Electronic Supplementary Material (ESI) for RSC Advances.

Reengineering the Programming of a Functional Domain of an Iterative Highly Reducing Polyketide Synthase

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Electronic Supplementary Information

1.0 Multiple alignment of SQTKS *vs* vertebrate FAS and other PKS

1.1 Alignment with Type I FAS and PKS for determination of approximate domain boundaries

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                                                KS                                            KS
MF SQTKS    1     MVPHYQQASSCESNTMTAMDEYQHHEDATIPIAIIGMSCRFPGNATSPEKLWELCAEGRSAWSKIPKSRFRQEGFYNPNA  80
C2 SQTKS    1     MVPYYQPASSCGSNTMAAMDEHQHNEDATIPIAIIGMSCRFPGNATSPEKLWELCAQGRSAWSSIPKSRFRQEGFYNPNA  80
MFAS        1     ME----------------------------EVVIAGMSGKLPESE-NLQEFWANLIGGVDMVTD--DDRRWKAGLYGL-P  48
PFAS        1     ME----------------------------EVVIAGMSGKLPESE-NLEEFWANLIGGVDMVTA--DDRRWKAGLYGL-P  48
TENS        1     MSPMKQNESESHS--------------VSEPIAIVGSAYRFPGGCNTPSKLWDLLQQPRDILKELDPERLNLRRYYHPDG  66

                  KS                                                                            KS
MF SQTKS    81    ERVGTSHVVGGHF-LEEDPSLFDASFFNLSAEAAKTMDPQFRLQLESVYEAMESAGITLEHIAGSDTSVYAGACFRDYHD  159
C2 SQTKS    81    ERVGTSHVVGGHF-LEEDPSLFDASFFNLSAEAAKTMDPQFRLQLESVYEAMESAGITLEHIAGSDTSVYAGACFRDYHD  159
MFAS        49    KRSGKL----------KDLSKFDASFFGVHPKQAHTMDPQLRLLLEVSYEAIVDGGINPASLRGTNTGVWVGVSGSEASE  118
PFAS        49    RRMGKL----------KDLSRFDASFFGVHSKQANTMDPQLRMLLEVTYEAIVDGGINPASLRGTSTGVWVGVSSSDASE  118
TENS        67    ETHGSTDVSNKAYTLEEDISRFDASFFGISPLEAASMDPQQRTLLEVVYESTETAGIPLDKLRGSLTSVHVGVMTTDWAQ  146

                  KS                                                                            KS
MF SQTKS    160   SLVRDPDLVPRFLLTGNGAAMSSNRISYFYDLHGASMTVDTGCSTTLTALHLACQGLRNRESKTSIVTGANVILNPDMFV  239
C2 SQTKS    160   SLVRDPDLVPRFLLTGNGAAMSSNRVSHFYDLRGASMTVDTGCSTTLTALHLACQGLRNRESKTSIVTGANVILNPDMFV  239
MFAS        119   ALSRDPETLLGYSMVGCQRAMMANRLSFFFDFKGPSIALDTACSSSLLALQNAYQAIRSGECPAALVGGINLLLKPNTSV  198
PFAS        119   ALSRDPETLVGYSMIGCQRAMMANRLSFFFDFKGPSITIDTACSSSLLALQSAYQAIRGGECSAAVVGGLNVLLKPNSSL  198
TENS        147   VQRRDPETMPQYTATGIASSIISNRISYIFDLKGASETIDTACSSSLVALHNAARALQSGDCEKAIVAGVNLILDPDPFI  226

                  KS                                                                            KS
MF SQTKS    240   TMSSLGLLGPEGKSHTFDARANGYGRGEGIATVIIKRLDEALAAQDPIRCIIRGTALNQDG-KTATLTSPSQTAQSDLIR  318
C2 SQTKS    240   TMSSLGLLGPEGKSHTFDARANGYGRGEGIATVIIKRLDDALRAQDPIRCIIRGTALNQDG-RTATLTSPSQTAQSDLIR  318
MFAS        199   QFMKLGMLSPDGTCRSFDDSGSGYCRSEAVVAVLLTKKSLARR----VYATILNAGTNTDGSKEQGVTFPSGEVQEQLIC  274
PFAS        199   QFMKLGMLSQDGTCRSFDAEGTGYCRAEAVVAVLLTKKSLARR----VYATILNAGTNTDGSKEQGVTFPSGDVQEQLIR  274
TENS        227   YESKLHMLSPDARSRMWDAAANGYARGEGAAAVVLKTLGHALRDGDRIEGVIRSTFVNSDG-LSSGLTMPSSAAQTALIR  305

                  KS                                                                            KS
MF SQTKS    319   ACYRAAALDP--NDTAFLAAHGTGTRTGDAVEIAAAAEVF---------GEKRLPDRPLWIGSLKTNIGHSEATSGLASV  387
C2 SQTKS    319   ACYRAAALDP--NDTAFLAAHGTGTRTGDAVEIAAAADVF---------GEKRSPERPLWIGSVKTNIGHSEATSGLASV  387
MFAS        275   SLYQPAGLAP--ESLEYIEAHGTGTKVGDPQELNGITRSL---------CAFR--QAPLLIGSTKSNMGHPEPASGLAAL  341
PFAS        275   SLYAPAGPDP--ESLEYIEAHGTGTKVGDPQELNGIVNAL---------CATR--REPLLIGSTKSNMGHPEPASGVAAL  341
TENS        306   QTYRKAGLDPVRDRPQFFECHGTGTRAGDPVEARAISDAFLPSHRTNGGGAATTVDDPLYVGSIKTVVGHLEGCAGLAGL  385

                  KS                                                                KS
MF SQTKS    388   IQAALALEKGLIPPNINFKEPNEKLSQVSSAVKVPSTLEKWP--LGSRVRRASVNNFGYGGANAHVILESGLTGSTQLAN  465
C2 SQTKS    388   IQAALALEKGLIPPNINFKEPNEKLGQVSAAVRVPSNLQKWP--SVSGVRRASVNNFGYGGANAHVILESGIPGHTPIAN  465
MFAS        342   TKVLLSLEHGVWAPNLHFHNPNP---EIPALLDGRLQVVDRP--LPVRGGNVGINSFGFGGSNVHVILQ---PNTRQAPA  413
PFAS        342   IKVLLSLEHGVWAPNLHYHTPNP---EIPALQDGRLQVVDRP--LPIRGGNVGINSFGFGGSNVHVILQ---PNSRPAPP  413
TENS        386   VKVLLSLKHGIIPPNLWFDKLNPEIARYYGPLQIPTKAIPWPELAPGTPLRASVNSFGFGGTNAHAIIER-YDASQSYCS  464


MF SQTKS    466   GNGHYETNGTTNGHKGANGTTNGHKGANGTTNGHNGTNGITNGHDITRGTIDYEPLESFVISLSAKEEAGTRSMMTNLGE  545
C2 SQTKS    466   GSGR--SNGTGNGHNGANGTTNGHNGTNGTTNGHFDATQATNGHYGTDETPDYAPLDSFVISISAKEEASARSMVTNLAD  543
MFAS        414   PTAH--------------------------------------------------------AALPHLLHASGRTL-----E  432
PFAS        414   PAQH--------------------------------------------------------AALPRLLQASGRTL-----E  432
TENS        465   QWRRDMTEEKTIARTQNNDDVEIPVPLVLTAKTGGALWRTVDAY--AQHLRQHPKLR--VANLSQFMHSRRSTHRVRA--  538

                                                                              AT                AT
MF SQTKS    546   YLRKNHVDDETKHFKSIAYTLGSHRSTFKWTAAKPITSLEELLAAAGGGQFQASRALERTRLGFVFTGQGAQWFAMGREL  625
C2 SQTKS    544   YLRTLQVQDETKHFKSIAHTLGSHRSMFKWTAAKSITGPEELIAAAEGGQFQASRALERTRLGFVFTGQGAQWFAMGREL  623
MFAS        433   AVQDL-LEQGRQHSQDLAFVSMLNDIAATPTAAMPFRGYTVLGVEGRVQEVQQVSTNKRP-LWFICSGMGTQWRGMGLSL  510
PFAS        433   AVQTL-LEQGLRHSRDLAFVGMLNEIAAVSPVAMPFRGYAVLGGEAGSQEVQQVPGSKRP-VWFICSGMGAQWQGMGLSL  510
TENS        539   ---SFSGASREELVENMANFVQAHAADAKSPASQNRIGYSPLLIDPK----------EVSGILGIFTGQGAQWPAMGRDM  605

                  AT                                                                            AT
MF SQTKS    626   INTYPVFRKSLDRANGYLKEFGCE----WSILDELSRDAETSNVNDMTLSPPLCTAVQISLVRLLESWGIVPTAVTGHSS  701
C2 SQTKS    624   INTYPVFRQSLDRADRYLKEFGCE----WSIIDELSRDAENSNVNDMTLSPPLCTAVQISLVQLLESWGIVPTAVTGHSS  699
MFAS        511   MRL-DSFRESILRSDEAVKPLGVK-------VSDLLLSTDERTFDDIVHAFVSLTAIQIALIDLLTSVGLKPDGIIGHSL  582
PFAS        511   MRL-DRFRDSILRSDQALKPLGLR-------VSDLLLSTDEAVLDDIVSSFVSLTSIQIALIDLLTSLGLQPDGIIGHSL  582
TENS        606   MHQSPLFRKTIADCESVLQALPLKDAPAWSLSEELKKDASTSRLGEAEISQPLCTAVQLALVNVLTASGVYFDAVVGHSS  685

                  AT                                                                            AT
MF SQTKS    702   GEIAAAYAAGALDFRSAMAVTYFRGEVGLACQDKIVGK----GGMIAVGLGPEEAED--RIARVQSGKIVIACINSQSSV  775
C2 SQTKS    700   GEIAAAYAAGALDFKSAMAVTYFRGEVGLACQDKIVGK----GGMIAVGLGPEDAED--RIARVQSGKIVVACINSQSSV  773
MFAS        583   GEVACGYADGCLSQREAVLAAYWRGQ---CIKDAHLPP----GSMAAVGLSWEECKQ--RCP----AGVVPACHNSEDTV  649
PFAS        583   GEVACGYADGCLTQEEAVLSSYWRGY---CIKEANVLP----GAMAAVGLSWEECKQ--RCP----PGIVPACHNSKDTV  649
TENS        686   GEIAATYASGIINLKAAMQIAYYRGL----YAKLARGQSDEAGGMMAAGLSMDDAVKLCRLPEFE-GRIQVAASNAPQSV  760

                  AT                                                                            AT
MF SQTKS    776   TVSGDLAGIVELEEGLKAEGVFARRVKV-QAAYHSHHMQVIANGYLTSLKDI---LK-PGKKFGE---IIYSSPTTGK--  845
C2 SQTKS    774   TVSGDLSGIVELEDLLKAEGVFARRVKV-QAAYHSHHMQVIANGYLTSLKDM---LK-PTKKFGK---IIYSSPTTGR--  843
MFAS        650   TISGPQAAVNEFVEQLKQEGVFAKEVRTGGLAFHSYFMEGIAPTLLQALKKV---IREPRPRSAR---WLSTSIPEAQ--  721
PFAS        650   TISGPQAAMSEFLQQLKREDVFVKEVRTGGIAFHSYFMESIAPTLLRQLRKV---ILDPKPRSKR---WLSTSIPEAQ--  721
TENS        761   TLSGDKEAIKAAKAKLDADGVFARELKV-DTAYHSHHMLPCAEPYLKALLACDIQVSAPTKTPGRKCMWSSSVRGDAELL  839

                  AT                                                                            AT
MF SQTKS    846   RETSAKLMASAQHWVNNMLSPVRFAESFQNMCFPTQKVSRSGELEQDVDIILEVGPHGMLQGPIQQMMSLPRFESARMPY  925
C2 SQTKS    844   RETNAKLMASAQHWVNNMLSPVRFAESFQNMCFSNRNSSQSEEIFQDVDIVLEVGPHGMLQGPIQQMMSLPIFERARLPY  923
MFAS        722   WQSSLARTSSAEYNVNNLVSPVLFQEALWHI--------------PEHAVVLEIAPHALLQAVLKRGVK------SSCTI  781
PFAS        722   WQGSLARTFSAEYSVNNLVSPVLFQEALQHV--------------PAHAVVVEIAPHALLQAVLKRSLE------SSCTI  781
TENS        840   RRDRNLDSLKGPYWVANMVQTVQFSRAIQSTIWHG----------GPFDLAVEVGPHPALKGPTEQTLKA--VYGSAPLY  907
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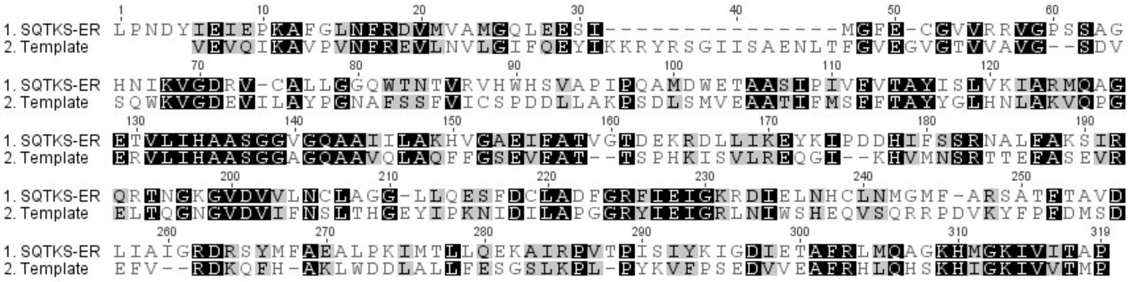
AT AT
MF_SQTKS 926 LSCLLRGQSA-VYTMQSLAAGLMG---------WGYRVDMAA----VNFPQGTHGARILHDLPSYPWNHDNSHWWEPRLN 991
C2_SQTKS 924 ISCLLRGQSA-VHTMQTVAAGLMG---------WGYRVDMVA----VNFPQGTYGVKILHDLPSYPWNHDNSHWWEPRLN 989
MFAS 782 IPLMKRDHKDNLEFFLTNLGKVHL---------TGINVNPNALFPPVEFP-APRGTPLIS--PHIKWDHSQT-WDVPVAE 848
PFAS 782 IPLMKKDHRDNLEFFLSNVGRLHL---------AGVSVNPNGLFPPVEFP-APRGTPLIS--PHXKWDHSQA-WDVPSAA 848
TENS 908 TGVLSRGAND-AVAFSTAIGNIWSHLGPAFVDITGYQSIFSGTCE----GHGGSEAPFISDLPLYPWDHDEEYWRESRIS 982
DH DH
MF_SQTKS 992 KAHRQRVHPPHDLLGSLIPGR-DLREPTWRHFIRVQDIPWIRDHVVQSQLVYPGAGFICMAIEAMVQLHDLKDSQSKKIA 1070
C2_SQTKS 990 KAHRQRVHPPHDLLGSLIVGR-DLREPTWRHFIRVQDIPWIRDHVVQSALVYPGAGFICMAMEAMVQLHELRDSQSRKVA 1068
MFAS 849 D------FPNGSSSSSATVYSIDASPESPDHYLV--------DHCIDGRVIFPGTGYLCLVWKTLARSLGLSLEETPVV- 913
PFAS 849 D------FPSGSSCSSVAVYKFDVSPESPDHYLV--------DHCIDGRVLFPGTGYLWLTWKTLARALSQNLEETPVV- 913
TENS 983 RRYRTGKDESHELLGRRMPDD-NEREIRWRNLLKVSELPWTQGHRVLGEVLLPGAAYISMAIEAGRR---LALDQGREVS 1058
DH DH
MF_SQTKS 1071 GYRLADVDILRAMLIPDTSEG---------LEAHISLRPCST--KLLLTNEWYDFCVSSVGEDDKFVDHCRGRIAVEFNT 1139
C2_SQTKS 1069 GYRLAEVDILRAMLIPDTSEG---------LEAHISLRPCST--KLLLTNEWYDFCVSSVGDDDKFVDHCRGRITIEFDT 1137
MFAS 914 ---FENVSFHQATILPKTGTVA---LEVRLLEASHAFEVSDT-GNLIVSGKVYLW----EDPNSKL-----------FDH 971
PFAS 914 ---FEDVTLHQATILPKTGTVS---LEVRLLEASHAFEVSDSNGSLIASGKVYQW----ESPDPKL-----------FDT 972
TENS 1059 LLEVSDVDILRPVVVADNKEGTETLFTVRLLDEYASTGKKSD-ELMTASFSFYIY---NSPASTSIVHTCEGRIAVHLGA 1134
DH DH
MF_SQTKS 1140 SSLSDAPKTTSRE---RSRGAGLTRSVDPSNLYSFLRAQGIYHGSIFQ-----NLKTISSRKNYSESSFVVADT---ASV 1208
C2_SQTKS 1138 SGSADTPRTSLRE---RSRSTGLMRSVDPSNLYSFLRAQGIYHGPIFQ-----NLKTISSRKDHSESSFVVANT---ASV 1206
MFAS 972 PEVPTPPESASVS---R---------LTQGEVYKELRLRGYDYGPQFQGICEATLEGEQGKLLWKDNWVTFMDTMLQVSI 1039
PFAS 973 RAAVDPADSTAEF---R---------LSQGDVYKDLRLRGYDYGPFFQLVLESDLEGNRGRLQWNDSWVSFLDAMLHMSI 1040
TENS 1135 KLGSEAAANSTPQLPPREPSVSNLQQLDCEKLYSVFETIGLEYSGAFRRIV----------SSSRCLGHATATASW 1200
DH DH
MF_SQTKS 1209 MPDGFQSAHVVHPTTLDSIFQGAYTALPSAGLDQ-KTAMIPRSIQEIYLSSALTSEVGQCLVSDTSLIRYDGQSFT-VNV 1286
C2_SQTKS 1207 MPNGFQSPHVIHPTTLDSIFQGAYTALPGAGLDQ-NTAMIPRSIQELYLSSALTSDVGQCLVSDTSLIRYDGQSFT-VNV 1284
MFAS 1040 LGSSQQSLQL--PTRVTAIY----------IDP-ATHR-----QKVYRLKEDTQVADVTTSRCLGITVSGGIHISRLQT 1100
PFAS 1041 LAPGQLGLYL--PTRFTSIR----------IDP-VTHR-----QKLYTLQDTTQAADVVVDRNLNTVVAGGALFLGAHS 1101
TENS 1201 PTADLNDCYLVHPAILDVAFQTIFVARAHPDSGQLSSALLPSRIERVRVVPSLAMGSKLQNNENFNAAI-DSWALNQTAS 1279
DH DH CMeT CMeT
MF_SQTKS 1287 GISSK-----ADSEC-TPVLE-IKGLRNQSVGQMAPQQGDSGNNDLCFKLEWALDISSVKQERLKEKFGFPLDPAEADII 1359
C2_SQTKS 1285 DVSSK-----ADSEH-TPVLE-IKGLRNQSVGQMAPQPGDSSNNDLCFKLDWAPDISSVKQERLKEKFGFPLDPTEADII 1357
MFAS 1101 TATSR-----RQQEQLVPTLEKFVFTPHMEAECLSESTALQKELQLCKGLARALQTKATQQ---------- 1156
PFAS 1102 SVAPR-----RPQEHLKPILEKFCFTPHVESGCLAGNTALQEELQLCRGLAQALQTKVAQQ---------- 1157
TENS 1280 SLTGNINVYDADSER---ALIQVEGFEVRAVG----EPDASKDRLLFYETVWGRDISIMGLSDPIRDE--TSDAMVQNLS 1350
CMeT CMeT
MF_SQTKS 1360 MGLRQACLYYIRQALTSLTPSARDQLDWHQKRFYDWMMLQMHLAEEDRLAPNSSAWLQCTSSDEQKLLENVRAASVNGQM 1439
C2_SQTKS 1358 MGLRQACIHFIHRSLQSLTAPDRDQLDWHQKRFYDWMVLQIQLAEEDRLAPNSSAWLQCSSSDEQKLLENVRASSVNGQM 1437
MFAS 1157 -GLKAA----------MLGQE------DPPQHGL---------PRLLAAACQLQLNGNL 1189
PFAS 1158 -GLKMV----------VPGLDGAQAPREAPQQSL---------PRLLAAACQLQLNGNL 1196
TENS 1351 EAIERVSLFYVRQLMGELSTADRRQANWYHTRMLAAFDHHLAKVHEETHLHLRPEWL-ADDWTVIQTIDEAYPDAVELQM 1429
CMeT CMeT
MF_SQTKS 1440 VVHVGESILAILRHEIAPLELMLQDKLLYRYYTDAIKWDRSYQQIDQLVKLHAHKCPSAKIIEIGAGTGGCTRAVLDALS 1519
C2_SQTKS 1438 VVHVGKSMLAILRHEIAPLELMLQDKLLYRYYTDAIKWDRSYQQIDQLVKLHAHKCPTAKIIEIGAGTGGCTRAVLDALS 1517
MFAS 1190 QLELGEAL--------AQERLLLPEDPLISGLLNSQALKACVD-----TALENLSTLKMKVAEVLAGEGHLYSRIPALLN 1256
PFAS 1197 QLELGQVL--------AQERPLLCDDPLLSGLLDAPALKACVD-----TALENMASPKMKVVEVLAGDGQLYSRIPALLN 1263
TENS 1430 LHAVGQNVADVIRGKKHLLEVLRVDNLLDRLYTEDKGMHMANLFLANALKEITFKFPRCKILEIGAGTGATTWAALSAIG 1509
CMeT CMeT
MF_SQTKS 1520 THGAARCAQYDFTDVSSGFFEAAQQKFTAFADVIRFQKLDIEKDIETQG---------FECGSYDLVIASQVLHATGKIE 1590
C2_SQTKS 1518 NQGIARCAQYDFTDVSSGFFEAAQQKFAAFDDVIRFQKLDIEKDIEMQG---------FECGSYDLVIASQVLHATGKME 1588
MFAS 1257 TQPMLQ-LEYTATDRHPQALKDVQTK--------------LQQHDVAQGQWNPSDPAPSSLGALDLLVCNCALATLGDPA 1321
PFAS 1264 TQPVMD-LDYTATDRNPQALEAAQAK--------------LEQLHVTQGQWDPANPAPGSLGKADLLVCNCALATLGDPA 1328
TENS 1510 EA----FDTYTYTDLSVGFFENAVERFSAFRHRMVFRALDIEKDPASQS---------FDLNSYDIIIATNVLHATRNLG 1576
CMeT CMeT
MF_SQTKS 1591 DTMANVRRLLKPGGKLLL-VETTRDEMDLQLVFGLLPGWWLSSEEERKMSPSLSTSSWEKVLKKTGFNGLDVELRDCDSD 1669
C2_SQTKS 1589 HTMANVRKLLKPGGKLLL-VETTRDEMDLQLVFGLLPGWWLSSEEERQMSPSLSTNSWEKVLKKTGFDGLDIELRDCDSD 1667
MFAS 1322 LALDNMVAALKEGG-FLL-VHTVLKGHALGETLACLPS------EVQPAPSLLSQEEWESLFSRKALHLVGLKR------ 1387
PFAS 1329 VAVGNMAATLKEGG-FLL-LHTLLAGHPLGEMVGFLTS------PEQGGRHLLSQDQWESLFAGASLHLVALKR------ 1394
TENS 1577 VTLGNVRSLLKPGGYLLLNEKTGPDSLRATFNFGGLEGWWLAEEKERQLSPLMSPDGWDAQLQKAQFSGVDHIVHDVQED 1656
YKR YKR
MF_SQTKS 1670 Q----------FYSFSVIMATASPTVPMNPVDFIILHGKSS----------IPDQWMNDLRTATSPF-TKSD 1720
C2_SQTKS 1668 E----------FYSFSVMMATASSTIASSSMAFAIVYGEVP----------LPDQFLDDMKTAISSS-AVSD 1718
MFAS 1388 S----------FYGTALFLCRRA--IPQEKPIFLSVEDTSF----------QWVDSLKS-TLAT-SSSQ 1432
PFAS 1395 S----------FYGSVLFLCRQQ--TPQDSPVFLSVEDTSF----------RWVDSLKD-ILAD-ASSR 1439
TENS 1657 QQDKQQNSMIMSQAVDDTFYARLSPLSEMANLLPMNEPLLIIGGQTTATLKMIKEIQKLLPRQWRHKVRLIASVNHLEAE 1736
YKR YKR
MF_SQTKS 1721 PVVGHINNADPTGKFCIFLEDPEEDILFHPDEKSYASIKRVITQCKGLLWISRGGSMHGTLPTSSLKTGLLRTLRLEYAE 1800
C2_SQTKS 1719 PVVGHLDSIDATGKFCIFIEDPETDILSSPDEKSYASIQKLVTRCKGLIWVSRGGAMHGTRPNSSLKTGLLRTLRLEYTE 1798
MFAS 1433 PV----------WLTAMDC-----PTSGV-VGLVNCLRKEPGG 1459
PFAS 1440 PV----------WLMAVGC-----STSGV-VGMVNCLRKEPGG 1466
TENS 1737 GVPAHSNVI------CL-QELDRGLFTTAMTSKCLDALKTLFINTRNLLWVTNAQHSSSMTPRASMFRGITRVLDGEIPH 1809
YKR YKR
MF_SQTKS 1801 KRF----ISLDLNPA----------RAPWAHESISTIREVLRGALAQTAEIPIRDSEFAENDGQLYVPRISSDIARN 1863
C2_SQTKS 1799 KRF----ISLDLDSA----------RPQWNHDSITTINEVLCGALAQNADSSIKDSEFAEQDGQLFVPRISCDIARN 1861
MFAS 1460 HRIRCILLSNLSNTS----------HAPKLDPGSPELQQVLKHDLVMN----------VYRD 1501
PFAS 1467 HRIRCVLVSNLSSTS----------PAPEMHPSSSELQKVLQGDLVMN----------VYRD 1508
TENS 1810 IRTQVLGIEPRATSSATARNLLEAFLRLRSDDGRHAANVDEDGADGSSQQVLWLHEPEAELLSN-GTMMIPRVKARKSLN 1888

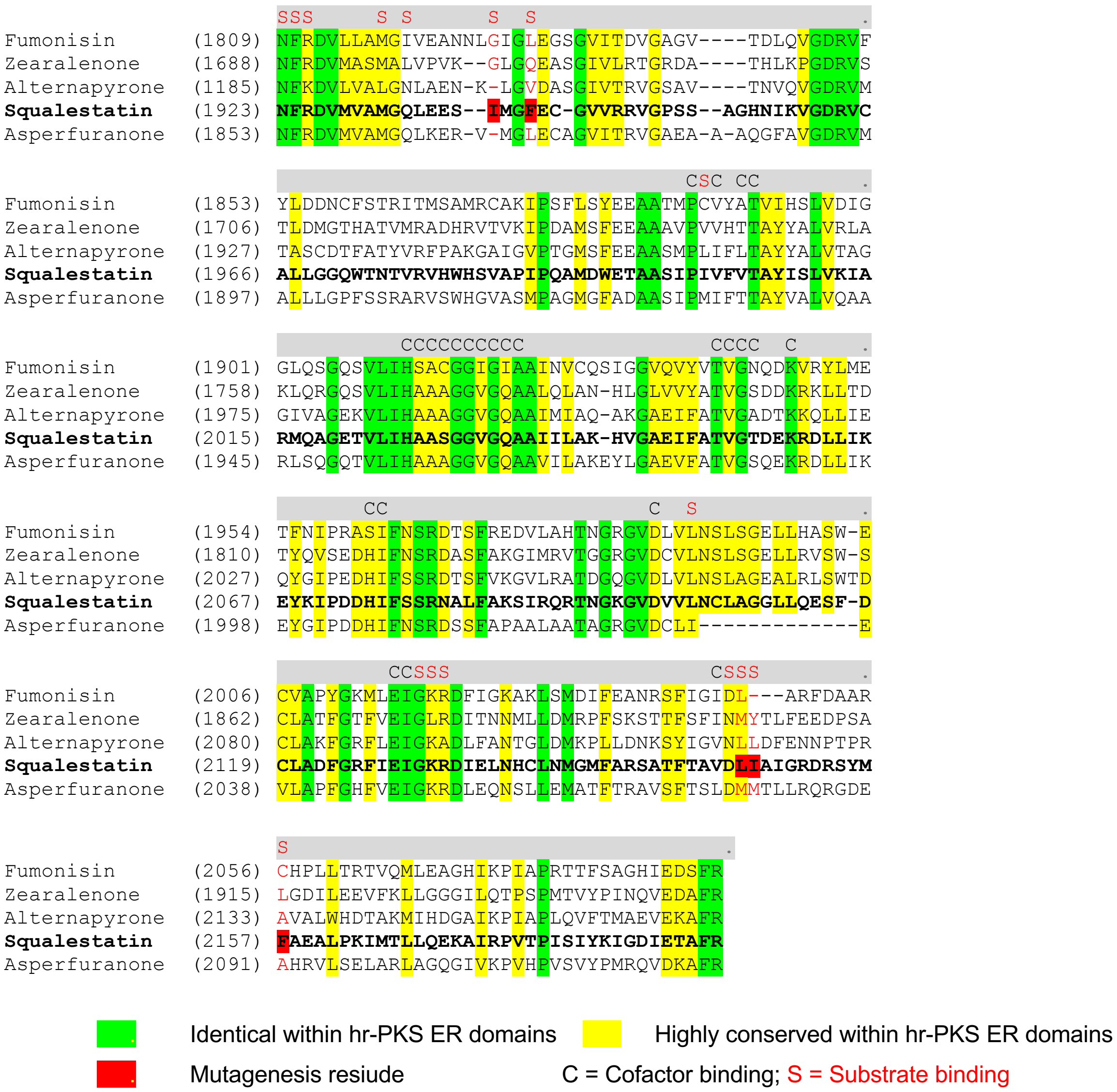
MF SQTKS - from Phoma MF5453
C2 SQTKS - from Phoma C2932
MFAS - vertebrate FAS from Mouse
PFAS - vertebrate FAS from Pig
TENS - Tenellin synthetase PKS from *Beauveria bassiana*

XX - conserved cofactor binding residues, active site catalytic residies
XX - Residues identified here as substrate-binding.

1.2 Sequence alignment of SQTKS ER domain with CurF ER domain (pdb 5dp2)



1.3 Sequence alignment of the SQTKS ER domain with other fungal ER domains.



2.0 Model Building and Docking

2.1 Model Building

SQTKS ER domain boundaries were previously determined as G1888-A2208.[1] This protein sequence was submitted to SwissModel and the template 5dp2 (CurF ER)[2] was selected for model construction using standard parameters.

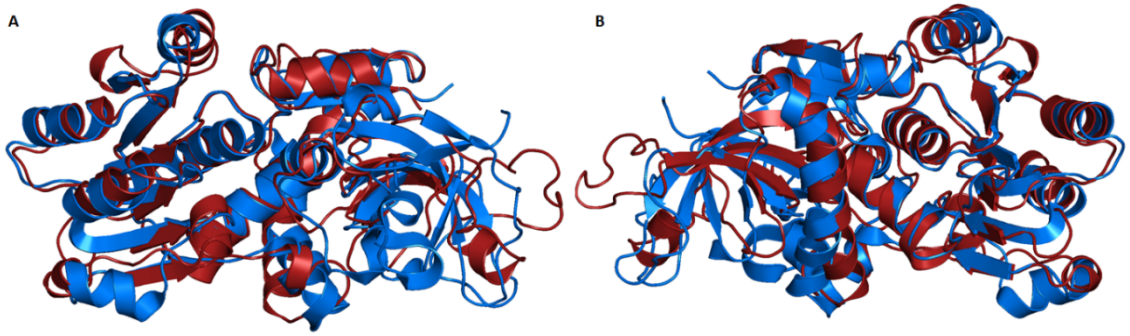


Figure S2.1A: Alignment of the SQTKS ER model (red) with the template CurF (PDB: 5dp2; blue) displayed in PyMOL. **A,** Front view; **B,** Back view.

2.2 Integration of the Cofactor

NADPH was integrated into the ER domain through the alignment of the model structure with the template in PyMOL (DeLano Scientific LLC, Version 1.8.2.0).[3,4] The cofactor coordinates were extracted from the template and then manually integrated into the structural model of the ER domain in PyMOL. The generated domain plus cofactor was then minimized in YASARA, to refine the protein-cofactor interaction.[5]

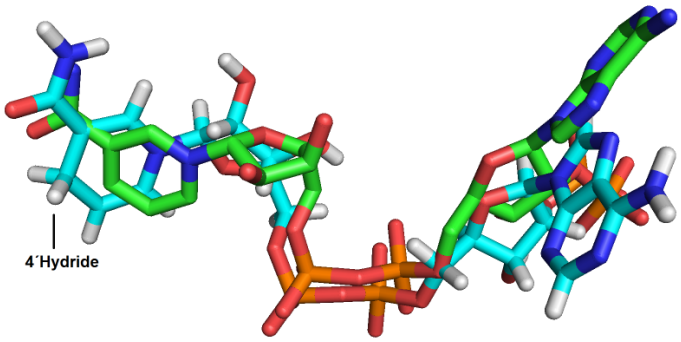


Figure S2.2A: Alignment of the cofactor **NADPH** from the SQTKS ER model (green) with the cofactor from the CurF (blue)

2.3 Investigation of the pocket volume

Pocket volumes were determined using the 3V web server.[6] As search parameters an outer probe radius of 10 Å and an inner probe radius of 2 Å were used.

2.4 Molecular Docking

Docking was performed by manually placing the specfic substrate in the active site of the *holo*-ER model using PyMOL. This method minimized the Grid Box, which has critical role in the speed of the docking calculations. Molecular docking was then done using using Autodock Vina (PyRx 0.8).[4,7,8] Python 3.6 was used as the underlaying programming language for performing the calculations with AutoDock Vina. All calculations were performd using. The protocol included the creation of pdbqt files of the respective substrate and protein. The degrees of freedom for the substrate were set as a default to maxium. The coordiantes of the Grid Box were customized for the specific protein-substrate complex. However, the size of the Gird Box that was used by default was 20 × 20 × 20 points. Afterwards, this information was saved in the corrosponding configuration file. For the subsequent calculations the AutoDock Vina file was used. Docking results, including the lowest binding energy and mean binding energy, were obtained from the docking log (dlg) file. The docked substrates were then compared using PyMOL. The best docked substrates were chosen and separate saved in a new pdb file then refined by YASARA. The visualization of the different models, after the refinement step was always done in PyMOL.

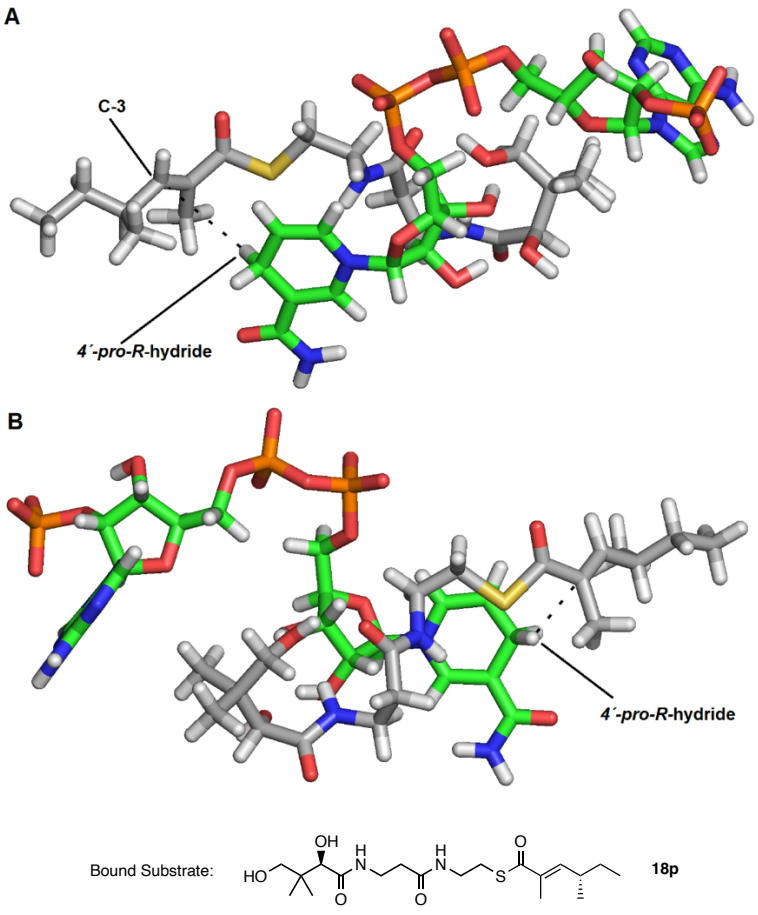


Figure S2.4A: Active site of the ER domain. Cofactor NADPH (green) and the modeled substrate squalestatin triketide 4*S*-2*E*-dimethylhex-2-enoylpantetheine **18p** (white). The hydride at the cofactor are marked. **A**, Back view; **B**, Front view.

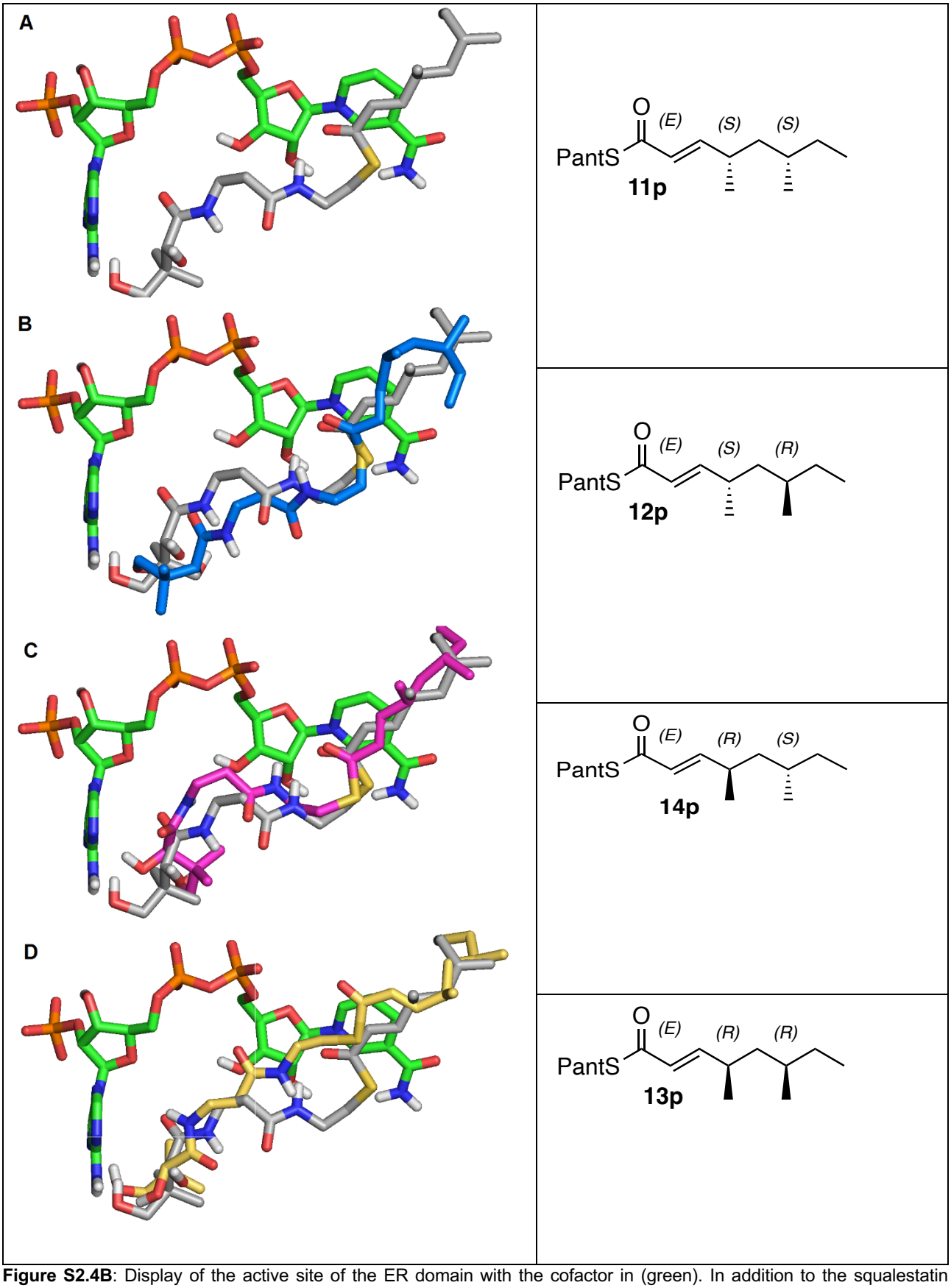


Figure S2.4B: Display of the active site of the ER domain with the cofactor in (green). In addition to the squalestatin tetraketide **11p** (4*S*,6*S*) are displayed the other docked diastereomers (grey). Blue (4*S,* 6*R*) **12p**, pink (4*R,* 6*S*) **14p** and in yellow (4*R*, 6*R*) **13p**.

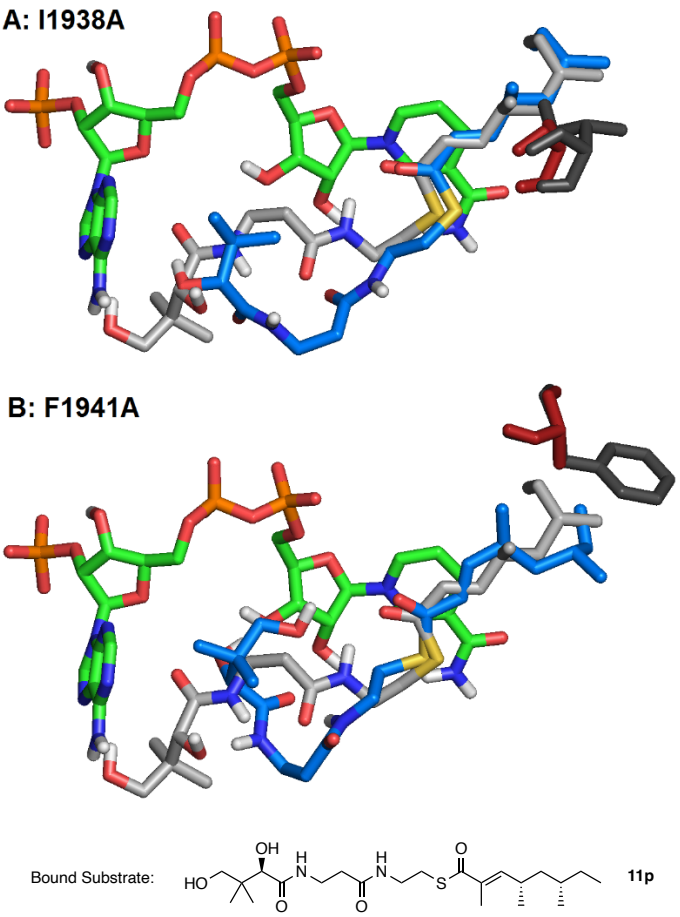


Figure S2.4C: Active site of the ER domain. In green the cofactor NADPH**.** Displayed are the docked tetraketide **11p** of the wild type (white) compared to the docked tetraketide **11p** in the mutant active site (blue).

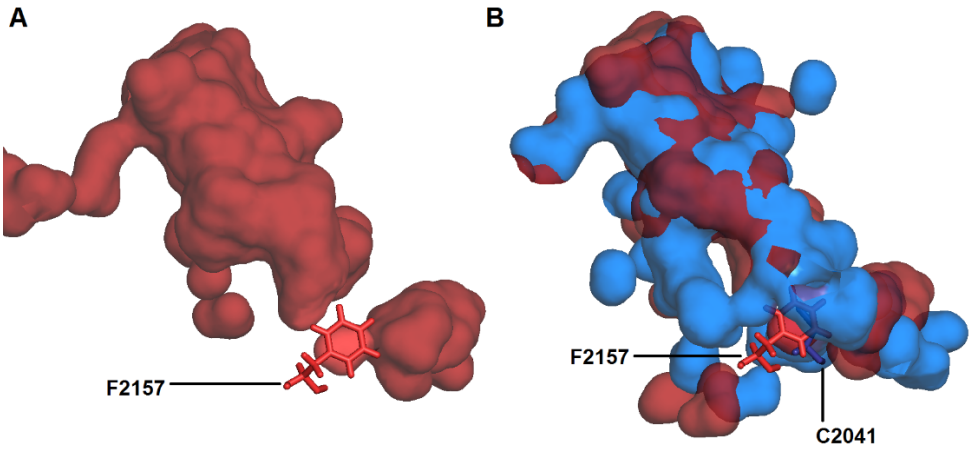


Figure S2.4D: Overlay of ER homology models: **A**, in red the pocket volume of SQTKS ER with the residue F2157 at the end of the pocket; **B**, in red the pocket volume of WT SQTKS ER (tetraketide), in blue pocket volume of FUM1 ER (nonaketide). In addition the residues F2157 of the SQTKS and C2041 of FUM1 at the end of the pocket are displayed. Interior surfaces of the proposed substrate binding pockets are shown.

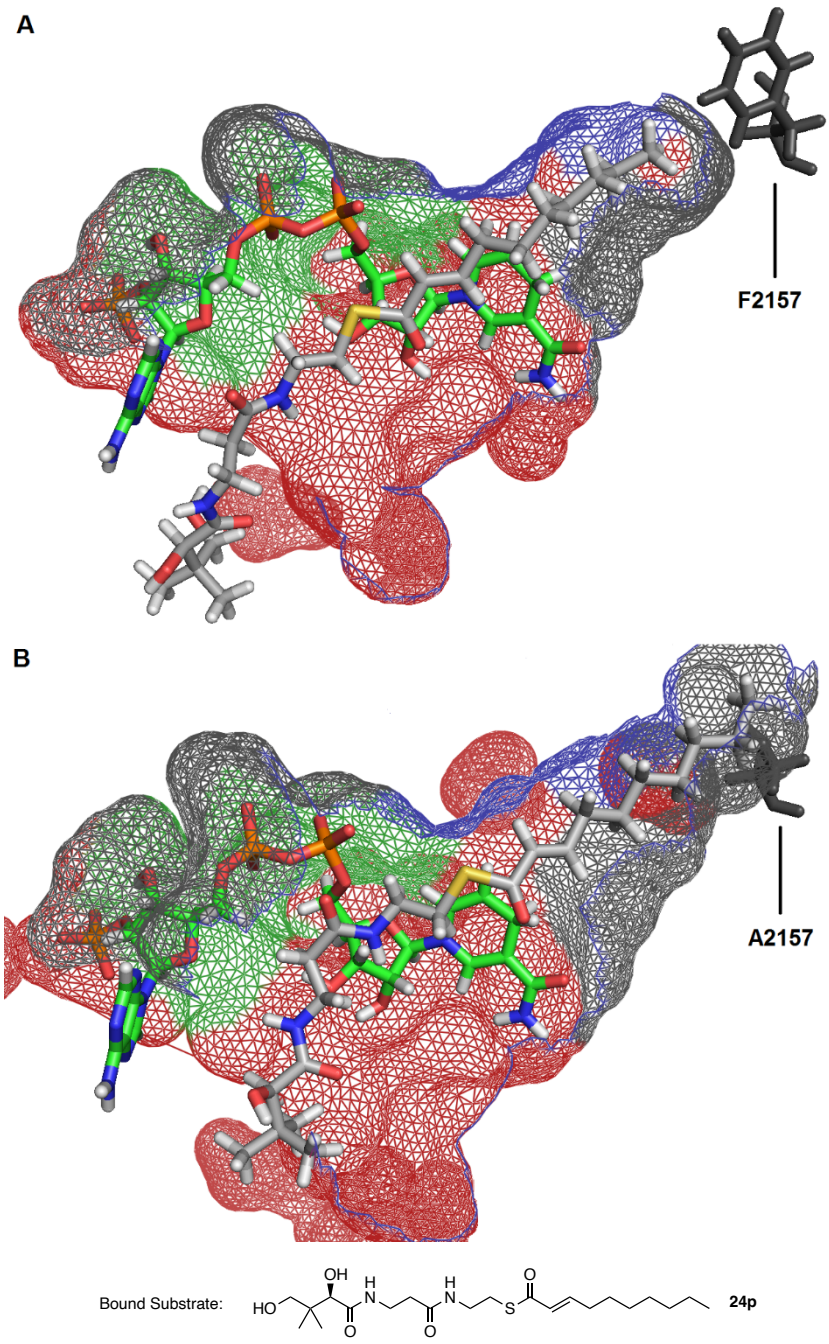


Figure S2.4E: Active site of the SQTKS ER domain with a mesh surface. In green the cofactor NADPH and in white the modeled pentaketide substrate **24p**. Modelled are: **A**, WT ER; **B**, F2157A ER.

2.5 Distance between the α-carbon and the catalytic hydrogen of the nicotinamide

	Model SQTKS *holo* ER	**Ligand**	**A-Carbon distance / Å**
1	WT	-	-
2	WT	Triketide **18p**	2.8
3	WT	4*S*,6*S* tetraketide **11p**	3.7
4	WT	4*R*,6*S* tetraketide **12p**	3.5
5	WT	4*R*,6*R* tetraketide **13p**	3.6
6	WT	4*S*,6*R* tetraketide **14p**	3.5
7	WT	Pentaketide **24p**	4.9
8	I1938A	4*S*,6*S* tetraketide **11p**	3.6
9	F1941A	4*S*,6*S* tetraketide **11p**	3.1
10	L2146A	Triketide **18p**	3.0
11	L2146A	Pentaketide **24p**	4.5
12	L2146V	Triketide **18p**	2.8
13	L2146V	Pentaketide **24p**	4.8
14	I2147A	Triketide **18p**	3.1
15	I2147A	Pentaketide **24p**	4.9
16	F2157A	Triketide **18p**	2.2
17	F2157A	Pentaketide **24p**	3.2
18	L2146A/I2147A	Triketide **18p**	2.9
19	L2146A/I2147A	Pentaketide **24p**	4.8
20	I2147A/F2157V	Triketide **18p**	2.0
21	I2147A/F2157V	Pentaketide **24p**	4.4
22	F1941A/F2157A	Triketide **18p**	2.3
23	F1941A/F2157A	Pentaketide **24p**	3.7
24	F1941A/I2147A/F2157V	Triketide **18p**	2.0
25	F1941A/I2147A/F2157V	Pentaketide **24p**	4.7
26	F1941A/I2147A/F2157V	Tetraketide **11p**	2.7

2.6 Data Deposition

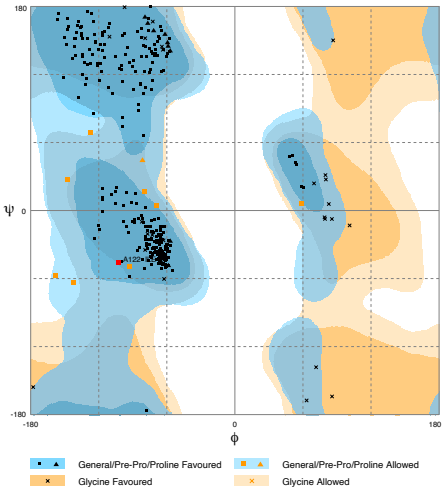
All model structures have been deposited at *figshare* as pdb coordinate files with the following DOI:

	Model SQTKS *holo* ER	**Ligand**	**DOI**
1	WT	–	10.6084/m9.figshare.11709966
2	WT	Triketide **18p**	10.6084/m9.figshare.11709567
3	WT	4*S*,6*S* tetraketide **11p**	10.6084/m9.figshare.11709579
4	WT	4*R*,6*S* tetraketide **12p**	10.6084/m9.figshare.11709585
5	WT	4*R*,6*R* tetraketide **13p**	10.6084/m9.figshare.11709591
6	WT	4*S*,6*R* tetraketide **14p**	10.6084/m9.figshare.11709600
7	WT	Pentaketide **24p**	10.6084/m9.figshare.11709621
8	I1938A	4*S*,6*S* tetraketide **11p**	10.6084/m9.figshare.11709639
9	F1941A	4*S*,6*S* tetraketide **11p**	10.6084/m9.figshare.11709651
10	L2146A	Triketide **18p**	10.6084/m9.figshare.11709657
11	L2146A	Pentaketide **24p**	10.6084/m9.figshare.11709669
12	L2146V	Triketide **18p**	10.6084/m9.figshare.11709672
13	L2146V	Pentaketide **24p**	10.6084/m9.figshare.11709681
14	I2147A	Triketide **18p**	10.6084/m9.figshare.11709684
15	I2147A	Pentaketide **24p**	10.6084/m9.figshare.11709690
16	F2157A	Triketide **18p**	10.6084/m9.figshare.11709702
17	F2157A	Pentaketide **24p**	10.6084/m9.figshare.11709711
18	L2146A/I2147A	Triketide **18p**	10.6084/m9.figshare.11709729
19	L2146A/I2147A	Pentaketide **24p**	10.6084/m9.figshare.11709744
20	I2147A/F2157V	Triketide **18p**	10.6084/m9.figshare.11709747
21	I2147A/F2157V	Pentaketide **24p**	10.6084/m9.figshare.11709756
22	F1941A/F2157A	Triketide **18p**	10.6084/m9.figshare.11709768
23	F1941A/F2157A	Pentaketide **24p**	10.6084/m9.figshare.11709882
24	F1941A/I2147A/F2157V	Triketide **18p**	10.6084/m9.figshare.11709897
25	F1941A/I2147A/F2157V	Pentaketide **24p**	10.6084/m9.figshare.11709903
26	F1941A/I2147A/F2157V	Tetraketide **11p**	10.6084/m9.figshare.11709963

2.7 Ramachandran Analysis

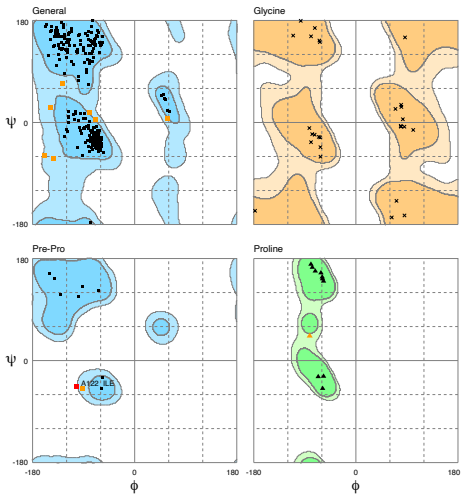
All protein models were analysed using RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).[9]

2.7.2 WT Triketide 18p



Number of residues in favoured region (~98.0% expected) : 309 (96.9%)
Number of residues in allowed region (~2.0% expected) : 9 (2.8%)
Number of residues in outlier region : 1 (0.3%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
Structure validation by Cα geometry: φ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450



Number of residues in favoured region (~98.0% expected) : 309 (96.9%)
Number of residues in allowed region (~2.0% expected) : 9 (2.8%)
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RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
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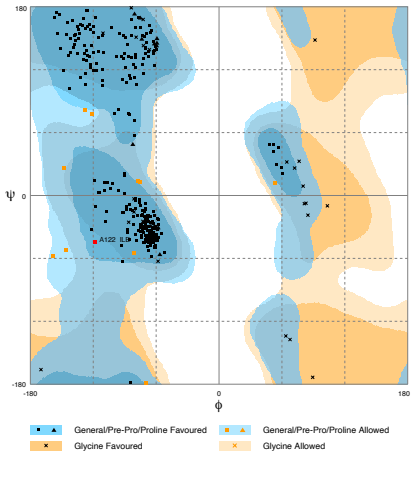
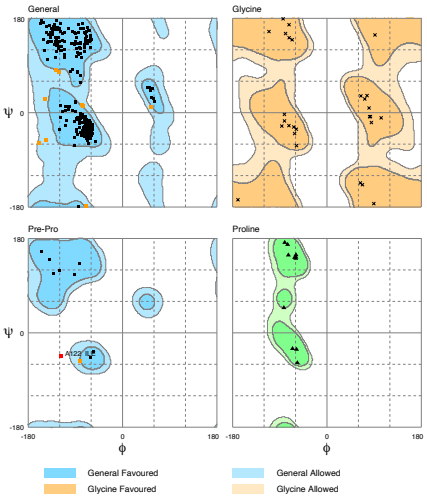
Structure validation by Cα geometry: φ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

2.7.3 WT 4S,6S tetraketide 11p



Number of residues in favoured region (~98.0% expected) : 308 (96.6%)
Number of residues in allowed region (~2.0% expected) : 10 (3.1%)
Number of residues in outlier region : 1 (0.3%)

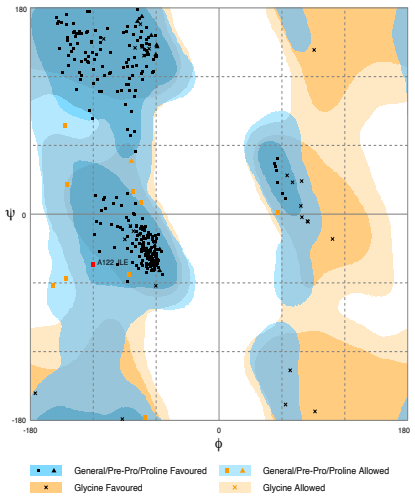
RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
Structure validation by Cα geometry: φ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

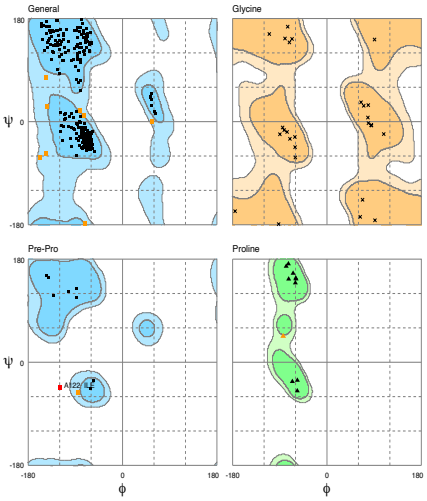


Number of residues in favoured region (~98.0% expected) : 308 (96.6%)
Number of residues in allowed region (~2.0% expected) : 10 (3.1%)
Number of residues in outlier region : 1 (0.3%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
Structure validation by Cα geometry: φ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

2.7.4 WT 4R,6S tetraketide 12p





General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected) : 308 (96.6%)
Number of residues in allowed region (~2.0% expected) : 10 (3.1%)
Number of residues in outlier region : 1 (0.3%)

Number of residues in favoured region (~98.0% expected) : 308 (96.6%)
Number of residues in allowed region (~2.0% expected) : 10 (3.1%)
Number of residues in outlier region : 1 (0.3%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/

Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002) Structure validation by Cα geometry: ϕ,ψ and Cβ deviation. Proteins: Structure, Function & Genetics. **50**:437-450

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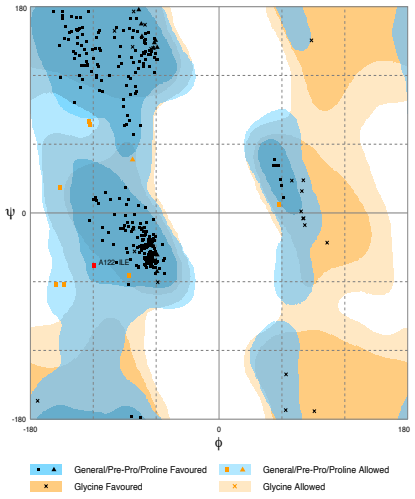
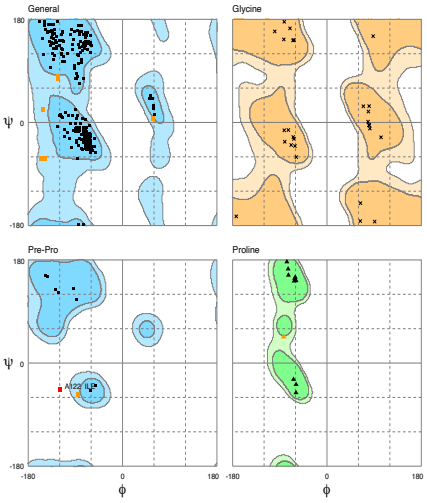
2.7.5 WT 4R,6R tetraketide 13p





General/Pre-Pro/Proline Favoured
Glycine Favoured
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Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected) : 310 (97.2%)
Number of residues in allowed region (~2.0% expected) : 8 (2.5%)
Number of residues in outlier region : 1 (0.3%)

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Number of residues in allowed region (~2.0% expected) : 8 (2.5%)
Number of residues in outlier region : 1 (0.3%)

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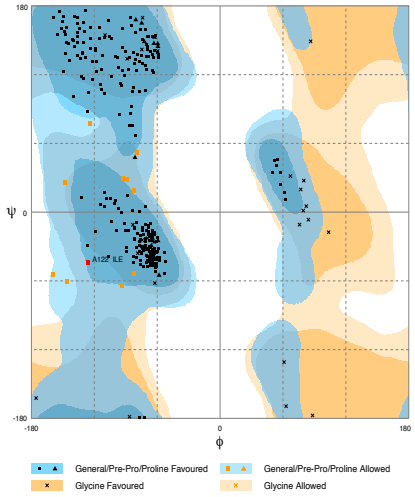
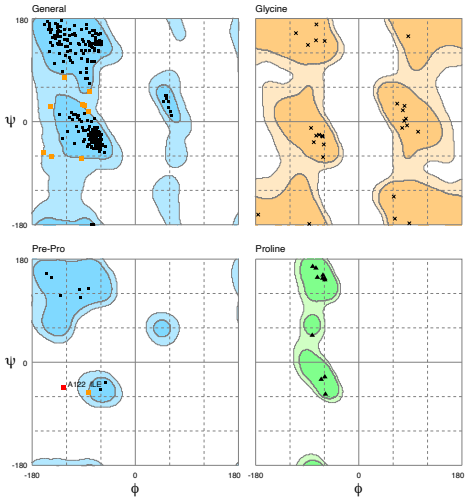
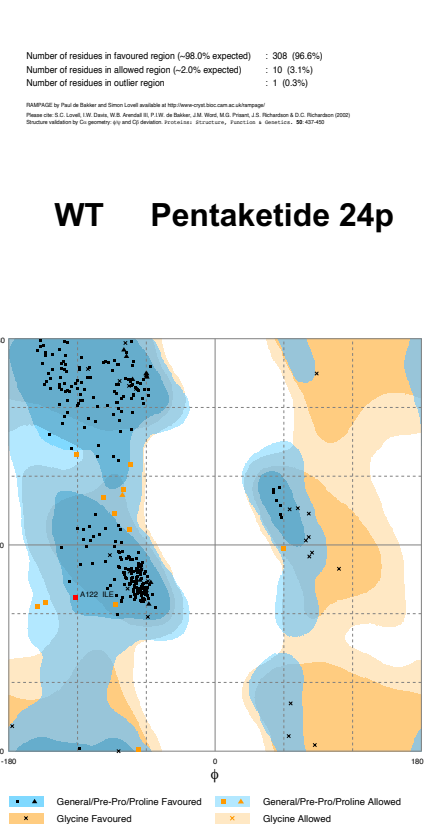
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002) Structure validation by Cα geometry: ϕ,ψ and Cβ deviation. Proteins: Structure, Function & Genetics. **50**:437-450

2.7.6 WT 4S,6R tetraketide 14p







Number of residues in favoured region (~98.0% expected) : 308 (96.6%)
Number of residues in allowed region (~2.0% expected) : 10 (3.1%)
Number of residues in outlier region : 1 (0.3%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
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Number of residues in allowed region (~2.0% expected) : 10 (3.1%)
Number of residues in outlier region : 1 (0.3%)

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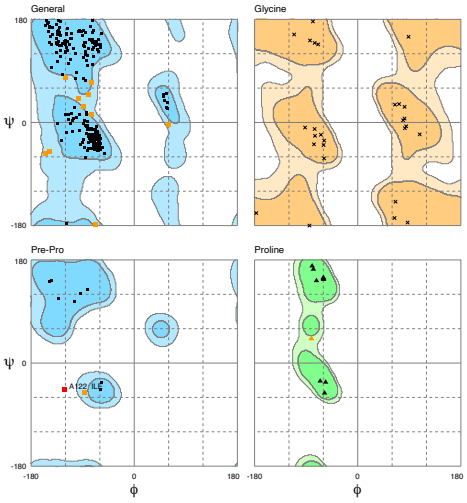
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

2.7.7 WT Pentaketide 24p



Number of residues in favoured region (~98.0% expected) : 306 (95.9%)
Number of residues in allowed region (~2.0% expected) : 12 (3.8%)
Number of residues in outlier region : 1 (0.3%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
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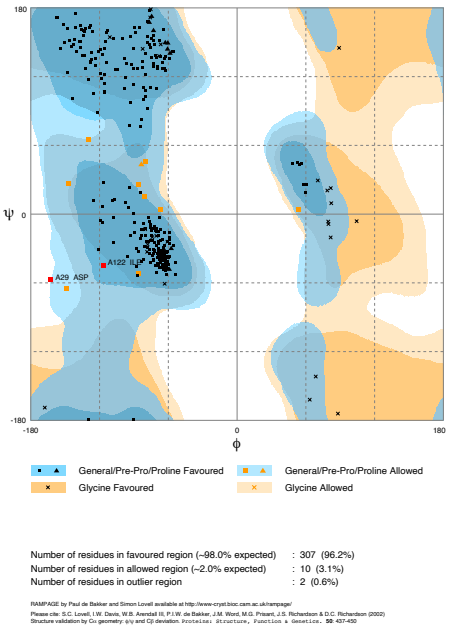
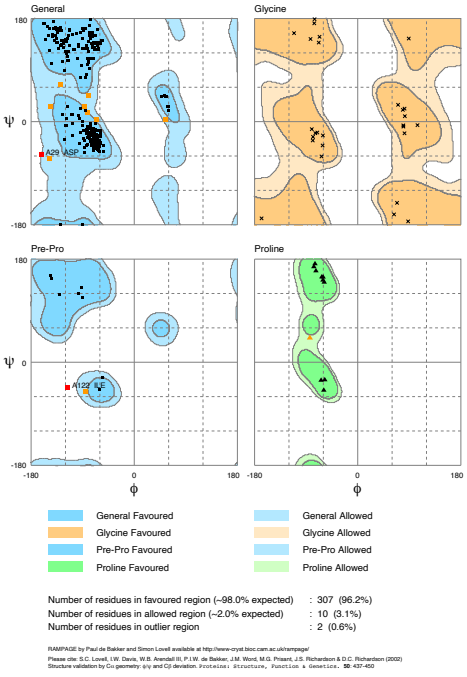
Number of residues in allowed region (~2.0% expected) : 12 (3.8%)
Number of residues in outlier region : 1 (0.3%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

2.7.8 I1938A 4S,6S tetraketide 11p





Number of residues in favoured region (~98.0% expected) : 307 (96.2%)
Number of residues in allowed region (~2.0% expected) : 10 (3.1%)
Number of residues in outlier region : 2 (0.6%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
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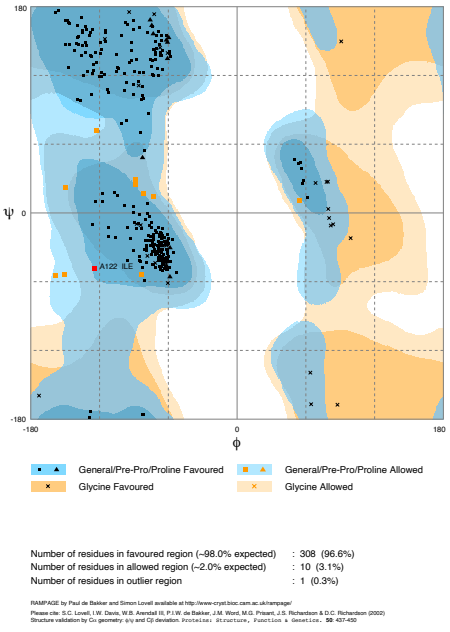
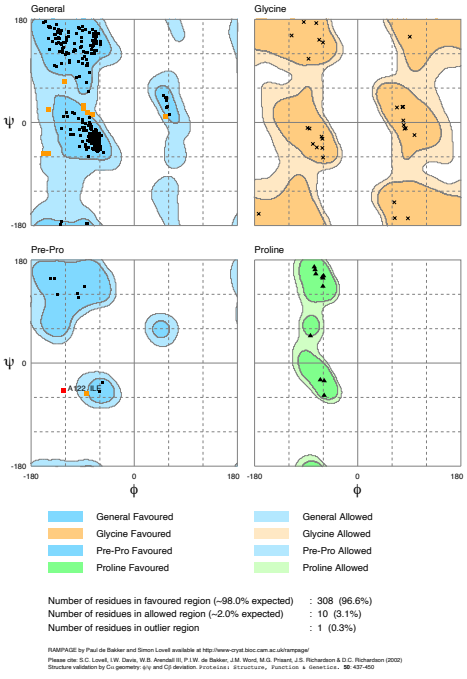
Number of residues in favoured region (~98.0% expected) : 307 (96.2%)
Number of residues in allowed region (~2.0% expected) : 10 (3.1%)
Number of residues in outlier region : 2 (0.6%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

2.7.9 F1941A 4S,6S tetraketide 11p





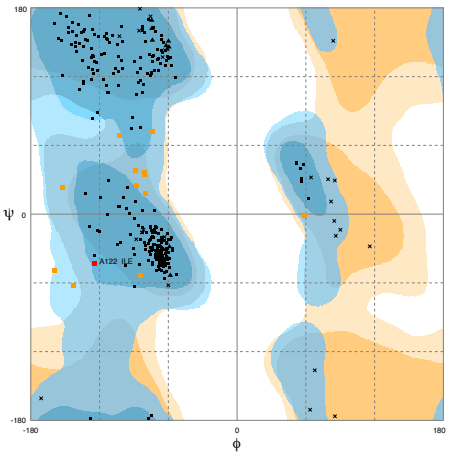
Number of residues in favoured region (~98.0% expected) : 308 (96.6%)
Number of residues in allowed region (~2.0% expected) : 10 (3.1%)
Number of residues in outlier region : 1 (0.3%)

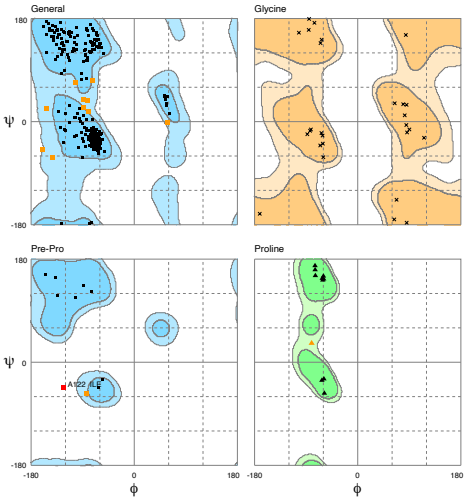
RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

Number of residues in favoured region (~98.0% expected) : 308 (96.6%)
Number of residues in allowed region (~2.0% expected) : 10 (3.1%)
Number of residues in outlier region : 1 (0.3%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

2.7.10 L2146A Triketide 18p





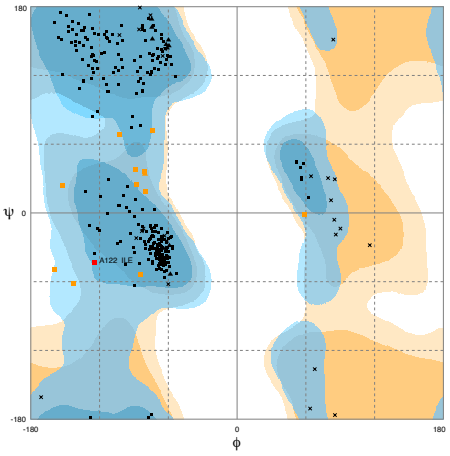
General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

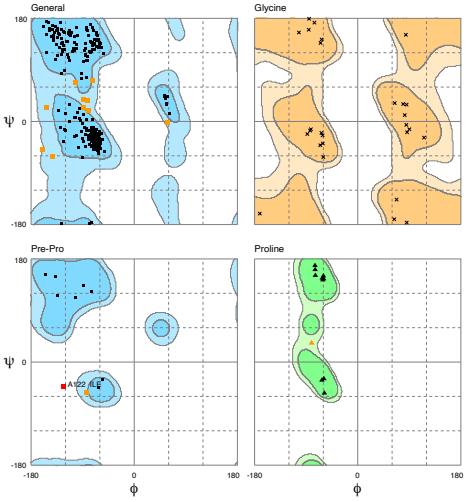
General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected) : 306 (95.9%)
Number of residues in allowed region (~2.0% expected) : 12 (3.8%)
Number of residues in outlier region : 1 (0.3%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. *Proteins: Structure, Function & Genetics*. **50**: 437-450

2.7.11 L2146A Pentaketide 24p





General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected) : 306 (95.9%)
Number of residues in allowed region (~2.0% expected) : 12 (3.8%)
Number of residues in outlier region : 1 (0.3%)

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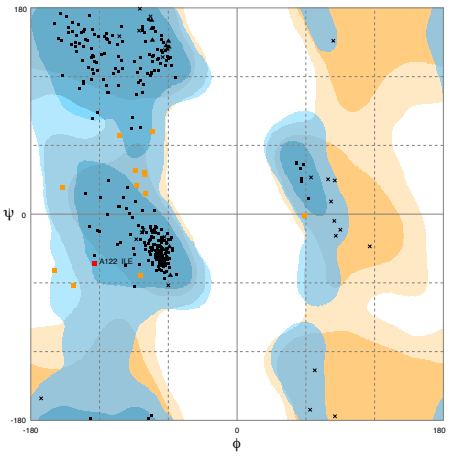
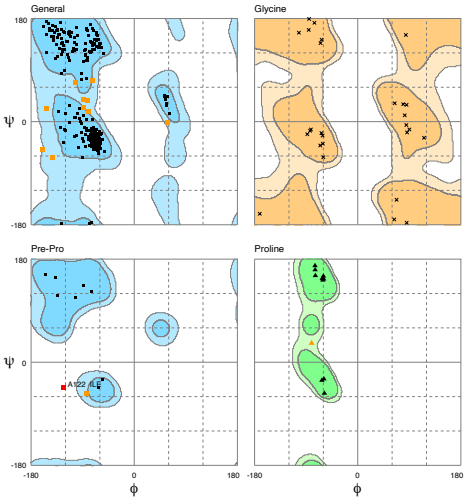
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. *Proteins: Structure, Function & Genetics*. **50**: 437-450

2.7.12 L2146V Triketide 18p





General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected) : 306 (95.9%)
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Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
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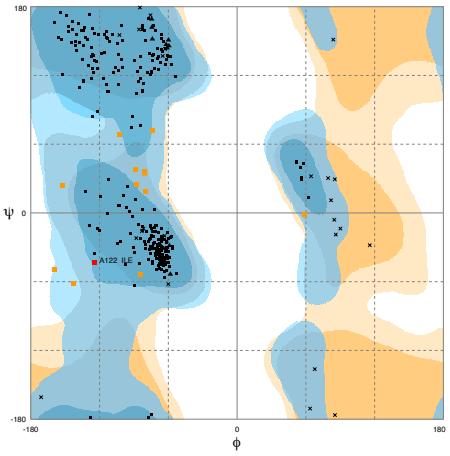
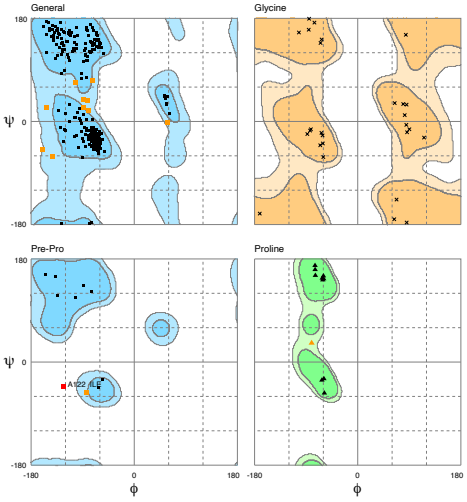
2.7.13 L2146V Pentaketide 24p





General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected) : 306 (95.9%)
Number of residues in allowed region (~2.0% expected) : 12 (3.8%)
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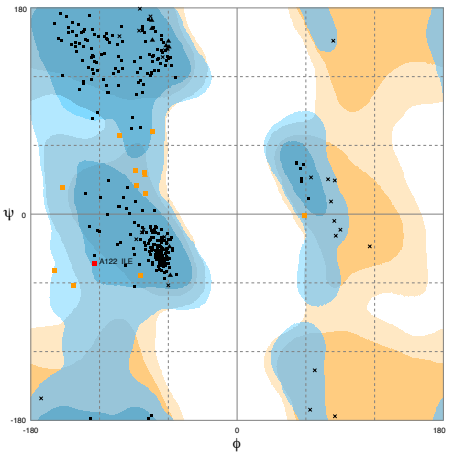
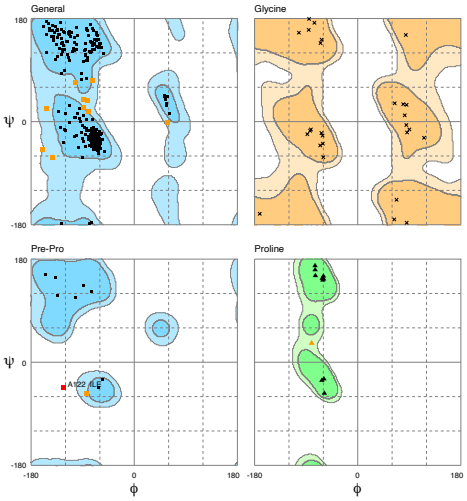
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. *Proteins: Structure, Function & Genetics*. **50**: 437-450

2.7.14 I2147A Triketide 18p





General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected)	: 306 (95.9%)
Number of residues in allowed region (~2.0% expected)	: 12 (3.8%)
Number of residues in outlier region	: 1 (0.3%)

Number of residues in favoured region (~98.0% expected)	: 306 (95.9%)
Number of residues in allowed region (~2.0% expected)	: 12 (3.8%)
Number of residues in outlier region	: 1 (0.3%)

RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/

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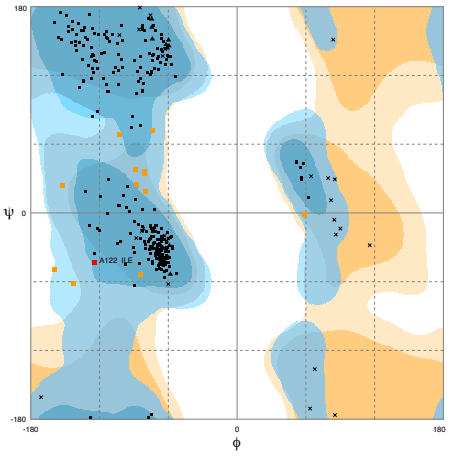
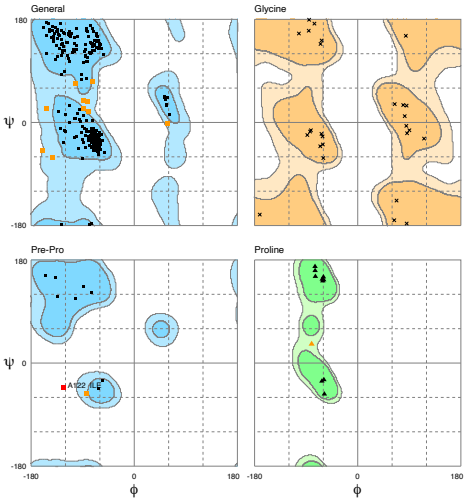
2.7.15 I2147A Pentaketide 24p





General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected)	: 306 (95.9%)
Number of residues in allowed region (~2.0% expected)	: 12 (3.8%)
Number of residues in outlier region	: 1 (0.3%)

Number of residues in favoured region (~98.0% expected)	: 306 (95.9%)
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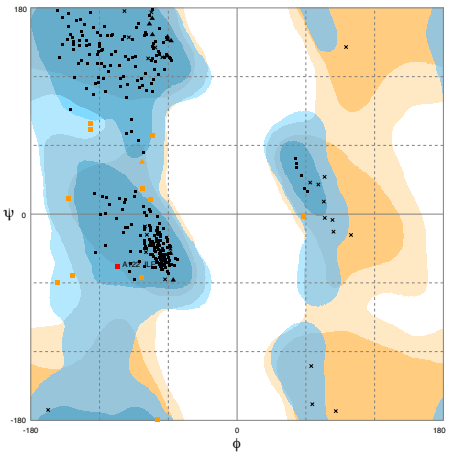
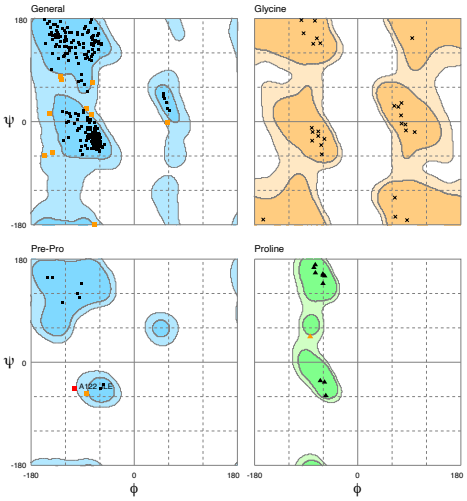
2.7.16 F2157A Triketide 18p





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RAMPAGE by Paul de Bakker and Simon Lovell available at http://www-cryst.bioc.cam.ac.uk/rampage/
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002)
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

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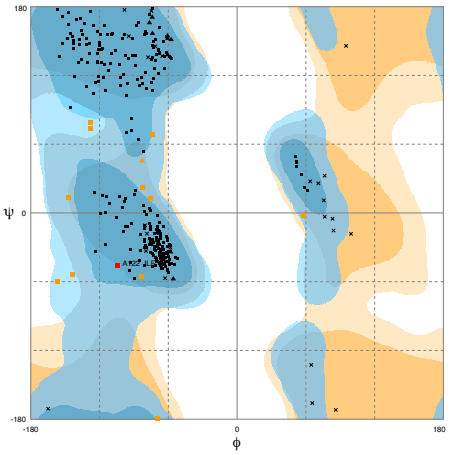
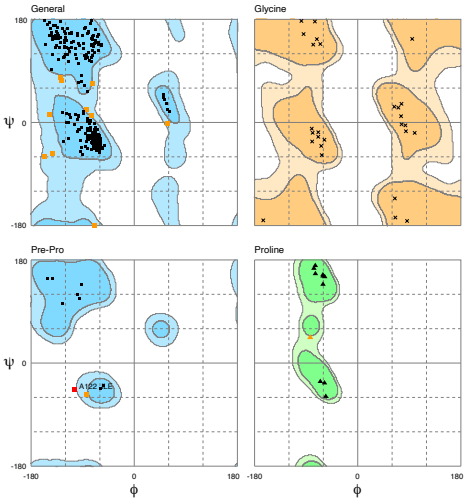
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

2.7.17 F2157A Pentaketide 24p





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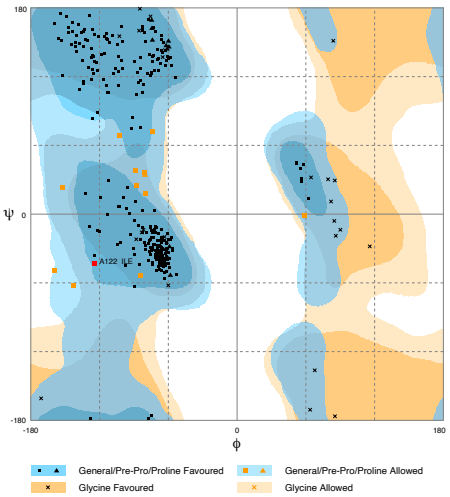
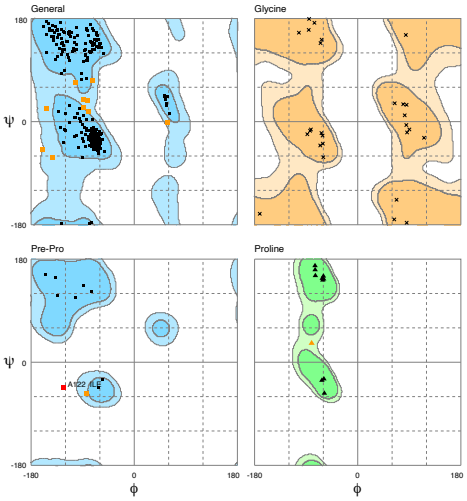
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. Proteins: Structure, Function & Genetics. 50: 437-450

2.7.18 L2146A/I2147A Triketide 18p





General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

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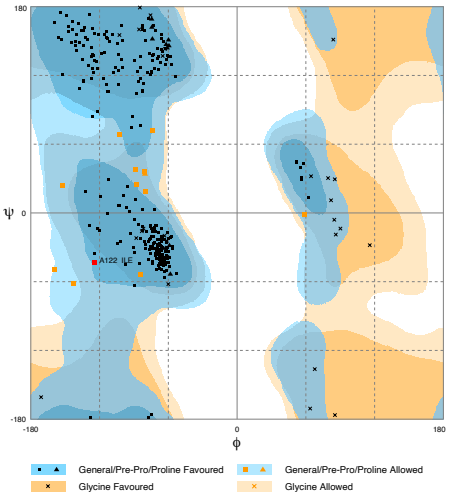
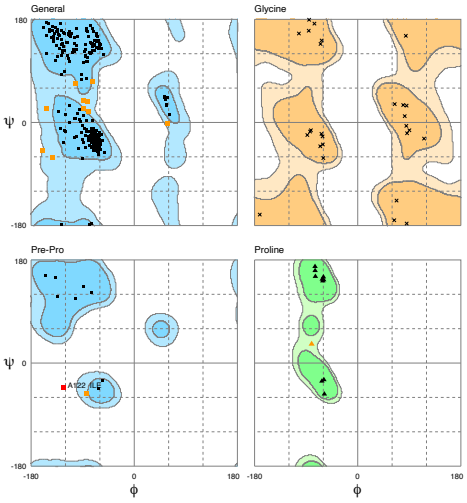
Please cite: S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson & D.C. Richardson (2002) Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. *Proteins: Structure, Function & Genetics*. **50**: 437-450

2.7.19 L2146A/I2147A Pentaketide 24p





General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected) : 306 (95.9%)
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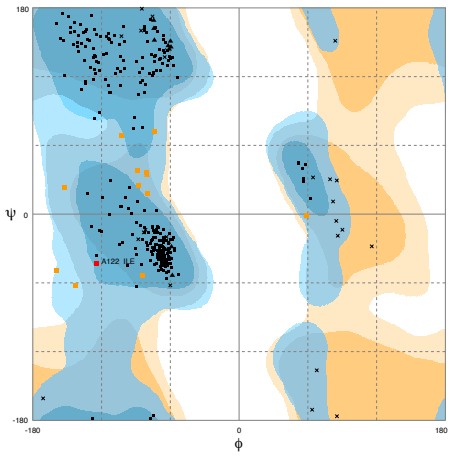
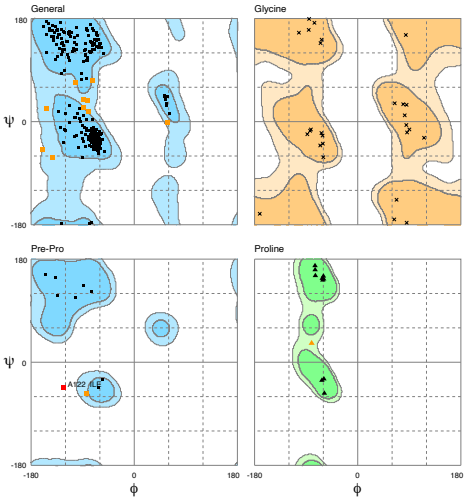
2.7.20 I2147A/F2157V Triketide 18p





General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected) : 306 (95.9%)
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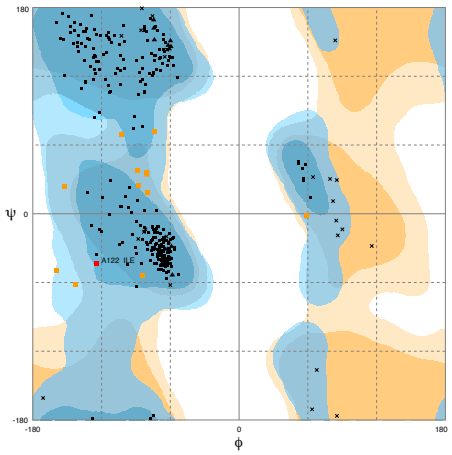
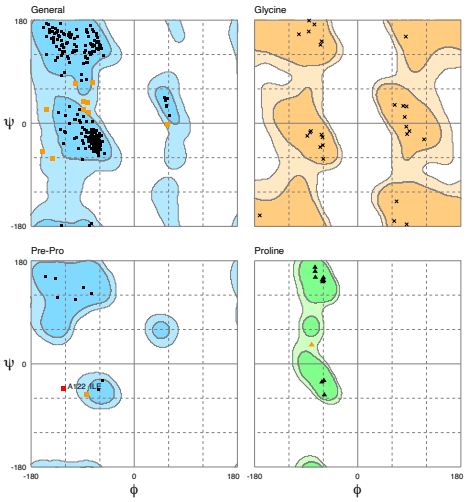
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. *Proteins: Structure, Function & Genetics*. **50**: 437-450

2.7.21 I2147A/F2157V Pentaketide 24p





General/Pre-Pro/Proline Favoured
Glycine Favoured
General/Pre-Pro/Proline Allowed
Glycine Allowed

General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected) : 306 (95.9%)
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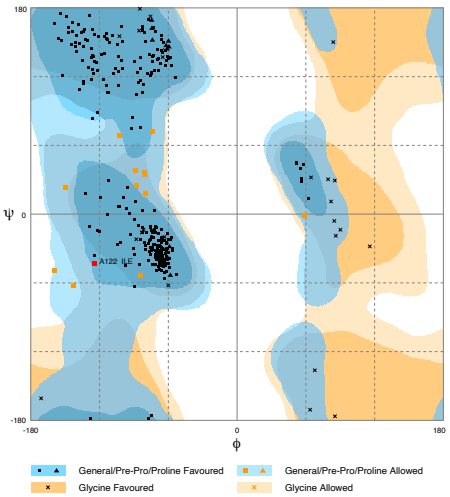
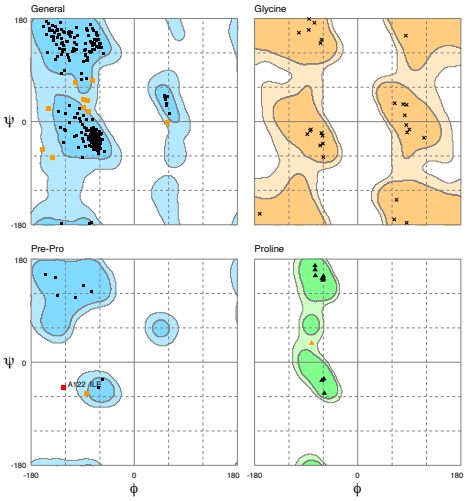
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. *Proteins: Structure, Function & Genetics*. **50**: 437-450

2.7.22 F1941A/F2157A Triketide 18p





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General Favoured
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Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected)	: 306 (95.9%)
Number of residues in allowed region (~2.0% expected)	: 12 (3.8%)
Number of residues in outlier region	: 1 (0.3%)

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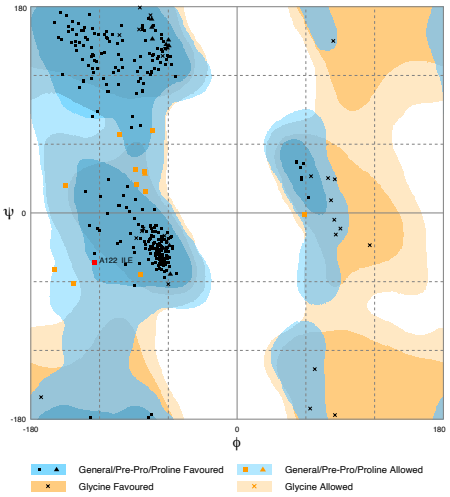
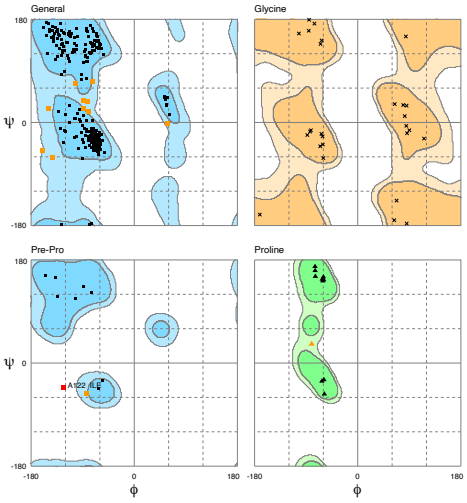
2.7.23 F1941A/F2157A Pentaketide 24p





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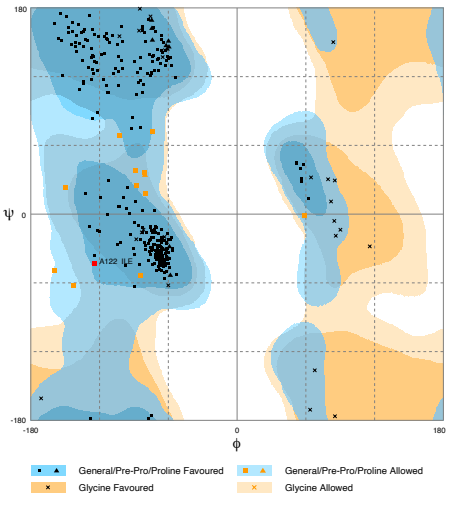
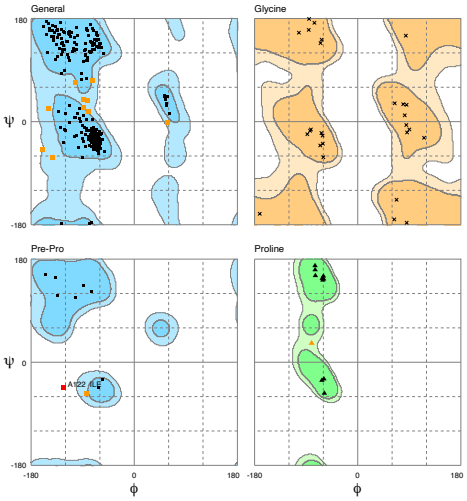
2.7.24 F1941A/I2147A/F2157V Triketide 18p





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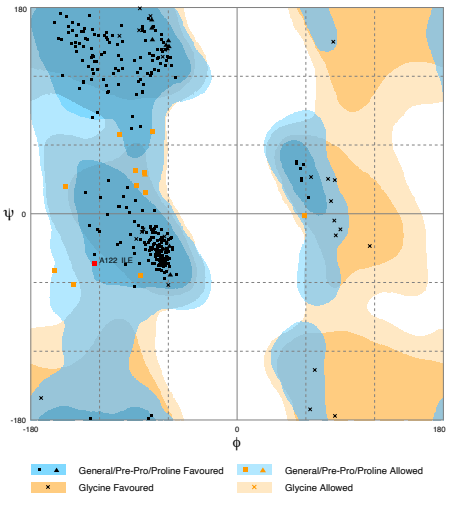
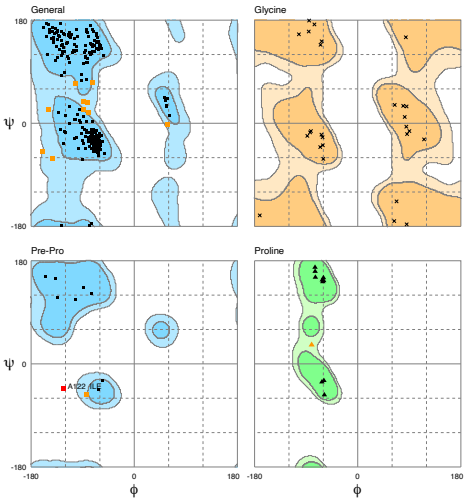
2.7.25 F1941A/I2147A/F2157V Pentaketide 24p





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General Favoured
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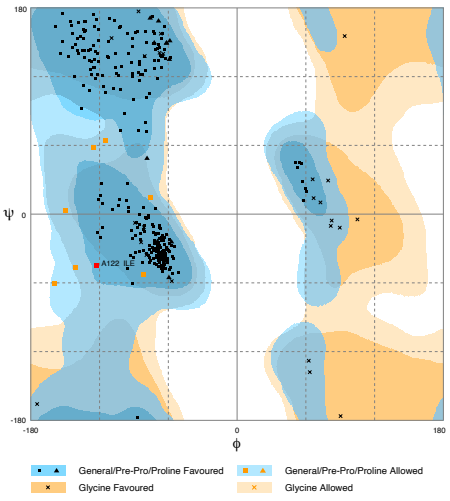
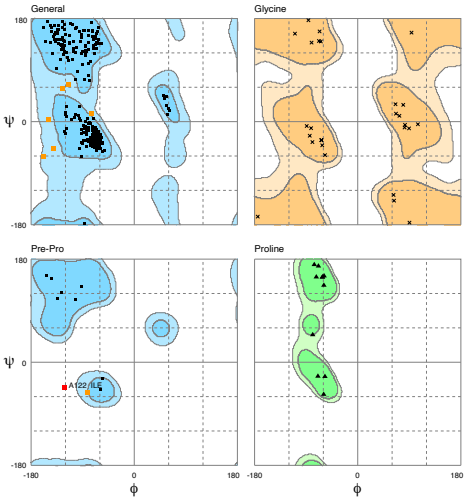
Structure validation by Cα geometry: ϕ/ψ and Cβ deviation. *Proteins: Structure, Function & Genetics*. **50**: 437-450

2.7.26 F1941A/I2147A/F2157V Tetraketide 11p





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General Favoured
Glycine Favoured
Pre-Pro Favoured
Proline Favoured
General Allowed
Glycine Allowed
Pre-Pro Allowed
Proline Allowed

Number of residues in favoured region (~98.0% expected)	: 311 (97.5%)
Number of residues in allowed region (~2.0% expected)	: 7 (2.2%)
Number of residues in outlier region	: 1 (0.3%)

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Number of residues in favoured region (~98.0% expected)	: 311 (97.5%)
Number of residues in allowed region (~2.0% expected)	: 7 (2.2%)
Number of residues in outlier region	: 1 (0.3%)

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3.0 General Methods and Equipment

3.1 NMR Analysis: ^{1}H-NMR analysis was performed using BRUKER DPX 200, Avance 400, DPX 400 and DRX 500 instruments. Signals are determined in some cases with two dimensional NMR ^{1}H, ^{1}H-COSY, ^{13}C-HSQC and ^{1}H, ^{13}C-J_3-HMBC. ^{13}C-NMR analysis was performed using BRUKER Avance 400, DPX 400 and DRX 500 instruments. Deuterated chloroform (ref. 7.26 ppm / 77.2 ppm)[10] and deuterated acetonitrile (ref. 1.94 ppm / 118.4 ppm) were used as solvents and served as internal references. All δ values are reportet in ppm. All *J* values are reported in Hz.

3.2 Column Chromatography: For column chromatography silicagel 60 (particle size 35-70 micron, Sigma-Aldrich or 40-63 micron, Macherey-Nagel) was used. Columns were packed wet under N_2 pressure. Products were eluted with the indicated solvent mixtures. Purified fractions were analysed by TLC and combined if same R_f was observed. Final products were evaporated in *vacuo*.

3.3 TLC: TLC analysis was preformed on TLC plates with a polyester backed 0.2 mm silica gel phase from Macherey and Nagel using the indicated solvent systems. Analysis of the plates were performed by ultraviolet light (254 nm) or with potassium permanganate (5 mmol) or *o*-anisaldehyde (anisaldehyde [15 g], EtOH [250 ml] and concentrated H_2SO_4 [2.5 ml]) solution.

3.4 Analytical LCMS: Analytical LCMS data were obtained with 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 600 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**.

3.5 Preparative LCMS: Purification of final 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 CH_3CN + 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 (vacuum centrifuge), weighed, and residues dissolved directly in solvent for use or analysis.

3.6 Protein Purification: The protein purification was performed with an FPLC ÄKTA Pure system (GE Healthcare). For FPLC analysis a combination with the software UNICORN 7.0 and different columns (Nickel column Protino Ni-NTA Columns 5 mL, Size exclusion column- HiLoad 26/600 Superdex 200pg (GE Healthcare), 320 mL) was used.

3.7 UV-Analysis: UV assays were measured at 340 nm with a JASCO-V630-spectrophotometer in quartz glass cuvettes with a depth of 10 mm. The temperature was controlled by the JASCO-V630-Spectrophotometer at 25 °C. The processed data (by JASCO/Spectramanager) was collected and then analysed in Microsoft EXCEL.

3.8 Buffers, Antibiotics, Media and Solutions

All enzymes used in this work were purchased from Thermo Fisher Scientific (Waltham, MA, USA), Takara Bio Inc. (Shiga, J), Invitrogen Life Technologies (Darmstadt, D), or Bioline (London, UK). All enzymes were used according to manufacturer's instructions with appropriate supplied buffers. Buffers and media used in this work were sterilised by autoclaving 15 min at 121 °C (Autoclave 2100 Classic, Prestige Medical) or by disposable sterile filter (0.45 µm pore size, Roth) and are summarized in tables S3.8A and S3.8B7.

Table S3.8A: Media used in this work

Media	Composition [% (w/v)]	Ingredients
2TY	1	Yeast extract
	0.5	Sodium chloride
	1.6	Tryptone
LB	0.5	Yeast extract
	1	Tryptone
	0.5	Sodium chloride
SOC	0.5	Yeast extract
	2	Tryptone
	0.06	Sodium chloride
	0.02	Potassium chloride
	25 mM	Magnesium chloride × 6 H_20
	1	D(+)-Glucose

Table S3.8B: Agar used in this work

Agar	Composition [% (*w/v*)]	Ingredients
LB agar	0.5	Yeast extract
	1	Tryptone
	0.5	Sodium chloride
	1.5	Agar

Antibiotics stock solutions were prepared in distilled water or ethanol. They were filter sterilized through 0.45 µm syringe filter and stored at −20 °C. Stock and working concentrations are listed in table S3.8C.

Table S3.8C: Agar used in this work

Antibiotic	Solvent	Stock concentration [mg / ml]	Working concentration [µg / ml]
Carbenicillin	H_2O	50	50
Kanamycin	H_2O	50	50

Information about E. *coli* strains used in this work are summarized in table S3.8D.

Table S3.8D: *E. coli* strains used in this work

Bacteria	Reference
BL21 (DE3)	Thermo Fisher Scientific
OneShot *ccdB* survival 2T1R	Thermo Fisher Scientific
OneShot Top10	Thermo Fisher Scientific

4.0 Preparation of WT and Mutant ER domains and Assay Procedures

4.1 SQTKS ER Domain

4.1.1 Transformation of pET28-SQTKS-ER into *E. coli* Top10

50 µl chemically competent *E. coli* Top10 cells were thawed on ice and incubated on ice for 30 min with 1 µl of the appropriate vector. Subsequently heat shock transformation was performed, therefore, the cells were incubated at 42 °C for 30 s, then immediately chilled on ice for 2 min. 250 µl SOC medium were added. The transformed cells were shaken (350 rpm) for 1 h at 37 °C. Positive clones were selected by plating 50 - 150 µl of the cells on solid LB medium containing the appropriate antibiotic. The cells were grown over night at 37 °C

4.1.2 Transformation of pET28-SQTKS-ER into *E. coli* BL21

50 µl chemically competent *E. coli* BL 21 cells were thawed on ice and incubated on ice for 30 min with 1 µl of the SQTKS-ER-domain. Subsequently heat shock transformation was performed, therefore, the cells were incubated at 42 °C for 10 s, then immediately chilled on ice for 2 min. 800 µl SOC medium were added. The transformed cells were shaken (350 rpm) for 1 h at 37 °C. Positive clones were selected by plating 50 - 150 µl of the cells on solid LB medium containing the appropriate antibiotic. The cells were grown over night at 37 °C.

4.1.3 Protein Expression and Purification

A starter culture was prepared by scraping the surface of a glycerol stock of *E.coli* BL21 transformed with pET28a-ER. The cells were grown in LB media with kanamycin (50 mg/mL) and incubated at 37 °C, 180 rpm, overnight. 0.5 ml of the starter culture was added to 100 mL 2TY media. The cells were incubated to an OD_{600} of 0.6, then the flask was cooled down to 16 °C and induced with 50 µl 1M IPTG solution. This solution was incubated over night at 16 °C, 180 rpm.

To isolate the protein, the media was centrifuged at 7000 rpm for 30 min and the pellet was collected. The cells could be used immediately or frozen at -20 °C. The cells were suspended in 50 ml of nickel column wash buffer (50 mM Tris pH 8, 150 mM NaCl, 10% glycerol (*v/v*) and 20 mM imidazole) and sonicated on ice for 6.5 minutes. Every 30 second the sonicator was switched on and of. The rest of the solution was centrifuged at 8500 rpm for 30 min, filtered and purified by a nickel column*. For this a linear gradient of elution buffer (50 mMTris pH 8, 150 mM NaCl, 10% glycerol (*v/v*) and 0.5 M imidazole) was used. The fractions were checked by SDS gel and the fractions with the correct mass were combined and concentrated. A second purification was done by a size exclusion column chromatography**. The protein solution was loaded onto the column and eluted with size exclusion elution buffer (Buffer: 50 mM Tris pH 8, 150 mM NaCl, 20% glycerol (*v/v*)). The fractions

were analyzed again by SDS gel and the protein with the correct mass and high purity was combined and concentrated. The concentration of protein was estimated by a calculated absorption coefficient ε= 0.69 at 280 nm. After that the protein was divided into several aliquots and stored at - 4 °C.

*Nickel column Protino Ni-NTA Columns 5 mL
**Size exclusion column- HiLoad 26/600 Superdex 200pg (GE Healthcare), 320 ml).

4.1.4 Colony Polymerase Chain Reaction

The successful transformation of BL21 cells was conFigured by Colony-PCR. For a Colony-PCR the template was added by transferring a small part of a single *E.coli* colony into the reaction mixture using a sterile toothpick. For each primer pair the specific annealing temperature was determined in a PCR temperature gradient. A typical Colony-PCR contained:

0.2 µl	Forward Primer
0.2 µl	Reverse Primer
–	Picked DNA
2.5 µl	One Taq Master Mix
7.1 µl	Water
Total: 10 µl	

4.1.5 Bradford Assay

Standard solutions of bovine serum albumin (0.1-2 ml/ml) in size exclusion buffer [50 mM Tris pH 8, 150 mM NaCl, 20% glycerol (*v/v*)] were prepared by serial dilution. 100 µl of the standards were mixed with Bradford dye reagent (1 ml) and incubated for 15 min at RT. The absorption of each sample was measured at 595 nm against a standard (size exclusion buffer 100 µl, Bradford dye reagent, 1 ml) to construct a standard concentration curve. A sample of the protein to be quantified (20 µl) was diluted in size exclusion buffer (80 µl) and treated with Bradford dye reagent (1ml). This was incubated at room temperature for 15 mins and then the absorption was measured at 595 nm. This was compared to the previously prepared concentration curve to calculate the amount of protein that had been produced.

4.2 SQTKS ER-Domain Enzyme Assay

ER activity assays are performed in a total volume of 400 µl. The concentration of NADPH (10 µL of 10 mM stock) and SQTKS-ER-domain (20 µL of a 1.0 $mg \cdot ml^{-1}$ stock solution) and the temperature at 25 °C were kept constant in all assays. The amount of buffer (50 mM Tris pH 8.0, 150 mM NaCl,

20% Glycerol, 290-365 µL) and substrate (5-80 µL of 5 mM Stock) varied between the assays. The assays are performed in cuvettes under measuring the UV absorption at 340 nm.

4.3 Mutagenesis

4.3.1 Plasmid Isolation

For the isolation of the plasmid first an overnight culture of *E-coli* Top10 cells with the desired plasmid were cultivated. The next day the cell culture was centrifuged in a 2 ml Eppendorf-tube at 13.000 g for 2 min and 4 °C. Afterwards the supernatant was discarded and the previous step was repeated with the remaining overnight culture, if necessary. The pellet was resuspended in 100 µl Solution (5 mM Glucose, 25 mMTris-HCl (pH 8.0) and 10 mM EDTA) and 5 µl RNAse. The sample was incubated for 5 min by RT. 200 µl of Solution II (0.2 M NaOH and 1% SDS) was added It was mixed carefully by inversion. Incubated 5 min by RT. 150 µl Solution III was added (60 ml 5M Potassium acetate, 11.5 ml conc. acetic acid and 20.5 ml H_2O) and mixed carefully by inversion. The mixture was centrifuged by 11 000 g for 5 min by 4 °C. The supernatant was transferred to a new 1.5 ml Eppendorf-tube. Then 800 µl EtOH (96%, bio quality) and 45 µl 3 M Na-Acetate were added, inverted and to precipitate the plasmid DNA 30 min by -20 °C incubated. Afterwards by 11 000 g for 5 min by 4 °C centrifuged. Then the supernatant was discarded and the pellet was washed with 70% EtOH (bio quality) (100 µl). Afterwards the suspension was centrifuged at max speed for 5 min at RT. Then the supernatant discarded and pellet was dried by 37 °C for 15 min until it was clear. The pellet was resuspended in 20 µl Water and could be stored at -20 °C for further use.

4.3.2 Site Directed Mutagenesis[11]

For the site directed mutagenesis, different Oligo-primers were ordered from Sigma Aldrich and used in different combinations. Hereby non-overlapping primer pairs were tested.

As a template the vector pET28a containing the ER sequence of SQTKS was chosen. Two different PCR approaches with different polymerases were tested. The first approach contained the Q5 High-Fidelity DNA polymerase the other the Phusion High-Fidelity DNA polymerase.

Q5 High-Fidelity DNA polymerase

12.5 µl	Q5 Master Mix
1.25 µl	Forward Primer
1.25 µl	Reverse Primer
1 µl	Plasmid DNA
(6.5–9 µl)	Water
(0–2.5 µl)	$MgCl_2$ (50 nM)
Total: 25 µl	

Phusion High-Fidelity DNA polymerase

5 µl	Phusion Buffer (5x)
1.25 µl	Forward Primer
1.25 µl	Reverse Primer
1 µl	Plasmid DNA
(9–11.25 µl)	Water
(0–2.5 µl)	$MgCl_2$ (50 nM)
5 µl	dNTP Mix (1.25 mM)
0.25 µl	Polymerase
Total: 25 µl	

Different annealing temperatures were tested to find the best condition for the different primer combination. For efficient DNA amplification, 22 PCR cycles with an elongation time of 6.30 min were applied.

The products of the PCR were checked on an Agarose-Gel. Afterwards the blunt ends of the primers were ligated through the use of the Quick Ligation Kit (New England BioLabs). Hereby to 10 µl of the PCR product were 10 µl of the 2 × Quick Ligation Buffer added and mixed. 1 µl Quick T4 DNA Ligase were added and mixed. The mixture was incubated by RT for 15 min. Then 0.5 µl of DpnI were added to the sample and incubated for 2 h. The enzyme cuts methylated DNA, therefore the parental DNA string will be cut. Afterwards 1 µl of the mixture were transformed into TOP10 Cells (see 8.4). The transformation were checked via Colony-PCR.

4.3.3 Sequencing

Sequencing was done by Eurofins Genomics. Plasmids were sent to sequencing at a concentration of 100-200 ng / µl. Sequence analysis was done in Geneious (Version 7.1.9) through alignment with the original DNA sequence.

5.0 Synthesis of Pantetheine Substrates

Solvents were used without further purification or drying unless otherwise stated. Anhydrous Tetrahydrofuran (THF), diethyl ether (Et_2O) and dichloromethane (DCM) and NADPH were purchased from Carl Roth. Other chemicals were obtained from Sigma Aldrich or TCI Deutschland GmbH. The syntheses were done using previously established protocols,1[1,12] and where this was not the case it was recorded.

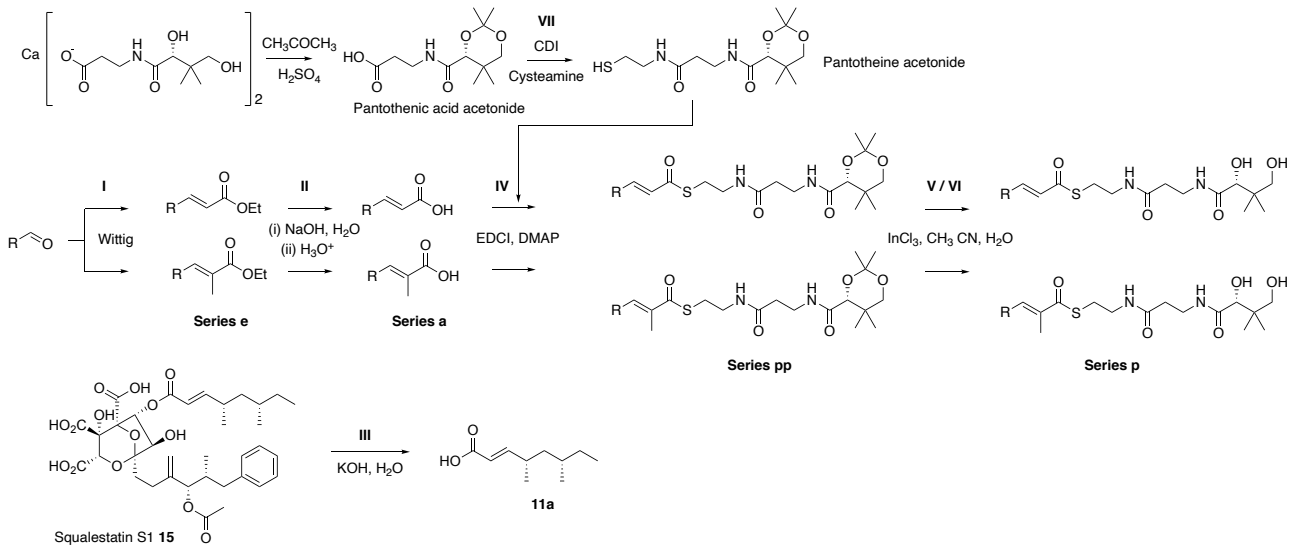


Figure S5.0A. Overall synthetic route to ER pantetheine substrates.

5.1 General Synthetic Methods

I. Wittig Reaction. A solution of dichloromethane (5 mL) and the respective aldehyde (1.00 mmol) was stirred at 0 °C. (Carbethoxyethylidene)triphenylphosphorane (0.72 g, 2.00 mmol) or Carbethoxy-methylidene)triphenylphosphorane (0.69 g, 2.00 mmol) was added to this solution warmed to 25 °C and stirred for 16-18 h. Then the solvent was evaporated under a nitrogen flow. The crude product was purified by column chromatography.

II. Ester Hydrolysis. To a solution of the corresponding ester in ethanol/water 5:1 (5 mL/ 1 mL) potassium hydroxide (22 mmol) was added to the solution. After stirring under reflux for 3 hours diethyl ether was added. The mixture was washed with $NaHCO_3$ (3 × 10 mL). Then the aqueous layer was acidified with 2 M HCl until pH 1 and extracted with ethyl acetate (3 × 10 ml). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*.

III. Squalestatin Hydrolysis. To a solution of squalestatin S1 (1.00 g, 1.40 mmol) in ethanol/water 5:1 (5 mL/ 1 mL) potassium hydroxide (22.00 mmol) was added to the solution. After stirring under

reflux for 3 hours, diethyl ether was added to the solution and the mixture was washed with $NaHCO_3$ (3 × 10 mL). The aqueous layer with the (4*S*, 6*S*)-2-4-dimethyloct-2-enoic acid was acidified with 2 M HCl until pH 1 and extracted with ethyl acetate (3 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*.

IV: Preparation of Acyl Pantetheine Acetonides[12]

Acid (1.00 mmol) and pantetheine acetonide (0.32 g, 1.00 mmol,) were dissolved in dichloromethane (8 ml). The mixture was cooled to 0 °C. Then *N*,*N*-dimethylaminopyridine (0.10 g, 0.80 mmol,) and *N*-(3-Diethylamino-propyl)-*N*-ethylcarbodiimide (0.38 g, 2.00 mmol) were added. The mixture was warmed to 25 °C and stirred for 4 hours. After that the mixture was quenched with 2M HCl (10 ml) and extracted with dichloromethane (3x 25 ml). The organic layer was washed with saturated $NaHCO_3$ (20 ml) and brine (20 ml). The product was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography

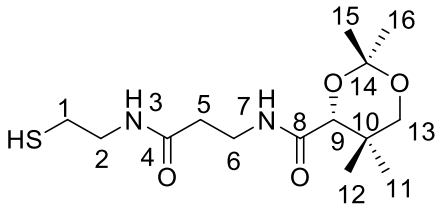
V. Synthesis of Pantetheine Substrates: Method 1[12]

The acyl pantetheine acetonide was stirred in a mixture of acetonitrile and water (1:1) and 10% TFA for 20 minutes. The reaction was followed by TLC and LCMS. The solvents were then lyophilized. The product was purified by preparative HPLC.

VI. Synthesis of Panthetine Substrates: Method 2

The corresponding Acyl pantetheine acetonide was dissolved in 8 ml acetonitrile. Indium chloride (2 eq.) and water (4 eq.) were added to the mixture. The reaction was stirred for 3 h at RT and completeness monitored by TLC and LCMS. The acetonitrile was evaporated and the product was purified by flash column chromatography (DCM / MeOH).

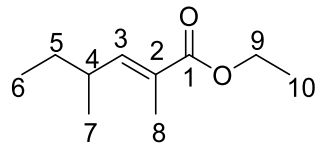
VII. Pantetheine Acetonide[1,12,13]



D-pantothenic acid hemicalcium salt (2.50 g, 10.50 mmol), p-toluensulfonic acid (2.30 g, 13.00 mmol) and 5 g molecular sieves were suspended in 125 mL dry acetone and stirred at 25 °C for 12 hours under a nitrogen atmosphere. The suspension was filtered with celite and washed with 200 ml acetone. The filtrate was concentrated to a colourless oil, redissolved in 200 ml ethyl acetate and washed two times with brine (25 ml) and dried over $MgSO_4$. After that the ethyl acetate was removed

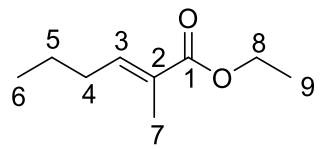
under vacuum and hexane was added to the flask to get a white solid that was dried under high vacuum. The corresponding D-pantothenic acetonide (1.90 g, 7.00 mmol) was dissolved in 40 mL dry THF with CDI (1.70 g, 11.00 mmol) and stirred for one hour at 25 °C. Then cysteamine (1.30 g, 11.00 mmol) was added to the solution and stirred for 12 hours. The solution was concentrated under vacuum and dichloromethane was added. The organic layer were washed with NH_4Cl (25 mL) and brine (25 mL), dried over $MgSO_4$ and concentrated *in vacuo*. After that the colourless oil was purified by column chromatography. The obtained product was a white solid (1.82 g, 5.74 mmol, 85% over to steps). R_f: 0.2 (EtOAc). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ[ppm]: 0.98 (s, 3H, 11-CH_3); 1.05 (s, 3H, 12-CH_3); 1.39 (t, 1H, SH); 1.43 (s, 3H, 15-CH_3); 1.47 (s, 3H, 16-CH_3); 2.40 (t, 2H, 5-CH_2); 2.64-2.70 (m, 2H, 1-CH_2); 3,29 (d, 1H, 13a-CH_2); 3.37-3.63 (m, 4H, 2-6-CH_2); 3,69 (d, 1H, 13b-CH_2); 4.09 (s,1H, 9-CH); 6.37 (bt, 1H, 3-NH); 7.03 (bt, 1H, 7-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ [ppm]: 18.7 (12-CH_3); 18.9 (11-CH_2); 22.1 (15-CH_3); 24.6 (1-CH_2); 29.5 (15-CH_3); 33.0 (10-C); 34.9 (6-CH_2); 36.2 (5-CH_2); 42.4 (2-CH_2); 71.4 (13-CH_2); 77.2 (9-CH); 99.1 (14-C); 170.3 (4-CO); 171.1 (8-CO). **ESMS:** *m/z*: 319 [M] H^+, 261 [M - $(CH_3)_2CO$]H^+

(±) E-Ethyl-2,4-dimethylhex-2-enoate 18e[14]



The obtained product was obtained from 2-methylbutanal as a colourless oil (0.29 g, 1.60 mmol, 85.3%). R_f: 0.78 (EtOAc / PE 1: 10). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.85 (t, 3H, 6-CH_3); 1.00 (d, 3H, 7-CH_3); 1.28-1.55 (m, 2H, 5-CH_2); 1.30 (t, 3H, 10-CH_3); 1.82 (s, 3H, 8-CH_3); 2.41 (m, 1H, 4-CH); 4.19 (q, 2H, 9-CH_2); 6.52 (d, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ11.9 (6-CH_3), 12.6 (7-CH_3), 14.3 (10-CH_3), 19.7 (8-CH_3), 29.7 (5-CH_2), 34.9 (4-CH), 60.4 (9-CH_2), 126.6 (2-C), 147.9 (3-CH), 168.4 (1-CO). **ESMS:** *m/z*: 193 [M + Na]$^+$, 171 [M]H^+

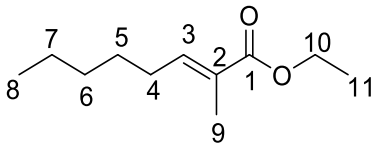
E-Ethyl 2-methylhex-2-enoate 20e[14]



The obtained product was obtained from butanal as a colourless oil (0,18 g, 1.15 mmol, 57.6%), Rf 0.21(EtOAc / PE 1: 10), **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.93 (t, 3H, 6-CH_3); 1.29 (t, 3H, 9-CH_3); 1.39-1.55 (m, 2H, 5-CH_2); 1.82 (s, 3H, 7-CH_3); 2.18 (q, 2H, 4-CH_2); 4.18 (q, 2H, 8-CH_2); 6.74 (t, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.4 (6-CH_3), 13.9 (9-CH_3), 14.3 (7-CH_3), 21.9 (5-CH_2); 30.7

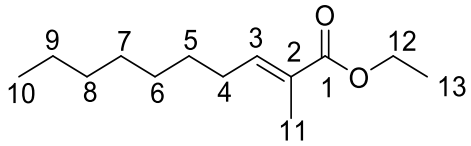
(4-CH_2); 60.4 (8-CH_2); 127.9 (2-C); 142.2 (3-CH); 168.4 (1-CO). **ESMS:** *m*/*z*: 179 $[M + Na]^+$, 157 $[M]H^+$, 129 $[M - CH_2CH_3]H^+$.

E-Ethyl-2-methyloct-2-enoate 23e[14]



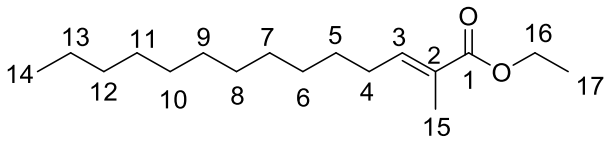
The obtained product was obtained from hexanal as a colourless oil (0.23 g, 1.25 mmol, 59.5%). R_f: 0.65 (EtOAc / PE 1: 10). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, 8-CH_3); 1.25-1.55 (m, 9H, 5-7-CH_2, 11-CH_3); 1.82 (s, 3H, 9-CH_3); 2.15 (q, 2H, 4-CH_2); 4.18 (q, 2H, 10-CH_2); 6.77 (t, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.3 (8-CH_3), 14.0 (9-CH_3), 14.3 (11-CH_3), 22.5 (7-CH_2), 28.3 (5-CH_2), 28.7 (4-CH_2), 31.6 (6-CH_2), 60.4 (10-CH_2), 127.7 (2-C), 142.5 (3-CH), 168.4 (1-C). **ESMS:** *m*/*z*: 207 $[M + Na]^+$, 185 $[M]H^+$.

E-Ethyl-2-methyldec-2-enoate 25e[14]



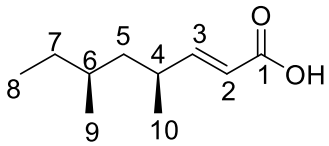
The obtained product was obtained from octanal as a colourless oil (0.27 g, 1.27 mmol, 63%). R_f: 0.85 (EtOAc / PE 1:7). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, 10-CH_3); 1.25-1.58 (m, 13H, 5-9-CH_2, 13-CH_3); 1.82 (s, 3H, 11-CH_3); 2.15 (q, 2H, 4-CH_2); 4.18 (q, 2H, 12-CH_2); 6.75 (t, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.3 (10-CH_3), 14.1 (11-CH_3), 14.3 (13-CH_3), 22.6 (9-CH_2), 28.6 (7-CH_2), 28.7 (6-CH_2), 29.1 (5-CH_2), 29.4 (4-CH_2), 31.8 (8-CH_2), 60.4 (12-CH_2), 127.6 (2-C), 142.5 (3-CH), 168.4 (1-CO). **ESMS:** *m*/*z*: 254 $[M + CH_3CN]H^+$, 213 $[M]H^+$.

E-Ethyl-2-methyltetradec-2-enoate 26e[15]



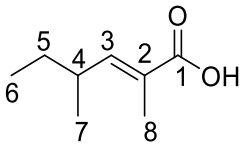
The obtained product was obtained from dodecanal as a colourless oil (0.34 g, 1.27 mmol, 63.4%). R_f: 0.9 (EtOAc / PE 1:7). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, 14-CH_3), 1.24-1.36 (m, 18H, 5-13-CH_2), 1.43 (t, 3H, 17-CH_3), 2.16 (q, 2H, 4-CH_2), 6,76 (d, 1H, 3-CH), 1.82 (s, 3H, 15-CH_3), 4.19 (q, 2H, 16-CH_2). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ 13.2 (15-CH3), 14.0 (14-CH_3), 14.0 (17-CH_3), 22.5 (13-CH_2), 28.1-31.8 (4-12-CH_2), 61.1 (16-CH_2), 142.8 (2-C), 126.5 (3-CH), 168.1 (1-CO). **ESMS:** *m*/*z*: 291 $[M+Na]H^+$

E-(4*S*,6*S*)-2-4-Dimethyloctenoic acid 11a[1,12]



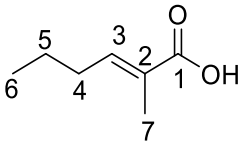
The obtained product was obtained by hydrolysis of squalestatin S1 **15** as a yellow solid (0.22 g, 1.29 mmol, 84%), **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (t, *J* = 7.4, 6H, 8-9-CH_3); 1.07 (d, *J* = 6.2, 3H, 10-CH_3); 1.27-1.46 (m, 5H, 7-5-CH_2-6-CH); 2.41-2.52 (m, 1H, 4-CH), 5.81 (d, *J* = 1.7, *J* = 16.0, 1H, 2-CH), 6.96 (dd, *J* = 8.8, *J* = 16.0, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): *δ* 11.1 (8-CH_3); 20.2 (9-CH_3); 20.7 (10-CH_3); 29.8 (7-CH_2); 31.9 (6-CH); 34.4 (4-CH); 43.1 (5-CH_2), 118.3 (2-CH); 164.6 (3-CH); 176.7 (1-CO). **ESMS:** *m*/*z*:193 $[M + Na]^+$, 171 $[M]H^+$

(±) E-2,4-Dimethylhex-2-enoic acid 18a[1,12]



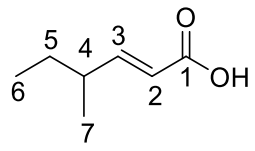
The product was obtained from **18e** as a red oil (0.17 g, 1.19 mmol, 70.8%), **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, 1-CH_3); 1.01 (d, 3H, 4-CH_3); 1.24-1.49 (m, 2H, 2-CH_2); 1.85 (s, 3H, 7-CH_3); 2.38-2.49 (m, 1H, 3-CH); 6.68 (qq, 1H, 5-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ11.8 (1-CH_3), 12.2 (7-CH_3), 19.5 (4-CH_3), 29.5 (2-CH_2), 35.1 (3-CH), 125.7 (6-C), 150.8 (5-CH), 173.3 (8-COOH). **ESMS:** *m*/*z*: 184 $[M + CH_3CN]H^+$, 143 $[M]H^+$

E-2-Methylhex-2-enoic acid 20a[1,12]



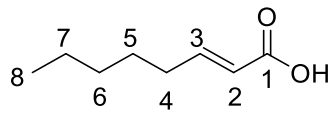
The obtained product was obtained from **20e** as a red oil (0.11 g, 0.85 mmol, 74.7%). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.91 (t, 3H, 6-CH_3); 1.32-1.50 (m, 2H, 5-CH_2); 1.85 (s, 3H, 7-CH_3); 2.22 (q, 2H, 4-CH_2); 6.93 (t, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.0 (6-CH_3), 13.8 (7-CH_3), 21.7 (5-CH_2), 30.6 (4-CH_2), 127.0 (2-C), 145.2 (3-CH), 173.1 (1-CO). **ESMS:** *m*/*z*: 152 $[M + Na]^+$, 129 $[M]H^+$; 111 $[M - H_2O]\ H^+$.

(±) E-4-Methylhexenoic acid 21a[1,12]



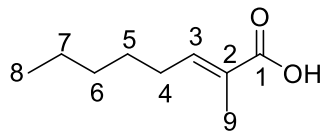
The product was obtained from **21e** as a yellow oil (1.43 g, 11.17 mmol, 47%). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, 6-CH_3); 1.04 (d, 3H, 7-CH_3); 1.36-1.44 (m, 2H, 5-CH_2); 2.17-2.27 (m, 1H, 4-CH); 5.78 (d, 1H, 2-CH); 6.87 (t, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ11.7 (6-CH_3), 18.9 (7-CH_3), 28.7 (5-CH_2), 38.1 (4-CH), 119.3 (2-CH), 154.8 (3-CH), 167.3 (1-CO). **ESMS:** *m*/*z*:152 [M + Na]$^+$, 129 [M]H$^+$

E-oct-2-enoic acid 22a[1,12]



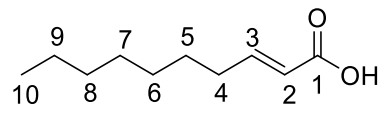
The product was obtained from *E*-ethyloct-2-enoate as a yellow oil.(1.69 g, 11.9, 49.5%). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.92 (t, 3H, 8-CH_3); 1.26-1.52 (m, 6H, 5-6-7-CH_2); 2.22 (q, 2H, 4-CH_2); 5.78 (dd, 1H, 2-CH); 6.94 (tq, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.0 (8-CH_3), 22.5 (7-CH_2), 28.1 (5-CH_2), 28.8 (4-CH_2), 31.5 (6-CH_2), 126.8 (2-C), 145.5 (3-CH), 173.1 (1-CO). **ESMS:** *m*/*z*: 143 [M-H]$^-$

E-2-Methyloct-2-enoic acid 23a[1,12]



The product was obtained from **23e** as a red oil (0.11 g, 0.7 mmol, 56.4%). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.92 (t, 3H, 8-CH_3); 1.26-1.52 (m, 6H, 5-6-7-CH_2); 1.86 (s, 3H, 9-CH_3); 2.22 (q, 2H, 4-CH_2); 6.94 (t, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.0 (8-CH_3), 14.0 (9-CH_3), 22.5 (7-CH_2), 28.1 (5-CH_2), 28.8 (4-CH_2), 31.5 (6-CH_2), 126.8 (2-C), 145.5 (3-CH), 173.1 (1-CO). **ESMS:** *m*/*z*: 179 [M + Na]$^+$, 157 [M]H$^+$

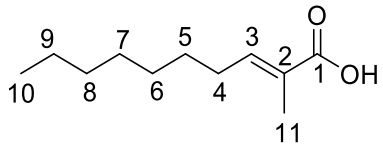
E-Dec-2-enoic acid 24a[1,12]



The product was obtained from *E*-ethyl-dec-2-enoate as a yellow oil (0.17 g, 1 mmol, 62.9%), **^{1}H-NMR** ($CDCl_3$, 400 MHz):δ 0.90 (t, 3H, 10-CH_3); 1.29-1.50 (m, 10H, 5-9-CH_2); 2.25 (dq, 2H, 4-

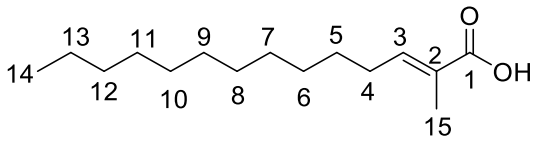
CH_2); 5.84 (d, 1H, 2-CH); 7.10 (dt, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ14.1 (10-CH_3), 22.6 (9-CH_2); 27.8 (8-CH_2); 29.0 (7-CH_2); 29.1 (6-CH_2); 31.7 (5-CH_2); 32.3 (4-CH_2); 120.5 (2-CH); 152.5 (3-CH); 171.9 (1-CO). **ESMS:** *m/z*: 212.4 $[M]H^+$, 193.2 $[M - Na]H^+$, 153.5 $[M - H_2O]H^+$

E-2-Methyldec-2-enoic acid 25a[1,12]



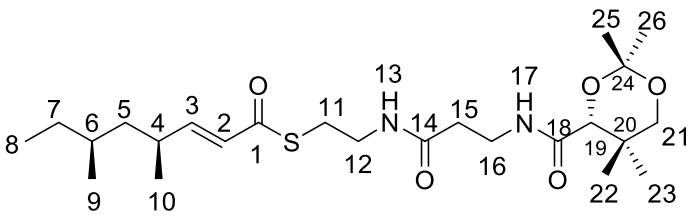
The product was obtained from **25e** as a red oil (0.17 g, 0.92 mmol, 62.9%). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.87-0.90 (m, 3H, 10-CH_3); 1.26-1.30 (m, 10H, 5-9-CH_2); 1.83 (s, 3H, 11-CH_3); 2.19 (q, 2H, 4-CH_2); 6.90 (t, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.3 (10-CH_3), 14.1 (11-CH_3), 22.6 (9-CH_2), 28.4 (7-CH_2), 28.9 (6-CH_2), 29.1 (5-CH_2), 29.3 (4-CH_2), 31.8 (8-CH_2), 126.8 (2-C), 145.6 (3-CH), 172.8 (1-CO). **ESMS:** *m/z*: 226 $[M + CH_3CN]H^+$, 167 $[M - H_2O]H^+$.

E-2-Methyltetradec-2-enoic acid 26a[15]



The product was obtained from **26e** as a red oil (0.29 g, 1.2 mmol, 85.3%). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, 14-CH_3), 1.24-1.36 (m, 18H, 5-13-CH_2), 2.16 (q, 2H, 4-CH_2), 6.76 (d, 1H, 3-CH), 1.82 (s, 3H, 15-CH_3). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ 13.2 (15-CH_3), 14.0 (14-CH_3), 22.5 (13-CH_2), 28.1-31.8 (4-12-CH_2), 142.8 (2-C), 126.5 (3-CH), 168.1 (1-CO). **ESMS:** *m/z*: 263 $[M + Na]^+$, 223 $[M - H_2O]H^+$

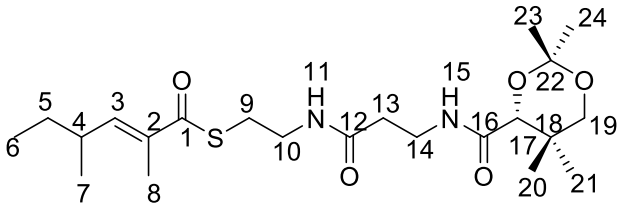
E-4*S*-6*S*-Dimethyl-octenoyl-pantethine acetonide 11pp[1,12,16]



The product was obtained from **11a** as a colorless oil (0.22 g, 0.47 mmol, 36.2%). R_f: 0.47 (EtOAc / PE, 9:1) **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.87 (m, 6H, 8-9-CH_3); 0.97 (s, 3H, 22-CH_3); 1.04 (s, 3H, 23-CH_3); 1.13-1.19 (m, 2H, 7-CH_3), 1.26-1.49 (m, 3H, 5-6-CH_2); 1.44 (s, 3H, 25-CH_3); 1.48 (s, 3H, 26-CH_3); 2.34 (m, 1H, 4-CH); 2.45 (t, *J* = 5.9, 1H, 14-CH); 3.10 (t, *J* = 6.5, 2H, 11-CH_2); 3.27 (d, *J* = 11.7, 1H, 21a-C*H*H); 3.42-3.62 (m, 4H, 12-16-CH_2); 3.70 (d, *J* = 11.6, 1H, 21b-CH*H*); 4.09 (s, 1H,

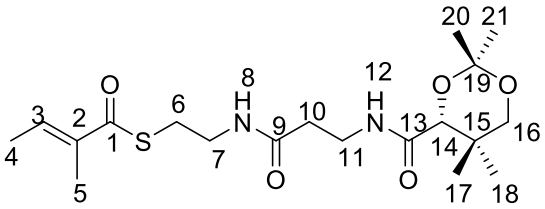
19-CH); 6.12 (dd, *J* = 1.0, 16.0, 1H, 2-CH); 6.28 (bt, *J* = 6.3, 1H, 13-NH); 6.80 (d, *J* = 8.2, 15.8, 1H, 3-CH); 7.06 (bt, *J* = 5.8, 1H, 17-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ11.1 (8-CH_3); 14.2 (9-CH_3); 18.7 (22-CH_3); 18.9 (23-CH_3); 22.1 (25-CH_3); 28.2 (11-CH_2); 29.5 (26-CH_3); 29.7 (7-CH_2); 32.9 (6-CH); 32.9 (18-C); 34.4 (4-CH); 34.7 (15-CH_2) 35.9 (16-CH_2); 39.7 (12-CH_2); 43-5 (5-CH) 71.5 (21-CH_2); 77.2 (19-CH); 99.1 (24-C); 126.6 (2-CH); 152.1 (3-CH); 170.1 (14-CO); 171.1 (18-CO); 190.1 (1-CO). **ESMS:** *m*/*z*: 471 $[M]H^+$, 493 $[M + Na]^+$

4-RS-E-2-4-Dimethylhex-2-enoyl pantetheine acetonide 18pp[1,12,16]



The product was obtained from **18a** as a colourless oil (0.38 g, 0.89 mmol, 89%). R_f: 0.45 (EtOAc). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.87 (t, *J* = 7.9, 3H, 6-CH_3); 0.97 (s, 3H, 20-CH_3); 1.04 (s, 3H, 21-CH_3); 1.03 (d, *J* = 7.5, 3H, 7-CH_3), 1.24-1.49 (m, 3H, 4-CH, 5-CH_2); 1.41 (s, 3H, 23-CH_3); 1.46 (s, 3H, 24-CH_3); 1.88 (s, 3H, 8-CH_3); 2.42 (t, *J* = 5.9, 2H, 13-CH_2); 3.05 (t, *J* = 6.4, 2H, 9-CH_2); 3.27 (d, *J* = 11.4, 1H, 19a-C*H*H); 3.39-3.64 (m, 4H, 10-14-CH_2); 3.68 (d, *J* = 11.7, 1H, 19b-CH*H*); 4.07 (s, 1H, 17-CH); 6.09 (bt, *J* = 5.4, 1H, 11-NH); 6.53 (dd, *J* = 1.4, 9.8, 1H, 3-CH); 7.03 (bt, *J* = 5.8, 1H, 15-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ11.9 (6-CH_3); 12.2 (7-CH_3); 18.7 (20-CH_3); 18.9 (21-CH_3); 19.9 (8-CH_3); 22.1 (23-CH_3); 28.5 (9-CH_2); 29.5 (24-CH_3); 29.6 (5-CH_2); 32.9 (18-C); 34.4 (13-CH_2); 35.0 (4-CH); 35.9 (14-CH_2); 39.7 (10-CH_2); 71.5 (19-CH_2); 77.2 (17-CH); 99.1 (22-C); 134.5 (2-C); 147.6 (3-CH); 170.0 (12-CO); 171.2 (16-CO); 193.9 (1-CO). **ESMS:** *m*/*z*: 471 $[M]H^+$, 493 $[M + Na]^+$

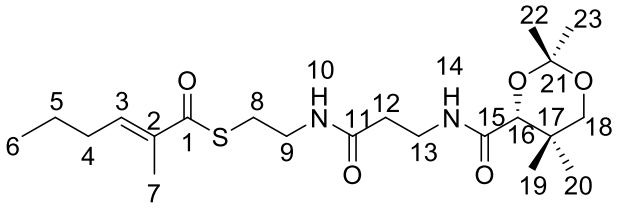
Tigloyl pantetheine acetonide 19pp[1,12,13]



The product was obtained from tiglic acid as a colourless oil (0.318 g, 0.795 mmol, 79.5%), R_f: 0.46 (EtOAc). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.99 (s, 3H, 17-CH_3); 1.04 (s, 3H, 18-CH_3); 1.44 (s, 3H, 21-CH_3); 1.48 (s, 3H, 22-CH_3); 1.85-1.87 (m, 6H, 4-5-CH_3); 2.44 (t, *J* = 6.5, 2H, 10-CH_2); 3.08 (t, *J* = 6.3, 2H, 6-CH_2); 3.28 (d, *J* = 11.7, 1H, 16a-C*H*H); 3.41-3.62 (m, 4H, 7-11-CH_2); 3.68 (d, *J* = 11.6, 1H, 16b-CH*H*); 4.08 (s, 1H, 14-CH); 6.13 (bt, *J* = 5.8, 1H, 8-NH); 6.93 (q, *J* = 1.4, 6.8, 1H, 3-CH);

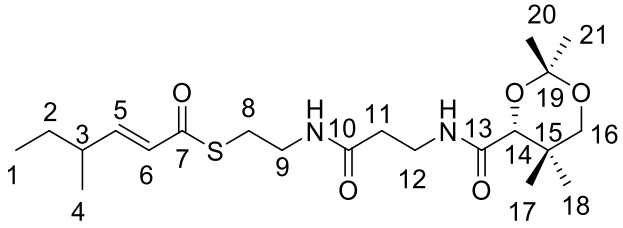
7.03 (bt, J = 5.9, 1H, 12-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.2 (4-CH_3); 14.4 (5-CH_3); 18.7 (17-CH_3); 18.9 (18-CH_3); 22.1 (21-CH_3); 28.3 (6-CH_2); 29.5 (22-CH_3); 32.9 (15-C); 34.8 (10-CH_2); 35.9 (11-CH_2); 39.7 (7-CH_2); 71.5 (16-CH_2); 77.2 (14-CH); 99.1 (19-C); 136.8 (2-C); 136.9 (3-CH); 170.1 (9-CO); 171.1 (13-CO); 190.2 (1-CO). **ESMS:** m/z: 423.3 $[M + Na]^+$, 401 $[M]H^+$

E-2-Methylhex-2-enoyl pantetheine acetonide 20pp[1,12,16]



The product was obtained from **20a** as a yellow oil (0.38 g, 0.88 mmol, 88%). R_f: 0.45 (EtOAc). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.96 (t, J = 7.6, 3H, 6-CH_3); 0.97 (s, 3H, 19-CH_3); 1.04 (s, 3H, 20-CH_3); 1.44-1.55 (m, 2H, 5-CH_2); 1.41 (s, 3H, 22-CH_3); 1.46 (s, 3H, 23-CH_3); 1.87 (s, 3H, 7-CH_3); 2.20 (q, J = 7.4, 2H, 4-CH_2); 2.42 (t, J = 6.1, 2H, 12-CH_2); 3.05 (t, J = 6.5, 2H, 8-CH_2); 3.27 (d, J = 11.2, 1H, 18a-C*H*H); 3.39-3.62 (m, 4H, 9-13-CH_2); 3.68 (d, J = 11.7, 1H, 18b-CH*H*); 4.07 (s, 1H, 16-CH); 6.10 (bt, J = 5.7, 1H, 10-NH); 6.77 (t, J = 1.3, 6.9, 1H, 3-CH); 7.03 (bt, J = 5.7, 1H, 14-NH). **^{13}*C*-NMR** ($CDCl_3$, 100 MHz): δ12.5 (6-CH_3); 13.9 (7-CH_3); 18.7 (19-CH_3); 18.9 (20-CH_3); 21.8 (5-CH_2); 22.1 (22-CH_3); 28.4 (8-CH_2); 28.8 (4-CH_2); 29.5 (23-CH_3); 32.9 (17-C); 34.8 (12-CH_2); 35.9 (13-CH_2); 39.7 (9-CH_2); 71.5 (18-CH_2); 77.2 (16-CH); 99.1 (21-C); 135.9 (2-C); 142.1 (3-CH); 170.0 (11-CO); 171.2 (15-CO); 193.7 (1-CO). **ES-MS:** m/z: 429.7 $[M]H^+$

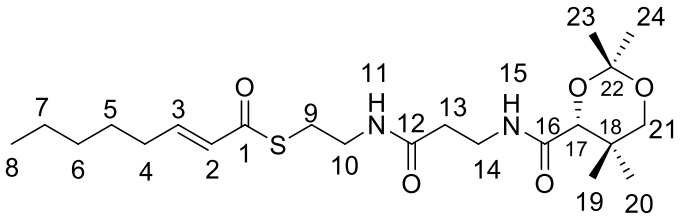
3-RS-E-4-Methylhex-2-enoyl pantetheine acetonide 21pp[1,12,16]



The product was obtained from **21a** as a colourless oil (0.35 g, 0.82 mmol, 82%). R_f: 0.47 (EtOAc). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (t, J = 7.2, 3H, 6-CH_3); 0.97 (s, 3H, 19-CH_3); 1.04 (s, 3H, 20-CH_3); 1.06 (d, J = 6.7, 3H, 7-CH_3), 1.40-1.45 (m, 2H, 5-CH_2); 1.41 (s, 3H, 22-CH_3); 1.46 (s, 3H, 23-CH_3); 2.17-2.26 (m, 1H, 4-CH); 2.42 (t, J = 6.3, 2H, 12-CH_2); 3.05 (t, J = 6.3, 2H, 8-CH_2); 3.27 (d, J = 11.9, 1H, 18a-C*H*H); 3.40-3.62 (m, 4H, 9-13-CH_2); 3.68 (d, J = 11.7, 1H, 18b-CH*H*); 4.07 (s, 1H, 16-CH); 6.08 (dd, J = 1.2, 15.6, 1H, 2-CH); 6.12 (bt, J = 5.1, 1H, 10-NH); 6.77 (dd, J = 7.5, 15.5, 1H, 3-CH); 7.03 (bt, J = 6.0, 1H, 14-NH). **^{13}*C*-NMR** ($CDCl_3$, 100 MHz): δ11.6 (6-CH_3); 12.2 (7-CH_3); 18.7 (19-CH_3); 18.9 (20-CH_3); 22.1 (22-CH_3); 28.3 (5-CH_2); 28.4 (8-CH_2); 29.5 (23-CH_3); 32.9

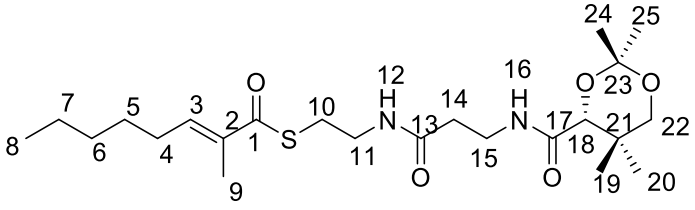
(17-C); 34.8 (12-CH_2); 35.9 (13-CH_2); 38.1 (4-CH); 39.7 (9-CH_2); 71.5 (18-CH_2); 77.2 (16-CH); 99.1 (21-C); 126.7 (2-CH); 151.7 (3-CH); 170.0 (11-CO); 171.2 (15-CO); 190.3 (1-CO). **ESMS:** *m*/*z* (%): 429.7 $[M]H^+$, 452 $[M + Na]^+$

E-Oct-2-enoic pantetheine acetonide 22pp[1,12,16]



The product was obtained from **22a** as a colorless oil (0.35 g, 0.79 mmol, 56.1%), R_f: 0.56 (EtOAc / PE, 9:1). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.90 (t, *J* = 7.4, 3H, 8-CH_3); 0.97 (s, 3H, 19-CH_3); 1.04 (s, 3H, 20-CH_3); 1.31-1.51 (m, 6H, 5-6-7-CH_2); 1.41 (s, 3H, 23-CH_3); 1.46 (s, 3H, 24-CH_3); 2.21 (g, *J* = 7.3, 2H, 4-CH_2); 2.42 (t, *J* = 5.8, 2H, 13-CH_2); 3.05 (t, *J* = 6.7, 2H, 9-CH_2); 3.27 (d, *J* = 11.8, 1H, 21a-C*H*H); 3.39-3.62 (m, 4H, 10-14-CH_2); 3.68 (d, *J* = 12.2, 1H, 21b-CH*H*); 4.07 (s, 1H, 17-CH); 6.08 (bt, *J* = 5.8, 1H, 11-NH); 6.92 (t, *J* = 1.5, 7.4, 1H, 3-CH); 7.03 (bt, *J* = 5.8, 1H, 15-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.5 (8-CH_3); 18.7 (19-CH_3); 18.9 (20-CH_3); 22.1 (24-CH_3); 22.5 (7-CH_2); 28.2 (10-CH_2); 28.4 (5-CH_2); 28.8 (4-CH_2); 29.5 (23-CH_3); 31.6 (6-CH_2); 32.9 (18-C); 34.8 (13-CH_2); 35.9 (14-CH_2); 39.7 (9-CH_2); 71.5 (21-CH_2); 77.2 (17-CH); 99.1 (22-C); 135.7 (2-C); 142.4 (3-CH); 170.0 (12-CO); 171.1 (16-CO); 193.7 (1-CO). **ESMS:** *m*/*z*: 443.62 $[M]H^+$

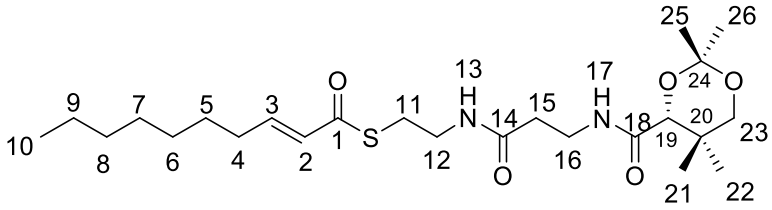
E-2-Methyloct-2-enoyl pantetheine acetonide 23pp[1,12,16]



The product was obtained from **23a** as a colourless oil (0.27 g, 0.60 mmol, 60%). R_f: 0.47 (EtOAc). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.90 (t, *J* = 7.4, 3H, 8-CH_3); 0.97 (s, 3H, 19-CH_3); 1.04 (s, 3H, 20-CH_3); 1.31-1.51 (m, 6H, 5-6-7-CH_2); 1.41 (s, 3H, 24-CH_3); 1.46 (s, 3H, 25-CH_3); 1.87 (s, 3H, 9-CH_3); 2.21 (g, *J* = 7.3, 2H, 4-CH_2); 2.42 (t, *J* = 5.8, 2H, 14-CH_2); 3.05 (t, *J* = 6.7, 2H, 10-CH_2); 3.27 (d, *J* = 11.8, 1H, 22a-C*H*H); 3.39-3.62 (m, 4H, 11-15-CH_2); 3.68 (d, *J* = 12.2, 1H, 22b-CH*H*); 4.07 (s, 1H, 18-CH); 6.08 (bt, *J* = 5.8, 1H, 12-NH); 6.92 (t, *J* = 1.5, 7.4, 1H, 3-CH); 7.03 (bt, *J* = 5.8, 1H, 16-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.5 (8-CH_3); 13.9 (1-CH_3); 18.7 (19-CH_3); 18.9 (20-CH_3); 22.1 (24-CH_3); 22.5 (7-CH_2); 28.2 (10-CH_2); 28.4 (5-CH_2); 28.8 (4-CH_2); 29.5 (25-CH_3); 31.6 (6-CH_2); 32.9 (21-C); 34.8 (14-CH_2); 35.9 (15-CH_2); 39.7 (11-CH_2); 71.5 (22-CH_2); 77.2 (18-CH); 99.1

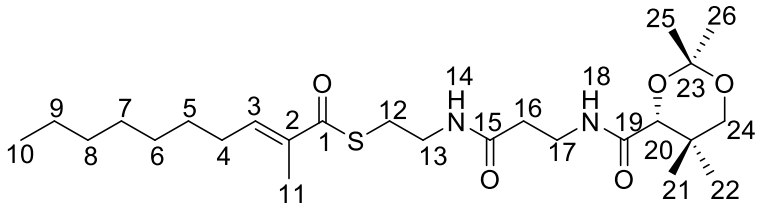
(23-C); 135.7 (2-C); 142.4 (3-CH); 170.0 (13-CO); 171.1 (17-CO); 193.7 (9-CO). **ESMS:** *m*/*z*: 457.7 $[M]H^+$

E-Dec-2-enoyl pantetheine acetonide 24pp[1,12,16]



The product was obtained from **24a** as a colorless oil (0.18 g, 0.38 mmol, 38.3%), R_f: 0.49 (EtAc / PE, 9:1), **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (t, *J* = 7.6, 3H, 10-CH_3); 0.97 (s, 3H, 21-CH_3); 1.04 (s, 3H, 22-CH_3); 1.30-1.47 (m, 10H, 5-6-7-8-9-CH_2); 1.41 (s, 3H, 25-CH_3); 1.46 (s, 3H, 26-CH_3); 2.21 (q, *J* = 7.1, 2H, 4-CH_2); 2.42 (t, *J* = 6.2, 2H, 15-CH_2); 3.05 (t, *J* = 6.2, 2H, 11-CH_2); 3.27 (d, *J* = 12.2, 1H, 23a-C*H*H); 3.39-3.62 (m, 4H, 12-16-CH_2); 3.68 (d, *J* = 12.2, 1H, 23b-CH*H*); 4.07 (s, 1H, 19-CH); 6.08 (bt, *J* = 5.0, 1H, 13-NH); 6.77 (t, *J* = 1.9, 6.9, 1H, 3-CH); 7.03 (bt, *J* = 5.5, 1H, 17-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): 14.0 (10-CH_3); 18.7 (21-CH_3); 18.9 (22-CH_3); 22.1 (25-CH_3); 22.6 (9-CH_2); 28.4 (5-CH_2); 28.5 (12-CH_2); 28.8 (6-CH_2); 29.1 (7-CH_2); 29.5 (26-CH_3); 31.8 (8-CH_2); 33.2 (4-CH_2); 34.8 (15-CH_2); 35.9 (16-CH_2); 39.7 (11-CH_2); 71.5 (23-CH_2); 77.2 (19-CH); 99.1 (20-C); 135.7 (2-C); 142.4 (3-CH); 170.0 (14-CO); 171.2 (18-CO); 193.7 (11-CO).
ESMS: *m*/*z*: 471.76 $[M]H^+$, 493 $[M + Na]^+$

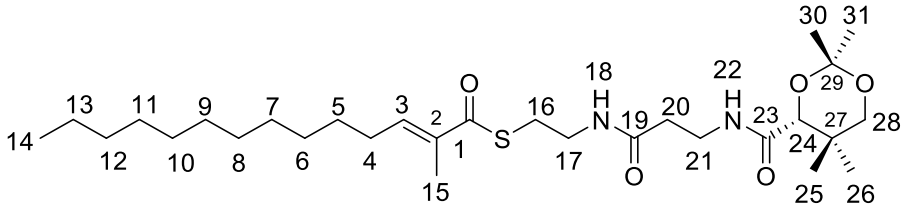
E-2-Methyldec-2-enoyl pantetheine acetonide 25pp[1,12,13]



The product was obtained from **25a** as a colourless oil (0.225 g, 0.53 mmol, 63%). R_f: 0.45 (EtOAc). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.87 (m, 6H, 8-9-CH_3); 0.97 (s, 3H, 22-CH_3); 1.04 (s, 3H, 23-CH_3); 1.13-1.19 (m, 2H, 7-CH_3), 1.26-1.49 (m, 3H, 5-6-CH_2); 1.44 (s, 3H, 25-CH_3); 1.48 (s, 3H, 26-CH_3); 2.34 (m, 1H, 4-CH); 2.45 (t, *J* = 5.9, 1H, 14-CH); 3.10 (t, *J* = 6.5, 2H, 11-CH_2); 3.27 (d, *J* = 11.7, 1H, 21a-C*H*H); 3.42-3.62 (m, 4H, 12-16-CH_2); 3.70 (d, *J* = 11.6, 1H, 21b-CH*H*); 4.09 (s, 1H, 19-CH); 6.12 (dd, *J* = 1.0, 16.0, 1H, 2-CH); 6.28 (bt, *J* = 6.3, 1H, 13-NH); 6.80 (t, *J* = 8.2, 15.8, 1H, 3-CH); 7.06 (bt, *J* = 5.8, 1H, 17-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ11.1 (8-CH_3); 14.2 (9-CH_3); 18.7 (22-CH_3); 18.9 (23-CH_3); 22.1 (25-CH_3); 28.2 (11-CH_2); 29.5 (26-CH_3); 29.7 (7-CH_2); 32.9 (6-CH); 32.9 (18-C); 34.4 (4-CH); 34.7 (15-CH_2) 35.9 (16-CH_2); 39.7 (12-CH_2); 43-5 (5-CH) 71.5 (21-CH_2); 77.2

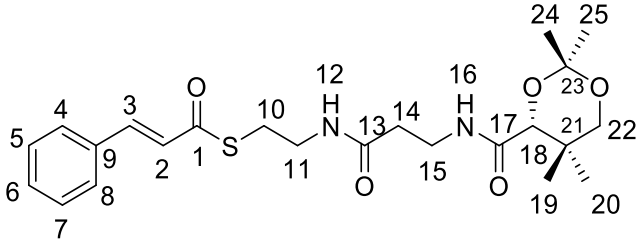
(19-CH); 99.1 (24-C); 126.6 (2-CH); 152.1 (3-CH); 170.1 (14-CO); 171.1 (18-CO); 190.1 (1-CO). **ESMS:** *m*/*z*: 485.7 $[M]H^+$

E-2-Methyltetradec-2-enoyl panthetine acetonide 26pp[1,12,16]



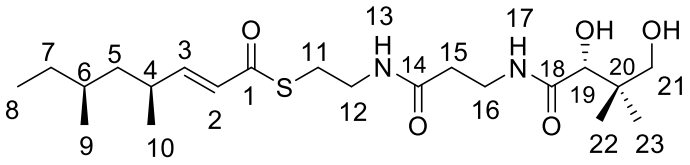
The product was obtained from **26a** as colourless solid (0.12 g, 0.22 mmol, 18.5%), R_f: 0.50 (EtOAc / PE, 9:1). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ [ppm]: 0.88 (t, 3H, 14-CH_3); 0.97 (s, 3H, 25-CH_3); 1.04 (s, 3H, 26-CH_3); 1.30-1.47 (m, 18H, 5-13-CH_2); 1.41 (s, 3H, 30-CH_3); 1.46 (s, 3H, 31-CH_3); 1.87 (s, 3H, 15-CH_3); 2.21 (q, 2H, 4-CH_2); 2.42 (t, 2H, 20-CH_2); 3.05 (t, *J* = 6.2 Hz, 2H, 16-CH_2); 3.27 (d, *J* = 12.2 Hz, 1H, 28a-CH_2); 3.39-3.62 (m, 4H, 17-21-CH_2); 3.68 (d, *J* = 12.2 Hz, 1H, 28b-CH_2); 4.07 (s,1H, 24-CH); 6.08 (bt, *J* = 5.0 Hz, 1H, 18-NH); 6.77 (t,1H, 3-CH); 7.03 (t, *J* = 5.5 Hz, 1H, 22-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ[ppm]: 12.5 (14-CH_3); 14.0 (15-CH_3); 18.7 (25-CH_3); 18.9 (26-CH_3); 22.1 (30-31-CH_3); 22.6 (13-CH_2); 28.5 (12-CH_2); 29.1-29.9 (5-11-CH_2); 31.6 (4-CH_2); 34.8 (21-CH_2); 39.7 (20-CH_2); 71.5 (28-CH_2); 77.2 (18-CH); 99.1 (29-C); 135.7 (29-C); 142.4 (2-CH); 170.0 (23-CO); 171.1 (19-CO); 193.7 (1-CO). **ESMS:** *m*/*z*: 541.3 $[M]H^+$, 573 $[M + Na]^+$

E-Cinnamoyl pantetheine acetonide 27pp[1,12,16]



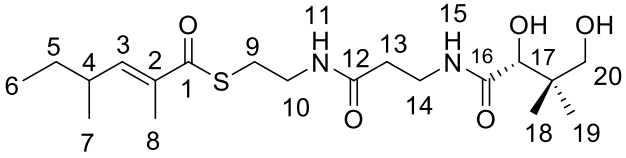
The product was obtained from cinnamic acid as a white solid (0.45, 0.73 mmol, 48.8%), R_f: 0.42 (EtOAc / PE, 9:1). **^{1}H-NMR** ($CDCl_3$, 400 MHz): 0.97 (s, 3H, 19-CH_3); 1.04 (s, 3H, 20-CH_3); 1.41 (s, 3H, 24-CH_3); 1.46 (s, 3H, 25-CH_3); 2.42 (t, *J* = 5.8, 2H, 14-CH_2); 3.05 (t, *J* = 6.7, 2H, 10-CH_2); 3.27 (d, *J* = 11.8, 1H, 22a-C*H*H); 3.39-3.62 (m, 4H, 11-15-CH_2); 3.68 (d, *J* = 12.2, 1H, 22b-CH*H*); 4.07 (s, 1H, 18-CH); 6.08 (bt, *J* = 5.8, 1H, 12-NH); 6.70 (d, 1H, 2-CH); 7.03 (bt, *J* = 5.8, 1H, 16-NH), 7.73 (dd, 3H, 5-,6-,7-CH); 7.52 (m, 2H, 4-,8-CH); 7.59 (d, *J* = 15.82, 1H, 3-CH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ 18.7 (19-CH_3); 18.9 (20-CH_3); 22.1 (24-CH_3);); 28.2 (10-CH_2); 29.5 (25-CH_3); 32.9 (21-C); 34.8 (14-CH_2); 35.9 (15-CH_2); 39.7 (11-CH_2); 71.5 (22-CH_2); 77.2 (18-CH); 99.1 (23-C); 123 (2-C) 127-128.5 (4-C, 5-C, 6-C, 7-C, 8-C) 135.7 (9-C); 143 (3-C), 170.0 (13-CO); 171.1 (17-CO); 193.7 (1-CO). **ESMS:** *m*/*z*: 449 $[M]H^+$, 472 $[M + Na]^+$

6*S*,4*S*-2*E*-Dimethyloct-2-enoylpantetheine 11p[15,17]



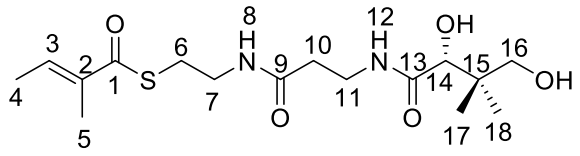
Method 2. The product was obtained from **11pp** as a yellow oil (0.16 g, 0.37 mmol, 73%), R_f: 0.42 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ0.81-0.90 (m, 6H, 8-9-CH_3), 0.92 (s, 3H, 21-CH_3), 1.03 (s, 3H, 22-CH_3), 1.05 (m, 3H, 10-CH_3), 1.10-1.44 (m, 6H, 5-7-CH_2, 6-CH), 2.42 (t, 2H, *J* = 6.0, 15-CH_2), 2.37-2.41 (m, 1H, 4-CH), 3.03-3.17 (m, 2H, 11-CH_2), 3.36-3.58 (m, 6H, 12-16-21-CH_2), 3.99 (s, 1H, 19-CH), 6.08 (dd, 1H, *J* = 1.4, *J* = 15.6, 2-CH), 6.23 (bt, *J* = 6.0, 1H, 13-NH), 6.79 (dd, *J* = 7.6, 1H, 3-CH); 7.37 (bt, *J* = 6.0, 1H, 17-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): 11.3 (8-CH_3), 18.8 (9-CH_3), 20.2 (10-CH_3), 20.5 (21-CH_3), 21.9 (22-CH_3), 28.3 (11-CH_2), 29.7 (7-CH_2), 31.9 (6-CH_2), 34.3 (4-CH), 35.1 (15-CH_2), 35.9 (16-CH_2), 39.5 (18-C), 39.8 (12-CH_2), 43.3 (5-CH_2), 71.1 (21-CH_2), 77.9 (19-CH), 126.2 (2-CH), 152.5 (3-CH), 171.8 (14-CO), 173.5 (18-CO), 190.9 (1-CO). **ESMS:** *m*/*z*: 453 $[M + Na]^+$, 431 $[M]H^+$, 413 $[M - H_2O]H^+$

4-*RS*-*E*-2,4-Dimethylhex-2-enoylpantetheine 18p[15,17]



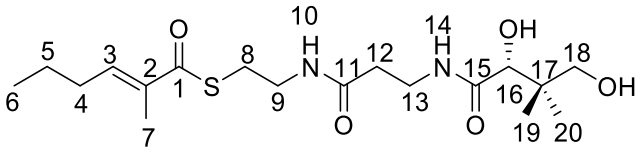
Method 2. The product was obtained from **18pp** as a colourless oil (0.22 g, 0.54 mmol, 88%). R_f: 0.42 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.86 (t, *J* = 7.4, 3H, 6-CH_3); 0.92 (s, 3H, 20-CH_3); 1.03 (s, 3H, 21-CH_3); 1.03 (d, *J* = 2.3, 3H, 7-CH_3); 1.30-1.51 (m, 4H, 5-CH_2); 1.88 (d, *J* = 1.1, 8-CH_3), 2.41 (t, *J* = 5.6, 2H, 13-CH_2); 2.40-2.49 (m, 1H, 4-CH); 2.99-3.14 (m, 2H, 9-CH_2); 3.35-3.59 (m, 6H, 10-14-19-CH_2); 3.99 (s, 1H, 17-CH); 6.19 (bt, *J* = 5.7, 1H, 11-NH); 6.54 (dq, *J* = 1, 3, *J* = 9.8, 1H, 3-CH); 7.36 (bt, *J* = 5.9, 1H, 15-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ11.9 (6-CH_3), 12.7 (7-CH_3), 19.5 (8-CH_3), 20.4 (20-CH_3); 21.7 (21-CH_3); 28.3 (9-CH_2); 29.6 (5-CH); 35.1 (13-CH_2); 35.6 (14-CH_2); 39.4 (18-C); 39.8 (10-CH_2); 71.0 (19-CH_2); 77.7 (17-CH); 134.4 (2-C); 147.8 (3-CH); 171.4 (11-CO); 173.3 (15-CO); 194.5 (1-COS). **ESMS:** *m*/*z*: 425 $[M + Na]^+$, 403 $[M]H^+$, 385 $[M - H_2O]H^+$

Tigloyl-pantethine 19p[1,12,16]



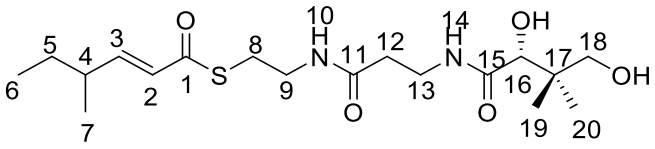
Method 1. The product was obtained from **19pp** as a colourless oil (0.167 g, 0.46 mmol, 90%), R_f: 0.44 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.92 (s, 3H, 17-CH_3); 0.92 (s, 3H, 18-CH_3); 1.82-1.87 (m, 6H, 4-5-CH_3); 2.41 (t, *J* = 5.9, 2H, 10-CH_2); 3.01-3.10 (m, 2H, 6-CH_2); 3.32-3.60 (m, 6H, 7-11-16-CH_2); 3.99 (s, 1H, 14-CH); 6.33 (bt, *J* = 5.8, 1H, 8-NH); 6.86 (q, *J* = 1.3, 6.8, 1H, 3-CH); 7.40 (bt, *J* = 5.6, 1H, 12-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ 12.2 (5-CH_3); 14.5 (4-CH_3), 20.4 (17-CH_3); 21.5 (18-CH_3); 28.2 (6-CH_2); 35.2 (11-CH_2); 35.6 (10-CH_2); 39.3 (15-C); 39.9 (7-CH_2); 70.9 (16-CH_2); 77.9 (14-CH); 136.8 (3-C); 137.3 (2-CH); 171.8 (9-CO); 173.5 (13-CO); 194.1 (1-COS). **ESMS:** *m*/*z*: 383 $[M + Na]^+$, 361 $[M]H^+$, 343 $[M - H_2O]H^+$

***E*-2-Methylhex-2-enoylpantetheine 20p**[15,17]



Method 2. The product was obtained from **20pp** as a colourless oil (0.2 g, 0.51 mmol, 90%). R_f: 0.44 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.93 (s, 3H, 1-CH_3); 0.95 (t, *J* = 7.4, 3H, 19-CH_3); 1.02 (s, 3H, 20-CH_3); 1.46-1.55 (m, 2H, 5-CH_2); 1.88 (d, *J* = 1.4, 7-CH_3) 2.20 (dq, *J* = 1.3, *J* = 7.0, 2H, 4-CH_2); 2.41 (t, *J* = 5.9, 2H, 12-CH_2); 3.00-3.13 (m, 2H, 8-CH_2); 3.35-3.59 (m, 6H, 9-13-18-CH_2); 3.99 (s, 1H, 16-CH); 6.23 (bt, *J* = 5.7, 1H, 10-NH); 6.77 (t, *J* = 1, 4, *J* = 7.4, 1H, 3-CH); 7.38 (bt, *J* = 5.9, 1H, 14-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.5 (6-CH_3), 13.9 (7-CH_3), 20.4 (19-CH_3); 21.7 (20-CH_3); 21.8 (5-CH_2); 28.3 (8-CH_2); 30.8 (4-CH_2); 35.1 (12-CH_2); 35.5 (13-CH_2); 39.4 (17-C); 39.8 (9-CH_2); 70.9 (18-CH_2); 77.7 (16-CH); 135.7 (2-C); 142.5 (3-CH); 171.6 (11-CO); 173.4 (15-CO); 194.2 (1-COS). **ESMS:** *m*/*z*:411 $[M + Na]^+$, 389 $[M]H^+$

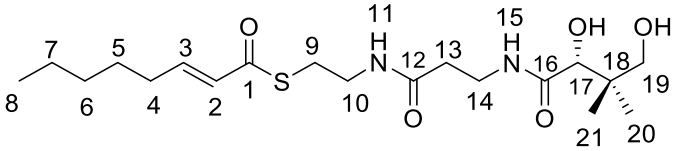
4-*RS*-*E*-4-Methylhex-2-enoylpantetheine 21p[15,17]



Method 2. The product was obtained from **21pp** as a colourless oil (0.19 g, 0.49 mmol, 94%). R_f: 0.46 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (t, *J* = 7.5, 3H, 6-CH_3); 0.98 (s, 3H, 19-CH_3); 1.01 (s, 3H, 20-CH_3); 1.05 (d, *J* = 6.9, 7-CH_3); 1.38-1.47 (m, 2H, 5-CH_2); 2.19-2.27 (m, 1H, 4-CH); 2.41 (t, *J* = 5.8, 2H, 12-CH_2); 2.94-3.15 (m, 2H, 8-CH_2); 3.33-3.58 (m, 6H, 9-13-18-CH_2);

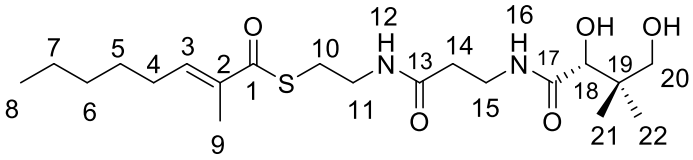
4.00 (s, 1H, 16-CH); 6.08 (dd, J = 1.4, J = 15.4, 1H, 2-CH); 6.40 (bt, J = 5.4, 1H, 10-NH); 6.83 (dd, J = 7.4, J = 15.4, 1H, 3-CH); 7.40 (bt, J = 5.4, 1H, 14-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ11.6 (6-CH_3), 18.7 (7-CH_3), 20.4 (19-CH_3); 21.7 (20-CH_3); 28.2 (5-CH_2); 28.8 (8-CH_2); 35.1 (12-CH_2); 35.8 (13-CH_2); 38.2 (4-CH); 39.4 (16-C); 39.7 (9-CH_2); 70.8 (18-CH_2); 77.6 (16-CH); 126.7 (2-CH); 152.1 (3-CH); 171.7 (11-CO); 173.6 (15-CO); 190.8 (1-COS). **ESMS:** m/z : 411 $[M + Na]^+$, 389 $[M]H^+$

E-Oct-2-enoylpantetheine 22p[15,17]



Method 2. The product was obtained from **22pp** as a colourless oil (0.17 g, 0.42 mmol, 83%). R_f: 0.45 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.91 (t, J = 6.7, 3H, 8-CH_3); 0.95 (s, 3H, 20-CH_3); 1.06 (s, 3H, 21-CH_3); 1.28-1.61 (m, 6H, 5-6-7-CH_2); 2.21 (dq, J = 1.4, J = 7.3, 2H, 4-CH_2); 2.43 (t, J = 6.0, 2H, 13-CH_2); 3, 05-3.20 (m, 2H, 9-CH_2); 3.38-3.62 (m, 6H, 10-14-19-CH_2); 4.01 (s, 1H, 17-CH); 6.13 (t, J= 4.07, 1H, 2-CH); 6.17 (dt, J = 1.4, J= 15.4, 1H, 11-NH); 6.96 (dt, J = 6, 9, J = 15.4, 1H, 3-CH); 7.34 (bt, J = 5.82, 1H, 15-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ 13.9 (8-CH_3), 20.4 (20-CH_3); 21.7 (21-CH_3); 22.4 (7-CH_2); 27.6 (6-CH_2); 28.1 (9-CH_2); 31.7 (5-CH_2); 32.3 (4-CH_2); 35.1 (13-CH_2); 35.5 (14-CH_2); 39.3 (18-C); 39.9 (10-CH_2); 70.9 (19-CH_2); 77.8 (17-CH); 128.1 (2-CH); 147.3 (3-CH); 171.6 (12-CO); 173.2 (16-CO); 190.6 (1-CO). **ESMS:** m/z: 425 $[M + Na]^+$, 403 $[M]H^+$

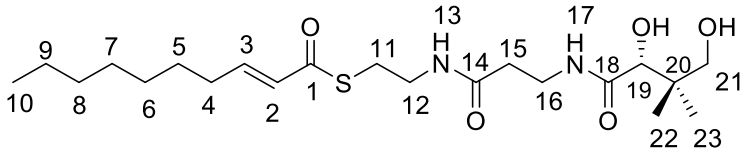
E-2-Methyloct-2-enoylpantetheine 23p[15,17]



Method 2. The product was obtained from **23pp** as a yellow oil. (0.19 g; 91.2%), R_f: 0.45 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.90 (t, J = 6.9, 3H, 8-CH_3); 0.91 (s, 3H, 21-CH_3); 1.01 (s, 3H, 22-CH_3); 1.25-1.36 (m, 4H, 6-7-CH_2); 1.41-1.50 (m, 2H, 5-CH_2); 1.85 (s, 3H, 9-CH_3); 2.20 (q, J = 7.5, 2H, 4-CH_2); 2.41 (t, J = 6.6, 2H, 14-CH_2); 2.99-3.12 (m, 2H, 10-CH_2); 3.35-3.58 (m, 6H, 11-15-20-CH_2); 3.99 (s, 1H, 18-CH); 6.37 (bt, J = 5.7, 1H, 12-NH); 6.77 (t, J = 1.3, J = 7.6, 1H, 3-CH); 7.41 (bt, J = 6.2, 1H, 16-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.5 (9-CH_3); 14.1 (8-CH_3); 20.4 (21-CH_3); 21.6 (22-CH_3); 22.5 (7-CH_2); 28.1 (5-CH_2); 28.2 (4-CH_2); 28.8 (10-CH_2); 31.6 (6-CH_2); 35.1 (14-CH_2); 35.6 (15-CH_2); 39.4 (19-C); 39.8 (11-CH_2); 70.9 (20-CH_2); 77.8 (18-CH); 135.6 (2-C);

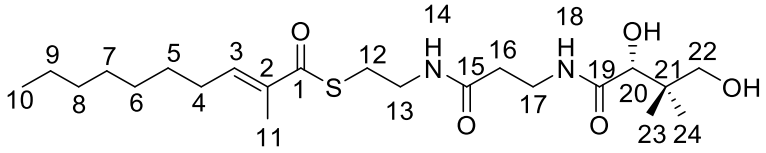
142.7 (3-CH); 171.7 (13-CO); 173.2 (17-CO); 194.2 (1-COS). **ESMS:** *m*/*z*: 439 $[M + Na]^+$, 417 $[M]H^+$, 399 $[M - H_2O]H^+$

E-Dec-2-enoyl-pantetheine 24p[15,17]



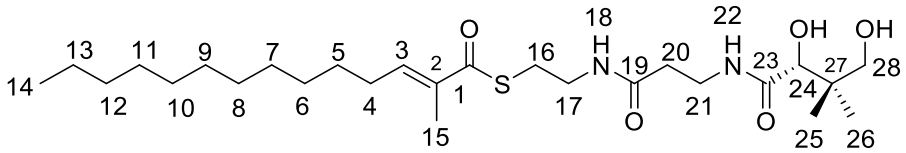
Method 2. The product was obtained from **24pp** as a colourless oil (0.16 g, 0.37 mmol, 89%). R_f: 0.48 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.91 (t, *J* = 7.1, 3H, 10-CH_3); 0.95 (s, 3H, 22-CH_3); 1.06 (s, 3H, 23-CH_3); 1.28-1.33 (m, 8H, 6, 7, 8, 9-CH_2); 1.45-1.53 (m, 2H, 5-CH_2); 2.23 (dq, *J* = 1.6, *J* = 7.2, 2H, 4-CH_2); 2.43 (t, *J* = 7.2, 2H, 15-CH_2); 3.05-3.20 (m, 2H, 11-CH_2); 3.37-3.62 (m, 6H, 12-16-21-CH_2); 4.01 (s, 1H, 19-CH); 6.13 (m, 1H, 2-CH); 6.17 (dt, *J* = 1.5, *J* = 15.4, 1H, 13-NH); 6.96 (dt, *J* = 7, 0, *J* = 15.5, 1H, 3-CH); 7.34 (bt, *J* = 6.0, 1H, 17-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ14.1 (10-CH_3), 20.4 (22-CH_3); 21.7 (23-CH_3); 22.6 (9-CH_2); 27.9 (5-CH_2); 28.6 (11-CH_2); 29.1 (7-CH_2); 29.2 (6-CH_2); 31.7 (8-CH_2); 32.3 (4-CH_2); 35.1 (15-CH_2); 35.6 (16-CH_2); 39.3 (20-C); 39.9 (12-CH_2); 70.9 (21-CH_2); 77.8 (19-CH); 128.1 (2-CH); 147.7 (3-CH); 171.6 (14-CO); 173.3 (18-CO); 190.9 (1-COS). **ESMS:** *m*/*z*: 453 $[M + Na]^+$, 431 $[M]H^+$

E-2-Methyldec-2-enoylpantetheine 25p[1,12,16]



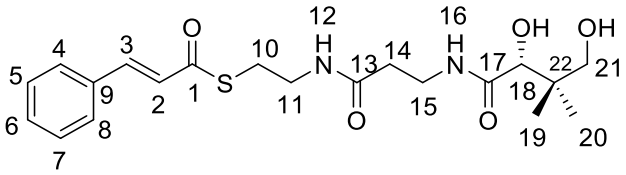
Method 1. The product was obtained from **25pp** as a colourless oil (0.01 g, 0.02 mmol, 17%). R_f: 0.48 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 0.88 (q, *J* = 7.2, 3H, 10-CH_3); 0.93 (s, 3H, 23-CH_3); 1.04 (s, 3H, 24-CH_3); 1.28-1.31 (m, 8H, 6-9-CH_2); 1.43-1.48 (m, 2H, 5-CH_2); 1.87 (s, 3H, 11-CH_3); 2.22 (q, *J* = 7.2, 2H, 4-CH_2); 2.41 (t, *J* = 6.2, 2H, 16-CH_2); 3.00-3.14 (m, 2H, 12-CH_2); 3.35-3.63 (m, 6H, 13-17-22-CH_2); 3.99 (s, 1H, 20-CH); 6.09 (bt, *J* = 5.6, 1H, 14-NH); 6.78 (tq, *J* = 1.3, *J* = 7.4, 1H, 3-CH); 7.38 (bt, *J* = 6.6, 1H, 18-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ12.5 (11-CH_3); 14.1 (10-CH_3); 20.4 (23-CH_3); 21.7 (24-CH_3); 22.6 (9-CH_2); 28.3 (5-CH_2); 28.5 (12-CH_2); 29.1 (7-CH_2); 29.4 (6-CH_2); 31.8 (8-CH_2); 35.1 (16-CH_2); 35.6 (17-CH_2); 39.4 (21-C); 39.8 (13-CH_2); 70.9 (22-CH_2); 77.8 (19-CH); 135.6 (2-C); 142.8 (3-CH); 171.7 (15-CO); 173.2 (19-CO); 194.2 (1-COS). **ESMS:** *m*/*z*: 467 $[M + Na]^+$, 445 $[M]H^+$, 427 $[M - H_2O]H^+$

E-2-Methyltetradec-2-enoylpantetheine 26p[1,12,16]



Method 1. The product was obtained from **26pp** as a colourless oil (0.01 g, 0.02 mmol, 16%), R_f: 0.3 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ [ppm]: 0.88 (t, 3H, 14-CH_3); 0.97 (s, 3H, 25-CH_3); 1.04 (s, 3H, 26-CH_3); 1.30-1.47 (m, 18H, 5-13-CH_2); 1.87 (s, 3H, 15-CH_3); 2.21 (q, 2H, 4-CH_2); 2.42 (t, 2H, 20-CH_2); 3.05 (t, *J* = 6.2 Hz, 2H, 16-CH_2); 3.27 (d, *J* = 12.2 Hz, 1H, 28a-CH_2); 3.39-3.62 (m, 4H, 17-21-CH_2); 3.68 (d, *J* = 12.2 Hz, 1H, 28-CH_2); 4.07 (s,1H, 24-CH); 6.08 (bt, *J* = 5.0 Hz, 1H, 18-NH); 6.77 (t,1H, 3-CH); 7.03 (t, *J* = 5.5 Hz, 1H, 22-NH). **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ[ppm]: 12.5 (14-CH_3); 14.0 (15-CH_3); 18.7 (25-CH_3); 18.9 (26-CH_3); 22.1 (30-31-CH_3); 22.6 (13-CH_2); 28.5 (12-CH_2); 29.1-29.9 (5-11-CH_2); 31.6 (4-CH_2); 34.8 (21-CH_2); 39.7 (20-CH_2); 71.5 (28-CH_2); 77.2 (18-CH); 142.4 (2-CH); 170.0 (23-CO); 171.1 (19-CO); 193.7 (1-CO). **ESMS:** *m*/*z*: 501.3 $[M]H^+$, 523 $[M + Na]^+$

E-Cinnamoyl pantetheine 27p[1,12,16]



Method 1. The product was obtained from **27pp** as a colourless oil (0.03 g, 0.07 mmol, 26%), R_f: 0.5 (DCM / MeOH). **^{1}H-NMR** ($CDCl_3$, 400 MHz): 0.97 (s, 3H, 19-CH_3); 1.04 (s, 3H, 20-CH_3); 2.42 (t, *J* = 5.8, 2H, 14-CH_2); 3.05 (t, *J* = 6.7, 2H, 10-CH_2); 3.27 (d, *J* = 11.8, 1H, 21a-C*H*H); 3.39-3.62 (m, 4H, 11-15-CH_2); 3.68 (d, *J* = 12.2, 1H, 21b-CH*H*); 4.07 (s, 1H, 18-CH); 6.08 (bt, *J* = 5.8, 1H, 12-NH); 6.70 (d, 1H, 2-CH); 7.03 (bt, *J* = 5.8, 1H, 16-NH), 7.73 (dd, 3H, 5-,6-,7-CH); 7.52 (m, 2H, 4-,8-CH); 7.59 (d, *J* = 15.82, 1H, 3-CH), **^{13}C-NMR** ($CDCl_3$, 100 MHz): δ 18.7 (19-CH_3); 18.9 (20-CH_3); 28.2 (10-CH_2); 70.2 (21-C); 34.8 (14-CH_2); 35.9 (15-CH_2); 39.7 (11-CH_2); 77.2 (18-CH); 48.1 (22-C); 123 (2-C) 127-128.5 (4-C, 5-C, 6-C, 7-C, 8-C) 135.7 (9-C); 143 (3-C), 170.0 (13-CO); 171.1 (17-CO); 193.7 (1-CO). **ESMS:** *m*/*z*: 432 $[M + Na]^+$, 409 $[M]H^+$

6.0 References

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