XL1* and *XL2* using *Beauveria bassiana* genomic DNA as the template. Yeast recombination was used to reassemble the vector fragment with *tenC*, and the resulting plasmid pTYGS-arg-*tenC* was sequenced. Then, the previously constructed plasmid YA-*tenS* was transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *tenS* with *tenC*.

9.5 Construction of hybrid plasmids

***TenS* (Δ *dmbS*-*C*MeT)**

The plasmid YA-*tenS* was digested with *Xba*I to excise the DNA sequences of the partial AT, DH, *C*MeT and partial ΨKR domains of *tenS* to produce the vector fragment VF1. Fragments F2 and F3 were amplified by PCR using *XL3* and *XL4*, *XL5* and *XL6* respectively as primer and YA-*tenS* as the template. Fragment F4 harbouring the swap sequence was amplified by PCR using primers *XL7* and *XL8* and the synthetic *dmbS*-*C*MeT as template. Yeast recombination was used to assemble the vector fragment VF1 with F2, F3 and F4. The resulting plasmid YA-*tenS* (Δ*dmbS*-*C*MeT) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-*C*MeT).

***TenS* (Δ *dmbS*-1-*C*MeT)**

Fragment F5 harbouring swap sequence was amplified using primers *XL7* and *XL9* and the synthetic *dmbS*-*C*MeT as template. Fragment F6 was amplified by PCR using *XL10* and *XL11* as primers and YA-*tenS* as the template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F5 and F6. The resulting plasmid YA-*tenS* (Δ*dmbS*-1-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using

Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-1-*C*MeT).

***TenS* (Δ *dmbS*-2-*C*MeT)**

Fragment F7 was amplified by PCR using *XL12* and *XL13* as primers and YA-*tenS* as the template. Fragment F8 harbouring swap sequence was amplified by PCR using primers *XL8* and *XL14* and the synthetic *dmbS*-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F7 and F8. The resulting plasmid YA-*tenS* (Δ*dmbS*-2-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-2-*C*MeT).

***TenS* (Δ *dmbS*-1A-*C*MeT)**

Fragment F9 harbouring swap sequence was amplified by PCR using primers *XL15* and *XL16* and the synthetic *dmbS*-*C*MeT as template. Fragment F10 was amplified by PCR using *XL17* and *XL18* as primers and YA-*tenS* as the template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F6, F9 and F10. The resulting plasmid YA-*tenS* (Δ*dmbS*-1A-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-1A-*C*MeT).

***TenS* (Δ *dmbS*-1B-*C*MeT)**

Fragment F11 was amplified by PCR using *XL12* and *XL19* as primers and YA-*tenS* as the template. Fragment F12 harbouring swap sequence was amplified by PCR using primers *XL20* and *XL21* and the synthetic *dmbS*-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F6, F11 and F12. The resulting plasmid YA-*tenS* (Δ*dmbS*-1B-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-1B-*C*MeT).

***Ten*S (Δ *dmb*S-2A-*C*MeT)**

Fragment F13 harbouring swap sequence was amplified by PCR using primers *XL14* and *XL22* and the synthetic *dmbS*-*C*MeT as template. Fragment F14 was amplified by PCR using *XL11* and *XL23* as primers and YA-*tenS* as the template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F7, F13 and F14. The resulting plasmid YA-*tenS* (Δ*dmbS*-2A-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-2A-*C*MeT).

***Ten*S (Δ *dmb*S-2B-*C*MeT)**

Fragment F15 was amplified by PCR using *XL24* and *XL25* as primers and YA-*tenS* as the template. Fragment F16 harbouring swap sequence was amplified by PCR using primers *XL8* and *XL26* and the synthetic *dmbS*-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F7, F15 and F16. The resulting plasmid YA-*tenS* (Δ*dmbS*-2B-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-2B-*C*MeT).

***TenS* (Δ *dmb*S-1A1-*C*MeT)**

Fragment F26 harbouring swap sequence was amplified by PCR using primers *XL38* and *XL39* and the synthetic *dmbS*-1A1-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F6, F10 and F26. The resulting plasmid YA-*tenS* (Δ*dmbS*-1A1-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-1A1-*C*MeT).

***TenS* (Δ *dmbS*-1A2-*C*MeT)**

Fragment F27 harbouring swap sequence was amplified by PCR using primers *XL38* and *XL40* and the synthetic *dmbS*-1A2-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F6, F10 and F27. The resulting plasmid YA-*tenS* (Δ*dmbS*-1A2-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-1A2-*C*MeT).

***TenS* (Δ *dmbS*-1B1-*C*MeT)**

Fragment F31 harbouring swap sequence was amplified by PCR using primers *XL47* and *XL48* and the synthetic *dmbS*-1B1-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F6, F11 and F31. The resulting plasmid YA-*tenS* (Δ*dmbS*-1B1-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-1B1-*C*MeT).

***TenS* (Δ *dmbS*-1B2-*C*MeT)**

Fragment F32 harbouring swap sequence was amplified by PCR using primers *XL47* and *XL48* and the synthetic *dmbS*-1B2-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F6, F11 and F32. The resulting plasmid YA-*tenS* (Δ*dmbS*-1B2-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-1B2-*C*MeT).

***Ten*S (Δ *dmb*S-2A1-*C*MeT)**

Fragment F24 harbouring swap sequence was amplified by PCR using primers *XL34* and *XL35* and the synthetic *dmbS*-2A1-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F7, F14 and F24. The resulting plasmid YA-*tenS* (Δ*dmbS*-2A1-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-2A1-*C*MeT).

***Ten*S (Δ *dmb*S-2A2-*C*MeT)**

Fragment F25 harbouring swap sequence was amplified by PCR using primers *XL36* and *XL37* and the synthetic *dmbS*-2A2-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F7, F14 and F25. The resulting plasmid YA-*tenS* (Δ*dmbS*-2A2-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-2A2-*C*MeT).

***TenS* (Δ *dmbS*-2B1-*C*MeT)**

Fragment F17 harbouring swap sequence was amplified by PCR using primers *XL27* and *XL28* and the synthetic *dmbS*-2B1-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F7, F15 and F17. The resulting plasmid YA-*tenS* (Δ*dmbS*-2B1-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-2B1-*C*MeT).

***TenS* (Δ *dmbS*-2B2-*C*MeT)**

Fragment F18 harbouring swap sequence was amplified by PCR using primers *XL27* and *XL28* and the synthetic *dmbS*-2B2-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F7, F15 and F18. The resulting plasmid YA-*tenS* (Δ*dmbS*-2B2-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-2B2-*C*MeT).

***TenS* (Δ *dmbS*-2A+2B1-*C*MeT)**

Fragment F19 harbouring swap sequence was amplified by PCR using primers *XL14* and *XL29* and the synthetic *dmbS*-*C*MeT as template. Fragment F20 was amplified by PCR using *XL6* and *XL30* as primers and YA-*tenS* as the template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F7, F19 and F20. The resulting plasmid YA-*tenS* (Δ*dmbS*-2A+2B1-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-2A+2B1-*C*MeT).

***TenS* (Δ *dmbS*-2A2+2B1-*C*MeT)**

Fragment F21 was amplified by PCR using *XL12* and *XL31* as primers and YA-*tenS* as the template. Fragment F22 harbouring swap sequence was amplified by PCR using primers *XL32* and *XL33* and the synthetic *dmbS*-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F20, F21 and F22. The resulting plasmid YA-*tenS* (Δ*dmbS*-2A2+2B1-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-2A2+2B1-*C*MeT).

***TenS* (Δ *dmbS*-2A2+2B-*C*MeT)**

Fragment F23 harbouring swap sequence was amplified by PCR using primers *XL8* and *XL32* and the synthetic *dmbS*-*C*MeT as template. Yeast recombination was used to reassemble the vector fragment VF1 with F2, F3, F21 and F23. The resulting plasmid YA-*tenS* (Δ*dmbS*-2A2+2B-*C*MeT) harbouring the domain swap was sequenced and then transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-2A2+2B-*C*MeT).

***TenS* (Δ *dmbS*-3-ΨKR)**

The plasmid YA-*tenS* was digested with *Kpn*I to excise the DNA sequences of the partial DH, *C*MeT, ΨKR, ER and partial KR domains of *tenS* to produce the vector fragment VF2. Fragment F28 harbouring the swap sequence was amplified by PCR using primers *XL41* and *XL42* and the synthetic *dmbS*-ΨKR-*C*MeT as template. Fragments F29 and F30 were amplified by PCR using *XL43* and *XL44, XL45* and *XL46* respectively as primer and YA-*tenS* as the template. Yeast recombination was used to assemble the vector fragment VF2 with F28, F29 and F30. The resulting plasmid YA-*tenS* (Δ *dmbS*-3-ΨKR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-3-ΨKR).

***TenS* (Δ *dmbS*-3a-ΨKR)**

Fragment F33 harbouring the swap sequence was amplified by PCR using primers *XL41* and *XL42* and the synthetic *dmbS*-1-ΨKR-*C*MeT as template. Yeast recombination was used to assemble the vector fragment VF2 with F29, F30 and F33. The resulting plasmid YA-*tenS* (Δ *dmbS*-3a-ΨKR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-3a-ΨKR).

***TenS* (Δ *dmbS*-3b-ΨKR)**

Fragment F34 harbouring the swap sequence was amplified by PCR using primers *XL41* and *XL42* and the synthetic *dmbS*-2-ΨKR-*C*MeT as template. Yeast recombination was used to assemble the vector fragment VF2 with F29, F30 and F34. The resulting plasmid YA-*tenS* (Δ *dmbS*-3b-ΨKR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ *dmbS*-3b-ΨKR).

***TenS* (Δ *dmbS*-4-KR)**

The plasmid pE-YA-*tenS* was digested with *XbaI* and *AgeI* to produce the vector fragment VF3. Fragment F35 was amplified by PCR (Q5) using tenSF1 and tenSR1 as primers and pE-YA-*tenS* as the template to create a patch for VF3. Fragments F36 and F37 were amplified by PCR (Q5) using TDSLk1-F and TDSLk1-R, T2A-F and TDSLk2-R respectively as primers and pE-YA-*tenS* as the template. Fragment F38 harbouring the swap sequence was amplified by PCR using D1A-

TLk-F and D1B-TLk-R as primers with pE-YA1-*dmbS* as the template. Yeast recombination was used to assemble the vector fragment VF3 with F35, F36, F37 and F38. The resulting plasmid pE-YA-*tenS*(Δ*dmbS*-4-KR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ*dmbS*-4-KR).

***TenS* (Δ *dmbS*-4A-KR)**

Fragment F39 was amplified by PCR using T1B-F and TDSLk2-R as primers and pE-YA-*tenS* as the template. Fragment F40 harbouring the swap sequence was amplified by PCR using D1A-TLk-F and D1A-TLk-R as primers with pE-YA1-*dmbS* as the template. Yeast recombination was used to assemble the vector fragment VF3 with F35, F36, F39 and F40. The resulting plasmid pE-YA-*tenS*(Δ*dmbS*-4A-KR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ*dmbS*-4A-KR).

***TenS* (Δ *dmbS*-4B-KR)**

Fragment F41 was amplified by PCR (Q5) using TDSLk1-F and T1A-R as primers and pE-YA-*tenS* as the template. Fragment F42 harbouring the swap sequence was amplified by PCR using D1B-TLk-F and D1B-TLk-R as primers with pE-YA1-*dmbS* as the template. Yeast recombination was used to assemble the vector fragment VF3 with F35, F37, F41 and F42. The resulting plasmid pE-YA-*tenS*(Δ*dmbS*-4B-KR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ*dmbS*-4B-KR).

***TenS* (Δ *dmbS*-5-KR)**

Fragment F43 and F44 were amplified by PCR (Q5) using TDSLk1-F and T1B-R, TDSLk2-F and TDSLk2-R respectively as primers and pE-YA-*tenS* as the template. Fragment F45 harbouring the swap sequence was amplified by PCR using D2A-TLk-F and D2B-TLk-R as primers with pE-YA1-*dmbS* as the template. Yeast recombination was used to assemble the vector fragment VF3 with F35, F43, F44 and F45. The resulting plasmid pE-YA-*tenS*(Δ*dmbS*-5-KR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ*dmbS*-5-KR).

***TenS* (Δ *dmbS*-5A-KR)**

Fragment F46 was amplified by PCR (Q5) using T2B-F and TDSLk2-R as primers and pE-YA-*tenS* as the template. Fragment F47 harbouring the swap sequence was amplified by PCR using D2A-TLk-F and D2A-TLk-R as primers with pE-YA1-*dmbS* as the template. Yeast recombination was used to assemble the vector fragment VF3 with F35, F43, F46 and F47. The resulting plasmid pE-YA-*tenS*(Δ*dmbS*-5A-KR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ*dmbS*-5A-KR).

***TenS* (Δ *dmbS*-5B-KR)**

Fragment F48 was amplified by PCR (Q5) using TDSLk1-F and T2A-R as primers and pE-YA-*tenS* as the template. Fragment F49 harbouring the swap sequence was amplified by PCR using D2B-TLk-F and D2B-TLk-R as primers with pE-YA1-*dmbS* as the template. Yeast recombination was used to assemble the vector fragment VF3 with F35, F44, F48 and F49. The resulting plasmid pE-YA-*tenS*(Δ*dmbS*-5B-KR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ*dmbS*-5B-KR).

***TenS* (Δ *milS*-KR)**

The plasmid pE-YA-*tenS* was digested with *XbaI* and *AgeI* to produce the vector fragment VF3. Fragment F35 was amplified by PCR (Q5) using tenSF1 and tenSR1 as primers and pE-YA-*tenS* as the template to create a patch for VF3. Fragments F51 and F52 were amplified by PCR (Q5) using TDSLk1-F and TDSLk1-R, T2A-F and TDSLk2-R respectively as primers and pE-YA-*tenS* as the template. Fragment F50 harbouring the swap sequence was amplified by PCR using TMS-F and TMS-R as primers with pE-YA1-*milS* as the template. Yeast recombination was used to assemble the vector fragment VF3 with F35, F50, F51 and F52. The resulting plasmid pE-YA-*tenS*(Δ*milS*-KR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ*milS*-KR).

***TenS* (Δ *milS*-3/4-KR)**

The plasmid pE-YA-*tenS* was digested with *XbaI* and *AgeI* to produce the vector fragment VF3. Fragment F35 was amplified by PCR (Q5) using tenSF1 and tenSR1 as primers and pE-YA-*tenS* as the template to create a patch for VF3. Fragments F53 and F54 were amplified by PCR (Q5) using TDSLk1-F and TDSLK1(3/4)-R, TDSLK1(3/4)-F and TDSLk2-R respectively as primers and pE-YA-*tenS* as the template. Fragment F55 harbouring

the swap sequence was amplified by PCR using TenS(3/4)-F and TenS(3/4)-R as primers with pE-YA1-*milS* as the template. Yeast recombination was used to assemble the vector fragment VF3 with F35, F53, F54 and F55. The resulting plasmid pE-YA-*tenS*(Δ*milS*-KR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ*milS-3/4*-KR).

***TenS* (Δ *milS-12m*-KR)**

The plasmid pE-YA-*tenS* was digested with *XbaI* and *AgeI* to produce the vector fragment VF3. Fragment F35 was amplified by PCR (Q5) using tenSF1 and tenSR1 as primers and pE-YA-*tenS* as the template to create a patch for VF3. Fragments F57 and F58 were amplified by PCR (Q5) using TDSLk1-F and LK1sub-R, LK2sub-F and TDSLk2-R respectively as primers and pE-YA-*tenS* as the template. Yeast recombination was used to assemble the vector fragment VF3 with F35, F57 and F58. The resulting plasmid pE-YA-*tenS*(Δ*milS-12m*-KR) harbouring the domain swap was sequenced and transferred to the expression vector pTYGS-arg-*tenC* using Gateway LR *in vitro* recombination (Invitrogen) leading to *TenS* (Δ*milS-12m*-KR).

9.6 Transformation of Aspergillus oryzae M-2-3

Plasmid DNA for fungal transformation was prepared using Fermentas Miniprep kits. *A. oryzae* M-2-3 was grown on MEA plate for 10 days. Spores washed by 4 mL sterile water were inoculated into 100 mL GNB liquid medium (2% glucose, 1% nutrient broth number 2(from Thermo Scientific)) and cultivated for 1 day at 30 °C, 250 rpm. Collect the mycelia on a sterile filter paper (autoclaved with a filter funnel) under vacuum and wash with sterile water, then 0.8 M NaCl. Put the mycelia in a sterile falcon centrifuge tube. Add 10 ml of filter-sterilized protoplasting solution (20 mg/ml lysing enzyme, Sigma L-1412, 10 ml/ml amount of driselase, 0.8 M NaCl, 10 mM Na phosphate buffer pH 6) and incubate at 30 °C, 100rpm for no longer than 3 hours. Filter the protoplasting solution through a syringe with glasswool inside. Centrifuge the filtrate at 3000 rpm for 10 min. Wash the pelleted protoplasts once with 0.8 M NaCl (ca. 15 ml) and then once with Solution 1 (0.8 M NaCl, 10 mM $CaCl_2$, 50 mM Tris HCl pH 7.5). Resuspend the protoplasts in Solution 1 to final concentration of 2.5×10^8/ml and add 1/5 volume of Solution 2 (PEG 4000 (60% w/v) in solution 1 but 50 mM $CaCl_2$). Put 0.2 ml portions into Falcon tubes. Add plasmid DNA (<20 μl) and place on ice for 30 min. Add 1 ml of Sol 2, mix well gently and place at room temperature for 20 min. 10 ml soft agar (0.8% agar containing 5% NaCl) was added to the transformation mixtures, and then poured onto two Czapek-Dox plates supplemented with sorbitol (1 M) and incubated at 28˚C for 5-10 days.

9.7 Transformation of Aspergillus oryzae NSAR1

A. oryzae was grown for 4 days on MEA solid medium at 30°C until sporulation occurred. Conidia harvested from a single plate were used to inoculate 50 ml of GNB liquid medium (1% (w/v) glucose, 2% (w/v) nutrient broth no. 2 (*Thermo Fisher*) and incubated at 28 °C with shaking at 200 rpm overnight. The germinated *A. oryzae* conidia were centrifuged at 8000 rpm for 10 min and the supernatant discarded. The pellet was washed once with H_2O and once with 0.8 M NaCl. The pellet was then resuspended in 10 ml of filter sterilised protoplasting solution (20 mg/ml *Trichoderma* lysing enzyme and 5 mg/ml Driselase in 0.8 M NaCl), and incubated at room temperature with gentle shaking for 1–1.5 hours. Protoplasts were released from hyphae by pipetting with a wide-bore 5 ml pipette, and then filtered through sterile miracloth. The protoplasts were then centrifuged at 1000 x g for 5 min and the supernatant discarded. The pellet was then washed with solution 1 (0.8 M NaCl, 10 mM $CaCl_2$, 50 mM Tris-HCl pH 7.5). The pellet was resuspended in 200-500 µl of solution 1, and, for each transformation, 100 µl transferred to a 50 ml centrifuge tube on ice. 5-10 µg (10 µl max) of plasmid DNA was added to the protoplasts and gently mixed. The tube was incubated on ice for 2 min, after which 1 ml of solution 2 (60% (w/v) PEG 3350, 0.8 M NaCl, 10 mM $CaCl_2$, 50 mM Tris-HCl pH 7.5) was added and the tube was incubated at room temperature for 20 min. 40 ml of molten (approx. 50 °C) CZD/S top medium with appropriate supplements (3.5% (w/v) Czapek Dox broth, 1 M sorbitol, 0.8% (w/v) agar) was added and gently mixed. 10 ml each of the mix was overlaid onto four plates prepared with appropriate supplements (15 ml of 3.5% (w/v) Czapek Dox broth, 1 M sorbitol, 1.5% (w/v) agar). The plates were then incubated at 28 °C for 3-5 days until colonies appeared.

10. Table S2. List of primers used in this study (Blue, *tenS* sequence; Red, *dmbS* sequence; Green, vector sequence; Black, *tenC* sequence).

Primer	template	direction	sequence 5' - 3'	amplification
XL1	*B. bassiana* gDNA	fwd	TCAACACAAGATCCCAAAGTCAAAGGCGCGATGGCAGCCATCTCTTCCC	*Ten*C
XL2	*B. bassiana* gDNA	rev	CTGGTAGACGTCATATAATCATACGGCGCGTCAGGGCAGCGCCTCCTCT	*Ten*C
XL3	*tenS*	fwd	TGCAGCAACCTATGCGAGC	F2 , F23
XL4	*tenS*	rev	TGCGGTCTGATTCAGAGCCC	F2
XL5	*tenS*	fwd	GCCGTGGATGACACGTTCTATGC	F3
XL6	*tenS*	rev	CACCCACAAGAGGTTTCGTGTATTG	F3, F20
XL7	*dmbS*-CMeT	fwd	ATTGATTCTTGGGCTCTGAATCAGACCGCATCATCCTTGACCGGGGATCTC	F4, F5
XL8	*dmbS*-CMeT	rev	GAGCCGGGCATAGAACGTGTCATCCACGGCTTGACTCATAATCATGGAGTTTTGCTGCTTG	F4, F8, F16, F23
XL9	*dmbS*-CMeT	rev	AGCATTCTCGAAAAAGCCAACCGAGAGATCGGTGTAAGTGTATGTATCGAATGTCTCATCAATTG	F5
XL10	*tenS*	fwd	GATCTCTCGGTTGGCTTTTTCGAGAATGC	F6
XL11	*tenS*	rev	GAGCCGGGCATAGAACGTGTCATCC	F6, F14
XL12	*tenS*	fwd	TTCAACGCCGCGATTGATTCTTG	F7, F11, F21
XL13	*tenS*	rev	TGTGTAAGTATACGTGTCGAATGCC	F7
XL14	*dmbS*-CMeT	fwd	GGTGAGGCATTCGACACGTATACTTACACAGATCTATCGGTTGGCTTCTTCG	F8, F13, F19
XL15	*dmbS*-CMeT	fwd	ATTGATTCTTGGGCTCTGAATCAGACCGCATCATCCTTGACCGGGGATC	F9
XL16	*dmbS*-CMeT	rev	GGCGTGAAGCATCTGCAACTCGACAGCATCTGGGTACGCCTCATCAATG	F9
XL17	*tenS*	fwd	GATGCTGTCGAGTTGCAGATGC	F10
XL18	*tenS*	rev	GCGGAAAATCTCTCGACAGC	F10
XL19	*tenS*	rev	TGGGTATGCCTCATCAATGG	F11
XL20	*dmbS*-CMeT	fwd	GTCATCCAAACCATTGATGAGGCATACCCAGATACTGTTGAGTTGCAGATGC	F12
XL21	*dmbS*-CMeT	rev	AGCATTCTCGAAAAAGCCAACCGAGAGATCGGTGTAAGTGTATGTATCGAATG	F12
XL22	*dmbS*-CMeT	rev	ACCAAAGTTAAAGGTAGCGCGAAGACTCTCTGGGCCAGTCTTTTCGTTC	F13
XL23	*tenS*	fwd	GAGAGTCTTCGCGCTACCTTTAACTTTG	F14
XL24	*tenS*	fwd	GCAATTGGTGAGGCATTCG	F15
XL25	*tenS*	rev	TGGGCCAGTCTTTTCGTTTAATAGC	F15

XL26	*dmbS*-*C*MeT	fwd	TATCTGCTATTAAACGAAAAGACTGGCCCAGAGAGTCTTCGCGCCACC	F16
XL27	*dmbS*-2B1-*C*MeT and *dmbS*-2B2-*C*MeT	fwd	AGCCCTCTTGAAGCCCGGC	F17, F18
XL28	*dmbS*-2B1-*C*MeT and *dmbS*-2B2-*C*MeT	rev	CATTTCGGAAAGCGGGGAGAGC	F17, F18
XL29	*dmbS*-*C*MeT	rev	TCGTGTACTATATGATCAACGCCAGAGAACGAGGCCTTTTGGAGCTGCGAATCCCAG	F19
XL30	*tenS*	fwd	GCCTCGTTCTCTGGCGTTGATC	F20
XL31	*tenS*	rev	TGGGTCTTTCTCAATATCGAGGGC	F21
XL32	*dmbS*-*C*MeT	fwd	TGGTCTTTAGAGCCCTCGATATTGAGAAAGACCCAGCCGCACAAAGCTTCGATCTCG	F22, F23
XL33	*dmbS*-*C*MeT	rev	TCGTGTACTATATGATCAACGCCAGAGAACGAGGCCTTTTGGAGCTGCGAATCCCAG	F22
XL34	*dmbS*-2A1-*C*MeT	fwd	GAGTGCAATTGGTGAGGCATTC	F24
XL35	*dmbS*-2A1-*C*MeT	rev	AGCCCACCAAAGTTAAAGGTAG	F24
XL36	*dmbS*-2A2-*C*MeT	fwd	CAATTGGTGAGGCATTCGAC	F25
XL37	*dmbS*-2A2-*C*MeT	rev	AGCCCACCAAAGTTAAAGGTAG	F25
XL38	*dmbS*-1A1-*C*MeT	fwd	ATTGATTCTTGGGCTCTGAATCAGAC	F26, F27
XL39	*dmbS*-1A1-*C*MeT	rev	GCCCAACGGCGTGAAGCATCTGCAACTCGACAGCATCTGGGTATGCCTCATCAATGGTTTG	F26
XL40	*dmbS*-1A2-*C*MeT	rev	GCCCAACGGCGTGAAGCATCTGCAACTCGACAGCATCTGGGTACGCCTCATCAATGATTTG	F27
XL41	*dmbS*-ΨKR-*C*MeT, *dmbS*-1-ΨKR-*C*MeT and *dmbS*-2-ΨKR-*C*MeT	fwd	ATGCCCAGCTCCAAAAGGCCTC	F28, F33, F34
XL42	*dmbS*-ΨKR-*C*MeT, *dmbS*-1-ΨKR-*C*MeT and *dmbS*-2-ΨKR-*C*MeT	rev	TCAATGCCAAGAACTTGGGTTCG	F28, F33, F34
XL43	*tenS*	fwd	ACTGCTACCTTATTCACCCTGCC	F29
XL44	*tenS*	rev	TTGACTCATGATCATGGAGTTTTGC	F29

XL45	*tenS*	fwd	CAAAACTCTAGCTCCATGACTCCCAGAGC	F30
XL46	*tenS*	rev	ACCCATTATGATGTTGTGGGAGCCGC	F30
XL47	*dmbS*-1B1-*C*MeT and *dmbS*-1B2-*C*MeT	fwd	AGACGACTGGGCCGTCATCCAAACCATTGATG	F31, F32
XL48	*dmbS*-1B1-*C*MeT and *dmbS*-1B2-*C*MeT	rev	TGCGGAAAATCTCTCGACAGCATTCTCG	F31, F32
tenSF1	*pE-YA-tenS*	fwd	GGTCCTTGTCTGAAGAGTTG	F35
tenSR1	*pE-YA-tenS*	rev	GGATATCACAAGCAAGAAGC	F35
TDSLk1-F	*pE-YA-tenS*	fwd	AGATCAGCAGGATAAGCAGC	F36, F41, F43, F48
TDSLk1-R	*pE-YA-tenS*	rev	AAGCCCACGGGTCTGGAG	F36
TDSLk2-F	*pE-YA-tenS*	fwd	GCCGCATCGGCGGTAGAC	F44
TDSLk2-R	*pE-YA-tenS*	rev	TCCTTTGGTGGTGGTGATG	F37, F39, F44, F46
T2A-F	*pE-YA-tenS*	fwd	GCCATTCTGAATAATACAGGCC	F37
T2A-R	*pE-YA-tenS*	rev	CTCAGAGACACTCATGACTCG	F48
T1B-F	*pE-YA-tenS*	fwd	ACTGTGGTGGACATGATTCG	F39
T1B-R	*pE-YA-tenS*	rev	AGCGCTCGAGCTTAGCAA	F43
T2B-F	*pE-YA-tenS*	fwd	ACGGATGTGCATCATGCCTT	F46
T1A-R	*pE-YA-tenS*	rev	CTGCACAGAGTCTTTGCTGC	F41
D1A-TLk-F	*pE-YA1-dmbS*	fwd	ACTGTACCGCCCCTCCAGACCCGTGGGCTTTTCAAGAGCGACAGGACCTA	F38, F40
D1A-TLk-R	*pE-YA1-dmbS*	rev	CATGGTGGCACGAATCATGTCCACCACAGTCTGCACAGAGTCTTTGTTGC	F40
D1B-TLk-F	*pE-YA1-dmbS*	fwd	ATGGACGCTTGCAGCAAAGACTCTGTGCAGACTGTCGTGGATACGATTCG	F42
D1B-TLk-R	*pE-YA1-dmbS*	rev	GTTTGACTGGCCTGTATTATTCAGAATGGCAGCGGCAGAACCAAGCAGAA	F38, F42
D2A-TLk-F	*pE-YA1-dmbS*	fwd	GACTTTTTTGTCTTGCTAAGCTCGAGCGCTGCCATCTTGAATAACATGGG	F45, F47
D2A-TLk-R	*pE-YA1-dmbS*	rev	CGCCTCAGCAAAGGCATGATGCACATCCGTCTCAGAGAGCCTCATAGCTC	F47
D2B-TLk-F	*pE-YA1-dmbS*	fwd	GGTACCACGCGAGTCATGAGTGTCTCTGAGACTGACGTGCATCACGCCTT	F49
D2B-TLk-R	*pE-YA1-dmbS*	rev	CGCTAGACTGTCTACCGCCGATGCGGCGGCTGCTTGCCCCGAGGCAATCA	F45, F49

Primer	template	direction	sequence 5' - 3'	amplification
tenSF1	*pE-YA-tenS*	fwd	GGTCCTTGTCTGAAGAGTTG	F35
tenSR1	*pE-YA-tenS*	rev	GGATATCACAAGCAAGAAGC	F35
TDSLk 1-F	*pE-YA-tenS*	fwd	AGATCAGCAGGATAAGCAGC	F36, F41, F43, F48, F51, F53, F58
TDSLk 1-R	*pE-YA-tenS*	rev	AAGCCCACGGGTCTGGAG	F36, F51
TDSLk 2-F	*pE-YA-tenS*	fwd	GCCGCATCGGCGGTAGAC	F44, F52
TDSLk 2-R	*pE-YA-tenS*	rev	TCCTTTGGTGGTGGTGATG	F37, F39, F44, F46, F52, F54, F57
T2A-F	*pE-YA-tenS*	fwd	GCCATTCTGAATAATACAGGCC	F37
T2A-R	*pE-YA-tenS*	rev	CTCAGAGACACTCATGACTCG	F48
T1B-F	*pE-YA-tenS*	fwd	ACTGTGGTGGACATGATTCG	F39
T1B-R	*pE-YA-tenS*	rev	AGCGCTCGAGCTTAGCAA	F43
T2B-F	*pE-YA-tenS*	fwd	ACGGATGTGCATCATGCCTT	F46
T1A-R	*pE-YA-tenS*	rev	CTGCACAGAGTCTTTGCTGC	F41
D1A-TLk-F	*pE-YA1-dmbS*	fwd	ACTGTACCGCCCCTCCAGACCCGTGGGCTTTTCAAGAGCGACAGGACCTA	F38, F40
D1A-TLk-R	*pE-YA1-dmbS*	rev	CATGGTGGCACGAATCATGTCCACCACAGTCTGCACAGAGTCTTTGTTGC	F40
D1B-TLk-F	*pE-YA1-dmbS*	fwd	ATGGACGCTTGCAGCAAAGACTCTGTGCAGACTGTCGTGGATACGATTCG	F42
D1B-TLk-R	*pE-YA1-dmbS*	rev	GTTTGACTGGCCTGTATTATTCAGAATGGCAGCGGCAGAACCAAGCAGAA	F38, F42
D2A-TLk-F	*pE-YA1-dmbS*	fwd	GACTTTTTTGTCTTGCTAAGCTCGAGCGCTGCCATCTTGAATAACATGGG	F45, F47
D2A-TLk-R	*pE-YA1-dmbS*	rev	CGCCTCAGCAAAGGCATGATGCACATCCGTCTCAGAGAGCCTCATAGCTC	F47
D2B-TLk-F	*pE-YA1-dmbS*	fwd	GGTACCACGCGAGTCATGAGTGTCTCTGAGACTGACGTGCATCACGCCTT	F49
D2B-TLk-R	*pE-YA1-dmbS*	rev	CGCTAGACTGTCTACCGCCGATGCGGCGGCTGCTTGCCCCGAGGCAATCA	F45, F49
TMS-F	*PE-YA1-*	fwd	ACTGTACCGCCCCTCCAGCCCGTGGGCTTT	F50

	milS		TCCAGAGCGACAAACCT	
TMS-R	*PE-YA1-milS*	rev	CGCTAGACTGTCTACCGCCATGCGGCGGCCGCTTGCTCAGAAGTAACCG	F50
TDSLK1(3/4)-R	*pE-YA-tenS*	rev	GTTTGACTGACCTATGTTGTTCGCGATAGTAGCGCTCGAGCTTAG	F53
TDSLK1(3/4)-F	*pE-YA-tenS*	fwd	GACGTCATGCGAGCCACGACACTCTCGGAGACGGATGTGCATCATG	F54
TenS(3/4)-F	*PE-YA1-milS*	fwd	ACTATCGCGAACAACATAG	F55
TenS(3/4)-R	*PE-YA1-milS*	rev	CTCCGAGAGTGTCGT	F55
LK2sub-F	*pE-YA-tenS*	fwd	CACAGCAACCGAGACGTCATGCGAGCCACGACACTCTCTGAGACGGATGTGC	F57
LK1sub-R	*pE-YA-tenS*	rev	GAGTGTCGTGGCTCGCATGACGTCTCGGTTGCTGTGCACCTTGGTGTCGTCA	F58

11. Table S3. swaps boundaries in TenS.

Swaps region	start	stop	Amino acid number
*C*MeT-ΨKR	S1279	A1784	505
*C*MeT	S1279	Q1670	391
1-*C*MeT	S1279	T1518	240
2-*C*MeT	D1519	Q1670	151
2A+2B1-*C*MeT	D1519	K1641	122
2A2+2B-*C*MeT	A1551	Q1670	119
2A2+2B1-*C*MeT	A1551	K1641	90
1A-*C*MeT	S1279	P1422	144
1B-*C*MeT	D1423	T1518	96
2A-*C*MeT	D1519	P1600	82
2B-*C*MeT	E1601	Q1670	69
1A1-*C*MeT	S1279	L1358	80
1A2-*C*MeT	F1359	P1422	64
1B1-*C*MeT	D1423	T1462	40
1B2-*C*MeT	E1463	T1518	56
2A1-*C*MeT	D1519	P1550	32
2A2-*C*MeT	A1551	P1600	50
2B1-*C*MeT	E1601	K1641	40
2B2-*C*MeT	A1642	Q1670	29
ΨKR	A1671	A1784	114
3A-ΨKR	A1671	L1727	57
3B-ΨKR	I1728	A1784	57
KR	F2204	A2481	278
4-KR	F2204	A2342	139
5-KR	A2343	A2481	139
4A-KR	F2204	Q2273	70
4B-KR	T2274	A2342	69
5A-KR	A2343	E2411	69
5B-KR	T2412	A2481	70

12. Table S4. The % identity and similarity between the *tenS* and *dmbS* amino acid sequences for the 14 different regions.

Expt.	AA sequence		Similarity /Identity (%)
1A1	*tenS*	SSLTGNINVYDAESGRALIQVEGFEVRAVGEPDASKDRLLFYETVWGRDISIMGLSDPIRDETSDAMVHNLSEAIERVSL	92/81
	dmbS	SSLTGDLNVYDTDTGIPLLQVEGFEVRAVGEPDASKDRLLFSETVWGRDISIMGLSDPIRNETTDAAVQSLAEAIERVSL	
1A2	*tenS*	FYVRQLMGELSTADRRQANWYHTRMLAAFDYHLAKVHEETHLHLRPEWLADDWAVIQTIDEAYP	92/77
	dmbS	FYVRQLMSELSTKDRREANWYHSRMLTAFEHHLARIHEDTHLHVRQEWLSDDWSVIQIIDEAYP	
1B1	*tenS*	DAVELQMLHAVGQNVADVIRGKKHLLEVLRVDNLLDRLYT	97/80
	dmbS	DTVELQMLHAIGQNMANVIRGEKHMLEVMRVNNLLDRLYT	
1B2	*tenS*	EDKGMHMANLFLANALEEITFKFPRCKILEIGAGTGATTWAALSAIGEAFDTYTYT	87/86
	dmbS	EDKGMQQGNHFLANALKEITFKFPRCKILEIGAGTGATTWAVLSAIDETFDTYTYT	
2A1	*tenS*	DLSVGFFENAVERFSAFRHRMVFRALDIEKDP	93/84
	dmbS	DLSVGFFETAVERFSAFRHKMIFKALDIEKSP	
2A2	*tenS*	ASQSFDLNSYDIIIATNVLHATRNLGVTLGNVRALLKPGGYLLLNEKTGP	96/90
	dmbS	AAQSFDLGSYDIIIATNVLHATRNLDITLGNVRSLLKPGGYLLLNEKTGP	
2B1	*tenS*	ESLRATFNFGGLEGWWLAEEKERQLSPLMSPDGWDAQLQK	100/93
	dmbS	ESLRATFNFGGLEGWWLAEEEERQLSPLLSPDGWDSQLQK	
2B2	*tenS*	ASFSGVDHIVHDVQEDQQDKQQNSMIMSQ	88/81
	dmbS	TQFSGVDHVVHDVQEEGKQQNSMIMSQ	
3A	*tenS*	AVDDTFYARLSPLSEMANLLPMNEPLLIIGGQTTATLKMIKEIQKLLPRQWRHKVRL	89/79
	dmbS	AVDDAFYARLSPLSEMASLLPTQEPLLLIGGQTNTTLRIIKEIQKQLPRKWRHKIRL	
3B	*tenS*	IASVDHVEAEGLPAHSDVICLQELDRGLFTTAMTSKCLDALKTLFINTRNLLWVTNA	92/82
	dmbS	IASVDQLEDEDLPAHSDVICVQELDRGLFTTAMTSKRLNALKSLFMNTKNLLWVTNA	
4A	*tenS*	FKSDRTYLMVGAAGGLGTSICRWMVRNGARHVVVTSRNPKADPEMLNEAERYGAAVQVVPMDACSKDSVQ	99/94
	dmbS	FKSDRTYLMVGAAGGLGTSLCRWMVRNGARHVVVTSRNPKADPEMLNEAERYGAIVRVVPMDACNKDSVQ	

4B	*tenS*	TVVDMIRATMPPIAGVCNAAMVLRDKLFLDMNVDHMKDVLGPKMQGTEHLDSIFAQEPLDFFVLLSSS	88/79
	dmbS	TVVDTIRATMPPIAGVCNAAMVLCDKLFLDMDVDQMNNTLGPKVDGTEYLDSIFAHEPLDFFILLGSA	
5A	*tenS*	AILNNTGQSNYHCANLYMDSLVTNRRSRGLAASIIHVGHVCDTGYVARLVDDTKVQMSLGTTRVMSVSE	88/77
	dmbS	AILNNMGQSNYHCANLYMDSLVKHRRSRGLAASIIHIGHVCDTGYVARMVDDNRIQSNIATMRAMRLSE	
5B	*tenS*	TDVHHAFAEAVRGGQPDSRSGSHNIIMGIEPPTKPLDLTKRKPVWISDPRLGPCLPFSTLENQMMASEQA	93/83
	dmbS	TDVHHAFAQAVRGGQLDSRSGSYNIIMGIEPPTKPLDLTRRQAVWLSDPRLGHMLPYSTLENQMIASGQA	
KR	*tenS*	FKSDRTYLMVGAAGGLGTSICRWMVRNGARHVVVTSRNPKADPEMLNEAERYGAAVQVVPMDACSKDSVQTVVDMIRATMPPIAGVCNAAMVLRDKLFLDMNVDHMKDVLGPKMQGTEHLDSIFAQEPLDFFVLLSSSAILNNTGQSNYHCANLYMDSLVTNRRSRGLAASIIHVGHVCDTGYVARLVDDTKVQMSLGTTRVMSVSETDVHHAFAEAVRGGQPDSRSGSHNIIMGIEPPTKPLDLTKRKPVWISDPRLGPCLPFSTLENQMMASEQA	84/71
	milS	FQSDRTYLMVGAAGGVGTSLCRWMVRHGARHVIVTSRNPKGDPTMLSEAKQYGATVRVVSMDVCDRRSVEAVVGMIRATMPPIAGVCNAAMVLCDKLFLDMDVDILNNTLGPKVDGTEILDSIFSEEALDFFILLGSTATIANNIGQSNYHCANLYMDSLVAQRRSRGLAASIIHIGYICDTGYVARLGDDAKVHSNRDVMRATTLSETDVHHAFAEAVRGGSPGSPIGSYNIIMGIDPPTKSLDSSRRKALWLSDPRLGHMVPYSASADQAVTSEQA	

13. Protein Modelling

Homolog modelling was done using the open free software SwissModel. In summary SwissModel uses four main steps, which are involved in building a homology model of a given protein structure: First the identification of structural template(s). Second the alignment of target sequence and template structure(s). Third building of the model and energy minimization and at least the assessment of the model's quality using QMEAN, a statistical potential of mean force. The proposed templates used for the-CMeT domain of TENS was CurJ (PDB: 5thz) and for the KR domain of TENS AmphB (PDB: 3mjv). This modelling resulted in a structure model of the *C*-MeT domain of Tenellin with a QMEAN value of -3.16. The KR domain of Tenellin had a QMEAN value of -2.82, which indicates that the quality of the generated structure model was good. For the generation of the chimeric mFAS/Tenellin structure, the single domains KR and *C*-MeT models were aligned with the mFAS structure. The alignment resulted in a RMDS value of KR (1.039) and *C*-MeT (2.71). Afterwards the coordinates of the PDB files of the KR and *C*-MeT domains were rewritten. The mFAS CMeT and KR domain were deleted and afterwards were this single structures in PyMOL (DeLano Scientific LLC, Version 1.8.2.0) were combined to give a new structure. Afterwards the chimeric mFAS/Tenellin structures were submitted for minimization in YASARA.

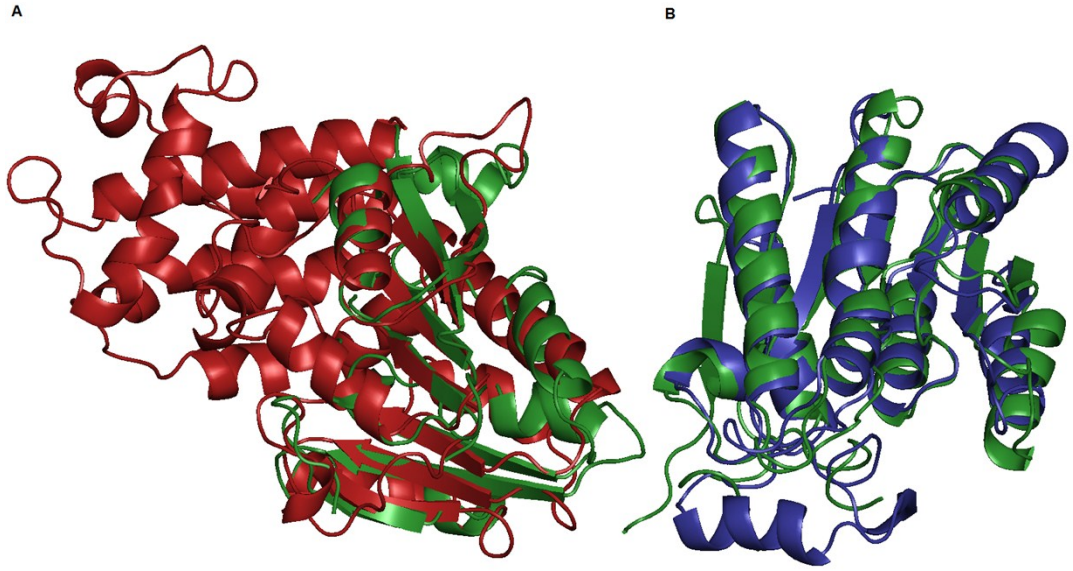
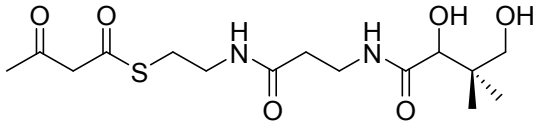


Figure 13.1: **A,** aligment of the Ψ*c*-met domain of mFAS (green) with the generated *C*-Met domain of TENS based on the CurJ template (red); **B**, aligment of the KR domain of mFAS(green) with the genereated KR domain of TENS based on the AmpB template (blue). Note the substrate-binding helix no present in mFAS.

Substrate Docking

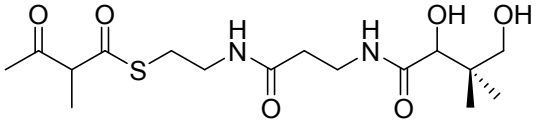
The substrate mimics (S-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) 3-oxobutanethioate **XX** and S-(2-(3-(2,4-dihydroxy-3,3-dimethyl-butanamido)propanamido)ethyl) 2-methyl-3-oxobutanethioate **ZZ** were docked into the active site of the homolog generated models of the CmeT and KR domain of Tenellin using Autodock Vina Vina (PyRx 0.8). The homolog models were generated with SwissModel. Docking results including lowest binding energy and mean binding energy were obtained from the docking log (dlg) file. Afterwards were the different docked poses submitted for the minimized in YASARA. Images of the best docked poses for each of the substrate mimics was captured with the PyMOL visualization software.

Substrate **XX**



S-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) 3-oxobutanethioate

Substrate **ZZ**



S-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) 2-methyl-3-oxobutanethioate

14. References

[1] F. H. Jin, J.-i. Maruyama, P. R. Juvvadi, M. Arioka, K. Kitamoto, *FEMS Microbiol. Lett.* **2004**, *239*, 79-85.