Electronic Supplementary Information

Heterologous biosynthesis of a fungal macrocyclic polylactone requires only two iterative polyketide synthases

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Pakorn Wattana-Amorn

Experimental

Table S1: Primers used for constructing *men1* and *men2*. Underlined nucleotides are regions of overlap with *E. coli*-yeast shuttle vectors (pEYA2eGFP and pEYA2dsRED)

gene	primer	Sequence 5′-3′
men1	*Fragment 1*	
	Men1-1-fwd	TAATGCCAACTTTGTACAAAAAAGCAGGCTATGGGCAGCGTCTACGATAG
	Men1-1-rev	AGCTCCGCGACGAGGGATGG
	Fragment 2	
	Men1-2-fwd	TCAATCGCGCCCATCTTACG
	Men1-2-rev	ATATCAACACGCGGGACGTG
	Fragment 3	
	Men1-3-fwd	TCCAAGCCCAGAAGAAGTCG
	Men1-3-rev	CTTGATGACGTCCTCGGAGGAGGCCATCTGCTTGGTGGTGGCGGCGGTAG
men2	*Fragment 1*	
	men2-1-fwd	TAATGCCAACTTTGTACAAAAAAGCAGGCTATGGCGGGCCCCGTTGCAAC
	men2-1-rev	AGGCGCCGAGCACCTGCTCG
	Fragment 2	
	men2-2-fwd	ATGCAGGAACGGTGCGAGTC
	men2-2-rev	TGAACAGCTCCTCGCCCTTGCTCACCATGTTGTCCATGGCGTCCTTAAGG

Results

MGSVYDSQSIAIVGLSCRLPGDADNAERFWNLMSEGRSAISSVPADRWNSKGFRDPTGKKRQNTSLTDRAHFVKGDISEFDANFFTISKAEADSM
DPQQRIMLEVAYEAFENAGLSMDSLAKSQTGCWVSSFSQDWKEMHFSDPDAAPKYAMSGMQPELLSNRVSYFFDLQGPSMTIETACSGSLVGLH
VACQSLRAGDCETALVGGANLFLNQNMFLALSNQSFLAPDGLCKAFDASANGYGRGEGFAAVILKPIEKAIRDGDHIRAVIRGTGTNQDGRTKGL
TMPNGHAQESLIRSTYAAAGLDLKDTAYFEAHGTGTQAGDFEELGAISRTVADARQKAGLEDLWVGSAKTNIGHLEAVAGLVGVLKAVLVLEN
GVIPPNLHFKNPNPRIPFGKWRIKVPTERIQWPSDGIRRVSVNSFGFGGSNAHAILDDADQYLSGRGIIRANGKSHHHHHQHQQQQLNGGNGGSNG
VNGTSEVNGTSGVNGTTTAITNGSTHVNGTAATAATAAAQQIIILNSYDQEGLGRQREALLRYAEKQQQQQQQQGGQGGADPEKLLGDLAFTLN
QKRSRLPWRTFFTASTLPELSRALEAASTFPAIRSGAATPRIAYVFTGQGAQWAQMGMDLLRFHVFRESVEAADRHLTQIGCPWSAVEELQRGDA
ESNIHISWYSQTLCTVIQVALVQLLESWNVRPRSVVGHSSGEMGAAFAIGALSREDAWTIAYWRGKLSSELTTIAPTQKGAMMAVGASHAQAQA
WVDGLTRGRCVVACVNSPSSVTVSGDESGLDELAAMLKEQGVFARKLKVSTAYHSHHMKAVAEAYLDALKGVRTRTVPAEGGAPQMFTSVSE
SLVDPAELGPAHWVANLISPVLFSNTVRELARPKGPDDEASGSAVDLMVEIGPHAALRGPVTQILQSHGLPALDYYSVLSRGANSVDTALAVVGE
LVCRGVPVDLGAVNRAHLTAEQQLQADRRPSLVAELPPYAWNHAKTYWSESRISRELKYRPAPQLGLIGAPMPNFAPNEHQWRGFLRLADAAWI
RDHKIQSSVIFPAGGFLAMAVEAAAQLAAAAQQEQPDRVVKGYKLRSVDISSAVRVADDSSVECIIQLRLSPGGAAAAEAAETWWDFSISTSPNA
GEALKRNCSGSVAVEFGALAIVDAAQASYASAASACTISQEVDVFYRQLDSVGLGYGPTFQAIKSILHDSRGQGCGVLEITETDSASPKDPDARPH
VVHPTTLESLFQMAYAAFGGRDGRVKRALMVTQIDELLVDATIPFAPGSRLLTSASAARQGFREIKADAFMLEAASESPKMAVKGLVCVEMPSAS
GMGGGGGGGLDADQASYSAMLSKFVWKPALELLSAPEQAKLLEDATRLPEDEAQRLASEATAELHAVKAVLESAQSKKIANLKLRNAAKWISQ
QLQASGIPGKPAENGAREGGSSSGFTAEVEKVLSGLAEADVLLGSKGSADHLVAQLPGMKMSLEKMYKLVNYMAHANPNLTVLEIVPGGAGVD
FSLPLSAKDIPSTIQYTYASPSADNVQQMQERLGGGSGDSALALALAPRFRVLEIEQDLADQGLDPGSFDIVIGCNLLSNAVNVEKTLSQAKSLLTE
GGKMALVELNKPSPAALPVLGILCDWWKRRDDGLRRPFTTDMVNESLAGQGFAIELATPDFTDPALQQSSLVLASCQPASAGKESAAQEVVSILV
RKDSSEAVNALASQLSQACNGAKTVTWEAGVDFKGQHLISLLEFDTPLLDRLTEEDFGLVKQLITQAASLQWVTAIPEPHASTVMGLARVARFEV
PSLRFQTVTLDPSSVLALDRAATLIIQAQKKSTSQDKEFKEVDSVLHVPRVDIDAPLNEQVTRLLLEEDVEPMPLGSGDAARKLCIRNPGMLNTLCF
EIDSLPSTVLAEDEVEMQVKASGLSPKDVAICLGQVSDTALGFEASGIVTRVGAGVAQFQAGDKICMMARGAHRTVLRSKSALCQRIPEGMSFEQ
AAAVPLAHGAVYHALVNIARARSGQKILVAVSDAVVSEAAVQLAKHLGLEAFVTTESQDRTPLIGTKEDYGISDDHIFYSRDPTYVKEITRLTNGA
GVDCVLSSVSGEALKHATSCLAPFGTFVDLGAKDVRSSAILDKHPEAMFAAINLERISELRPDMAGRIMDGTFALLREGAIKPVKLLAAYPASDLE
TAMQALHARSRQDKIVIAYSADQVVPVLHNPRESLRLPGDKTYLIAGGLGGIGRNIANLLVECGARHLAFVSRSGVTSEAQQKLVDNLTQRGAKI
AVYRCNIGDAQSLEQTLARCSAEMPPVKGVIHSAVVFRDAVIHNMTYAQWHELMESKLGGSWNLHALTTSYDLDFFLCIGSFMAIIGGLSQSNYA
AGGAFQDGLAHMRQSMGLPAATIDLGIVKGFGAVEEQGAVGHTLEWREPFGVDEDAVFALIKKALLGQMDKDGPGVPPQMINTVPTGGMVRES
GVGQPYYFEDPRFAIMAAIGTRNADGADGQASVALKEQLAQAESPEEAARLVSAAVAAKVAKLMQVGAEEIDAGKPLHAYGVDSLVAIEYVHW
AKKEVAAEITVFDVMASVPISAFASDLAKKGEWGTTAATTKQ

Fig. S1: Amino acid sequence of Men1. Domains were identified using SMART and highlighted as follows: KS (red), AT (dark green), DH (cyan), ER (bright green), KR (magenta) and ACP (dark grey).

MAGPVATSDRQQRLIFFGDQTVDALPCIKILTAQAHRLPALRRFLRDAADVIQVLLSSLEFDDHDHYRRFETICELAEIYSKQDGTHETIACALWTT
AQFGDLVMRAELNPSILTGGQQSADPTYVVGICGGLLPAAATATARDINELLDIGRKLVAVAFRLGVAQWRRAMDIEGKPGRWAVTIVNVPAKQ
IRTILDAFNEDMEIPKHRQFYISFLAKGWVTVSGPPSLFPELWAYSSTLNAASKMQLPLGTPAHAAHLPPVNVSEIVGTGDVLDLTVRENFFTVSTS
TCQPFQCQDLGSLLQESLRDITGKTLNIAGVNDYVISALDRNTPVRVSSFGPASQIASFKKTLEEAGYQVELDLGDPSLGNPEYPIDADSRDGSNLIA
VVGQSVRFPGSEDVETFWENIKAGRSFETEIPASRFDLAHHYDATGSKTSSVTTKYGSFLENPGLFDNRLFNVSPREAKQMDPIQRILMMCSYEAL
QAAGYSPDGSLSTNSMRIATYFGQSGDDWRQVRASQEVDIYYIPGTVRSFAPGKLNYHYKWGGGNYAVDSACAASTTTMMLASQALLARDCD
MALAGGGQLNAAPEPYAGLSRAGFLSKTTGGCKTFREDADGYCRGEGVGVVVLKRLEDALAENDNVLAVIRGADRNFSWDATSITHPSVSAQV
KLVKSVLRNTGVEPEEIGFVEMHGTGTQAGDGVEMETVTTVFGSRPKDNPLYVGAVKANIGHGEAAAGVASVIKAIEVLRHRTIPTQAGFPGPRD
PKFNHLDGMNIRIPESVVPFQPAPAPFSSDGKRKVLVNNFDASGGNNCVLLEEAPARDRETTADPRGVYTVAVSARTTNSLKNNISRLLGYLQSHP
DASVADVAYTTTARRMHEDLKKAYTVQTASELVSLLQADLKKDLTAVQYRSPHSVVFAFTGQGAQYAGMGKQLFDTSAAFRESVQAFHELAV
WQGFPEFLHLIADDQADVKAADPVQLQLAVVVLEMALANLWKSWGVEPGLVVGYSLGEYPALYVAGVLSVHDVIFLVGNRARLMQERCESGS
YAMLATQSSPQDLEQVLGAYPSCAVACKGAPRSTVVSGPTEDITQLHSELKEKNINGTLLNVPYGFHCAQVDPILDDFRDMADGIQFNKPRIPVAS
SLEGTVVTEEGVFSSAYLVRQTREPVNFIGAVKAAESSGRADNTTVWIEIGPKRVLSSLVKSTLSADQGRLLHTIDDKDSNWKTIAAAMTAGYTEG
MSIKWPKFHKLFSKHLTLLELPTYAFDLKDYWIPPAVPVTAAAPVAAPAADPSLPVIPVVPGFPTASLQQVRSEQINGDEAKVTFETVISHPALLAVI
RGHRVGGVDLFPASGFMDMAFSAAKYIHHRTKSGQPVPEISMKHLAITHPLTPSSGQSRQIVIVTASKRSGSSVVDVSFRSRDGSAEQDHGDCKLH
FDKRGSWDAEWAQTAHFINAAKKNVIANGTSPAGTGHRLPKSVVYKLFSSLVEYSGAFRAMEEVYVTDDFQKEAVASVVLPGGSSEFYVNPYW
SDALIHVCGFLLNSSPNLPSQDCFLFNGLEEMRLLSDDLQPGIPYTSYVYLTEPGSSPDSQAPRPKHARGDVYVFQGEKIVGVAKGVVFQRLTRRV
LATVLGGKLPGAAAPVREIAAPAPIRAVAPAPAPVAPRPVEMYRVPGVVGDEKADAAIGKILARAGANPAHITDATTFAEIGFDSLEWIELVREIRT
SLDLEVPASFFFEYPKVNGLRRAISELSLDYQGPASGSVSVSSSATTTHGMTTPSSTSSAQSSQSSQTPDGPGIYANAVIDIVLSQTGFDKADLLPTTR
FDDMGLDSLCTMEVVGVVREQTGLDLPASFFHQNPTVAHVRRALGSDSDGDSKPKSAPAPPAPEPVVEVAAPAPAVQAPPAILDGDLASYHCDFF
LMQGSSDSTKTPLFFLPDGTGYPAVLLKLPPIFEGDNALFTCKSPLLNVAEGREVRCTIEGLAAAYAAAIQRARPHGPYLLAGYSLGGAYAFEVAKI
LADAGEVVQGLLFVDFNMAASVGKLHRDRNPVPVDLTVGAMEKTGWMQGIQNDDKDFNIPPAPPKIKFHALSVFKSLISYYPTPMTPSQRPRNT
YALWAGIGMQDLLGTKNAGFLPAYGIIDWQMGDRHENNGPAGWEEYIGGPVKCATMPCDHLSLLMSHHWIPKSAEVIKGLLKDAMDN

Fig. S2: Amino acid sequence of Men2. Domains were identified using SMART and are highlighted as follows: SAT (yellow), KS (red), AT (dark green), PT (cyan), ACP (dark grey) and TE (magenta).

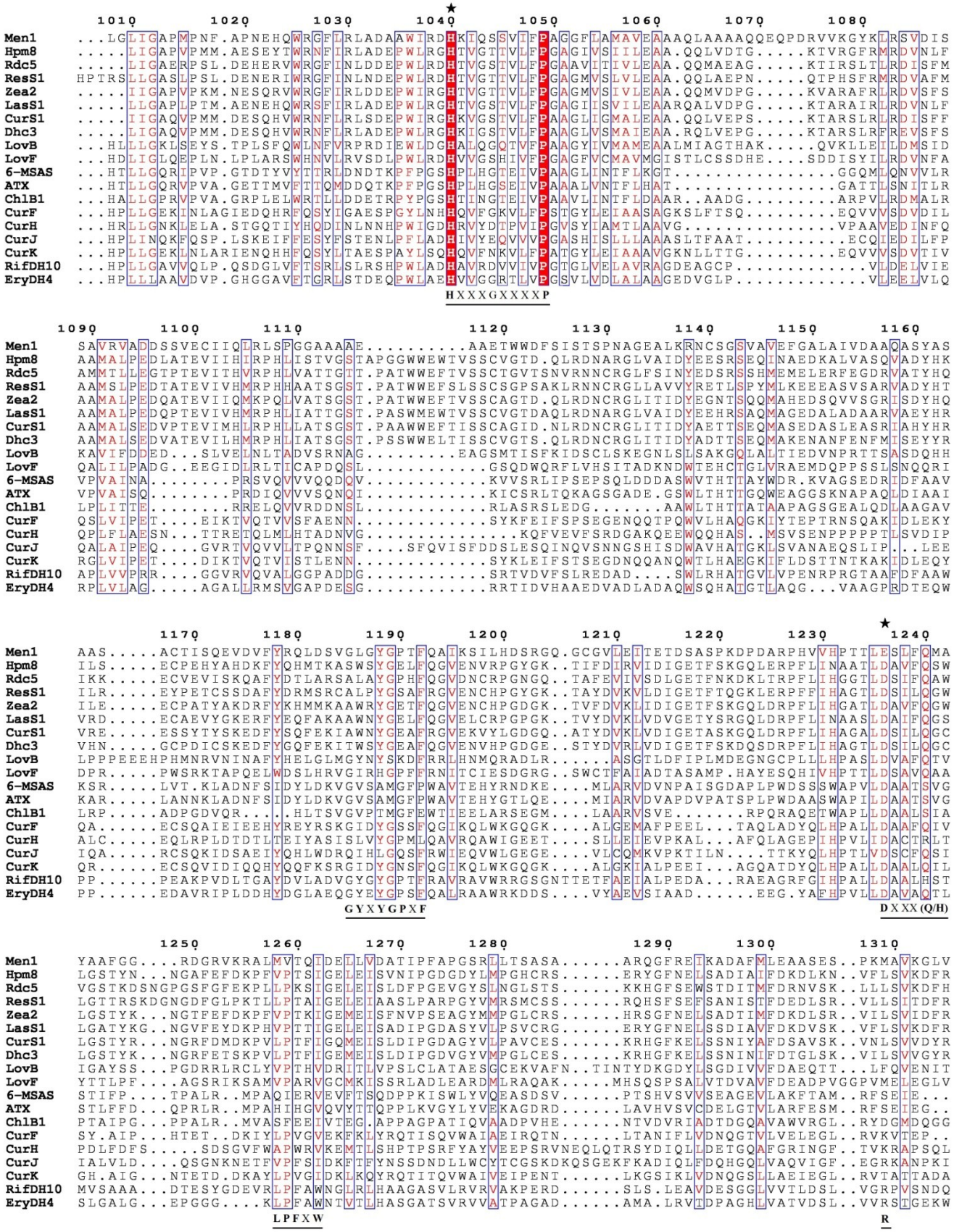

1010 1020 1030 1040 1050 1060 1070 1080
Men1 ...LGLIGAPMPNF.APNEHQWRGFLRLADAAWIRDHKIQSSVIFPAGGFLAMAVEAAAQLAAAAQQEQPDRVVKGYKLRSVDIS
Hpm8 .....LIGAPVPMM.AESEYTWRNFIRLADEPWLRGHTVGTTVLFPGAGIVSIILEAA.QQLVDTG.....KTVRGFRMRDVNLF
Rdc5 .....LIGAERPSL.DEHERVWRGFINLDDEPWLRDHTVGSTVLFPGAAVITIVLEAA.QQMAEAG.....KTIRSLTLRDISFM
ResS1 HPTRSLLGASLPSL.DENERIWRGFINLNDEPWLRDHTVGTTVLFPGAGMVSLVLEAA.QQLAEPN.....QTPHSFRMRDVAFM
Zea2 .....IIGAPVPKM.NESQRVWRGFIRLDDEPWIRGHTVGTTVLFPGAGMVSIVLEAA.QQMVDPG.....KVARAFRLRDVSFS
LasS1 .....LLGAPLPTM.AENEHQWRSFIRLADEPWLRGHTVGSTVLFPGAGIISVILEAARQALVDPG.....KTARAIRLRDVNLF
CurS1 .....IIGAQVPMM.DESQHVWRNFLRLSDEPWIRGHKVGSTVLFPAAGLIGMALEAA.QQLVEPS.....KTARSLRLRDISFF
Dhc3 .....LIGAQVPMM.DESQHVWRNFLRLADEPWLRGHKIGSTVLFPAAGLVSMAIEAA.RQLVEPG.....KTARSLRFREVSFS
LovB ...HLLLGKLSEYS.TPLSFQWLNFVRPRDIEWLDGHALQGQTVFPAAGYIVMAMEAALMIAGTHAK.....QVKLLEILDMSID
LovF ...HDLIGLQEPLN.LPLARSWHNVLRVSDLPWLRDHVVGSHIVFPGAGFVCMAVMGISTLCSSDHE....SDDISYILRDVNFA
6-MSAS ...HTLLGQRIPVP.GTDTYVYTTRLLDNDTKPFPGSHPLHGTEIVPAAGLINTFLKGT.....GGQMLQNVVLR
ATX ...HTLLGQRVPVA.GETTMVFTTQMDDQTKPFPGSHPLHGSEIVPAAALVNTFLHAT.....GATTLSNITLR
ChlB1 ...HALLGPRVPVA.GRPLELWRTLLDDETRPYPGSHTINGTEIVPAAVLINTFLDAAR..AADG.....ARPVLRDMALR
CurF ...HPLLGEKINLAGIEDQHRFQSYIGAESPGYLNHHQVFGKVLFPSTGYLEIAASAGKSLFTSQ.....EQVVVSDVDIL
CurH ...HRLLGNKLELA.STGQTIYHQDINLNNHPWIGDHRVYDTPVIPGVSYIAMTLAAVG.....VPAAVEDINFQ
CurJ ...HPLINQKFQSP.LSKEIFFESYFSTENLPFLADHIVYEQVVVPGASHISLLLAAASLTFAAT.....ECQIEDILFP
CurK ...HPLLGEKLNLARIENQHHFQSYLTAESPAYLSQHQVFNKVLFPATGYLEIAAAVGKNLLTTG.....EQVVVSDVTIV
RifDH10 ...HPLLGAVVQLP.QSDGLVFTSRLSLRSHPWLADHAVRDVVIVPGTGLVELAVRAGDEAGCP.....VLDELVIE
EryDH4 ...HPLLLAAVDVP.GHGGAVFTGRLSTDEQPWLAEHVVGGRTLVPGSVLVDLALAAGEDVGLP.....VLEELVLQ
HXXXGXXXXP
1090 1100 1110 1120 1130 1140 1150 1160
Men1 SAVRVADDSSVECIIQLRLSPGGAAAAE.....AAETWWDFSISTSPNAGEALKRNCSGSVAVEFGALAIVDAAQASYAS
Hpm8 AAMALPEDLATEVIIHIRPHLISTVGSTAPGGWWEWTVSSCVGTD.QLRDNARGLVAIDYEESRSEQINAEDKALVASQVADYHK
Rdc5 AMMTLLEGTPTEVITHVRPHLVATTGTT.PATWWEFTVSSCTGVTSNVRNNCRGLFSINYEDSRSSHMEMELERFEGDRVATYHQ
ResS1 AAMSLPEDTATEVIVHMRPHHAATSGST.PATWWEFSLSSCSGPSAKLRNNCRGLLAVVYRETLSPYMLKEEEASVSARVADYHT
Zea2 AAMALPEDQATEVIIQMKPQLVATSGST.PATWWEFTVSSCAGTD.QLRDNCRGLITIDYEGNTSQQMAHEDSQVVSGRISDYHQ
LasS1 AAMALPEDQPTEVIVHMRPHLIATTGST.PASWMEWTVSSCVGTDAQLRDNARGLVAIDYEEHRSAQMAGEDALADAARVAEYHR
CurS1 AAMALSEDVPTEVIMHLRPHLLATSGST.PAAWWEFTISSCAGID.NLRDNCRGLITIDYAETTSEQMASEDASLEASRIAHYHR
Dhc3 AAMALSEDVATEVILHMRPHLIATSGST.PSSWWELTISSCVGTS.QLRDNCRGLITIDYADTTSEQMAKENANFENFMISEYYR
LovB KAVIFDDED...SLVELNLTADVSRNAG.....EAGSMTISFKIDSCLSKEGNLSLSAKGQLALTIEDVNPRTTSASDQHH
LovF QALILPADG..EEGIDLRLTICAPDQSL.....GSQDWQRFLVHSITADKNDWTEHCTGLVRAEMDQPPSSLSNQQRI
6-MSAS VPVAINA.....PRSVQVVVQQDQV.....KVVSRLIPSEPSQLDDDASWVTHTTAYWDR.KVAGSEDRIDFAAV
ATX VPVAISQ.....PRDIQVVVSQNQI.....KICSRLTQKAGSGADE.GSWLTHTTGQWEAGGSKNAPAQLDIAAI
ChlB1 LPLITTE.....RRELQVVRDDNSL.....RLASRSLEDG.....AAWLTHTTATAAPAGSGEALQDLAAGAV
CurF QSLVIPET....EIKTVQTVVSFAENN.....SYKFEIFSPSEGENQQTPQWVLHAQGKIYTEPTRNSQAKIDLEKY
CurH QPLFLAESN...TTRETQLMLHTADNVG.....KQFVEVFSRDGAKQEEWQQHAS..MSVSENPPPPPTLSVDIP
CurJ QALAIPEQ....GVRTVQVVLTPQNNSF....SFQVISFDDSLESQINQVSNNGSHISDWAVHATGKLSVANAEQSLIP...LEE
CurK RGLVIPET....DIKTVQTVISTLENN.....SYKLEIFSTSEGDNQQANQWTLHAEGKIFLDSTTNTKAKIDLEQY
RifDH10 APLVVPRR....GGVRVQVALGGPADDG.....SRTVDVFSLREDAD....SWLRHATGVLVPENRPRGTAAFDFAAW
EryDH4 RPLVLAG.....AGALLRMSVGAPDESG.....RRTIDVHAAEDVADLADAQWSQHATGTLAQG...VAAGPRDTEQW
1170 1180 1190 1200 1210 1220 1230 1240
Men1 AAS.....ACTISQEVDVFYRQLDSVGLGYGPTFQAIKSILHDSRGQ.GCGVLEITETDSASPKDPDARPHVVHPTTLESLFQMA
Hpm8 ILS.....ECPEHYAHDKFYQHMTKASWSYGELFQGVENVRPGYGK..TIFDIRVIDIGETFSKGQLERPFLINAATLDAVFQSW
Rdc5 IKK.....ECVEVISKQAFYDTLARSALAYGPHFQGVDNCRPGNGQ..TAFEVIVSDLGETFNKDKLTRPFLIHGGTLDSIFQAW
ResS1 ILR.....EYPETCSSDAFYDRMSRCALPYGSAFRGVENCHPGYGK..TAYDVKVLDIGETFTQGKLERPFFIHAGTLDSILQGW
Zea2 ILE.....ECPATYAKDRFYKHMMKAAWRYGETFQGVENCHPGDGK..TVFDVKLIDIGETFSKGQLDRPFLIHGATLDAVFQGW
LasS1 VRD.....ECAEVYGKERFYEQFAKAAWNYGELFQGVELCRPGPGK..TVYDVKLVDVGETYSRGQLDRPFLINAASLDAIFQGS
CurS1 VRE.....ESSYTYSKEDFYSQFEKIAWNYGEAFRGVEKVYLGDGQ..ATYDVKLVDIGETASKGQLDRPFLIHAGALDSILQGC
Dhc3 VHN.....GCPDICSKEDFYGQFEKITWSYGEAFQGVENVHPGDGE..STYDVRLVDIGETFSKDQSDRPFLIHAGTLDSILQGC
LovB LPPPEEEHPHMNRVNINAFYHELGLMGYNYSKDFRRLHNMQRADLR.....ASGTLDFIPLMDEGNGCPLLLHPASLDVAFQTV
LovF DPR.....PWSRKTAPQELWDSLHRVGIRHGPFFRNITCIESDGRG..SWCTFAIADTASAMP.HAYESQHIVHPTTLDSAVQAA
6-MSAS KSR.....LVT.KLADNFSIDYLDKVGVSAMGFPWAVTEHYRNDKE....MLARVDVNPAISGDAPLPWDSSSWAPVLDAATSVG
ATX KAR.....LANNKLADNFSIDYLDKVGVSAMGFPWAVTEHYGTLQE....MIARVDVAPDVPATSPLPWDAASWAPILDAATSVG
ChlB1 LRP.....ADPGDVQR.....HLTSVGVPTMGFEWTIEELARSEGM....LAARVSVE.....RPQRAQETWAPLLDAALSIA
CurF QA.....ECSQAIEIEEHYREYRSKGIDYGSSFQGIKQLWKGQGK....ALGEMAFPEEL...TAQLADYQLHPALLDAAFQIV
CurH ALC.....EQLRPLDTDTLTEIYASISLVYGPMLQAVRQAWIGEET....SLLEIEVPKAL...AFQLAGEPIHPVLIDACTRLT
CurJ IQA.....RCSQKIDSAEIYQHLWDRQIHLGQSFRWIEQVWLGEGE....VLCQMKVPKTILN....TTKYQLHPTLVDSCFQSI
CurK QR.....ECSQVIDIQQHYQQFKSRGIDYGNSFQGIKQLWKGQGK....ALGKIALPEEI...AGQATDYQLHPALLDAALQIL
RifDH10 PP.....PEAKPVDLTGAYDVLADVGYGYGPTFRAVRAVWRRGSGNTTETFAEIALPEDA...RAEAGRFGIHPALLDAALHST
EryDH4 PP.....EDAVRIPLDDHYDGLAEQGYEYGPSFQALRAAWRKDDS....VYAEVSIAAD.....EEG.YAFHPVLLDAVAQTL
GYXYGPXF
DXXX(Q/H)
1250 1260 1270 1280 1290 1300 1310
Men1 YAAFGG....RDGRVKRALMVTQIDELLVDATIPFAPGSRLLTSASA.....ARQGFREIKADAFMLEAASES..PKMAVKGLV
Hpm8 LGSTYN...NGAFEFDKPFVPTSIGELEISVNIPGDGDYLMPGHCRS.....ERYGFNELSADIAIFDKDLKN..VFLSVKDFR
Rdc5 VGSTKDSNGPGSFGFEKPLLPKSIGELEISLDFPGEVGYSLNGLSTS.....KKHGFSEWSTDITMFDRNVSK..LLLSVKDFH
ResS1 LGTTRSKDGNGDFGLPKTLLPTAIGELEIAASLPARPGYVMRSMCSS.....RQHSFSEFSANISTFDEDLSR..VLLSIIDFR
Zea2 LGSTYK...NGTFEFDKPFVPTKIGEMEISFNVPSEAGYMMPGLCRS.....HRSGFNELSADTIMFDKDLSR..VILSVIDFR
LasS1 LGATYKG..NGVFEYDKPHVPTTIGELEISADIPGDASYVLPSVCRG.....ERYGFNELSSDIAVFDKDVSK..VFLSVKDFR
CurS1 LGSTYR...NGRFDMDKPVLPTFIGQMEISLDIPGDAGYVLPAVCES.....KRHGFKELSSNIYAFDSAVSK..VNLSVVDYR
Dhc3 LGSTYK...NGRFETSKPVLPTFIGEMEISLDIPGDVGYVMPGLCES.....KRHGFKELSSNINIFDTGLSK..VILSVVGYR
LovB IGAYSS...PGDRRLRCLYVPTHVDRITLVPSLCLATAESGCEKVAFN..TINTYDKGDYLSGDIVVFDAEQTT...LFQVENIT
LovF YTTLPF....AGSRIKSAMVPARVGCMKISSRLADLEARDMLRAQAK.....MHSQSPSALVTDVAVFDEADPVGGPVMELEGLV
6-MSAS STIFP....TPALR..MPAQIERVEVFTSQDPPKISWLYVQEASDSV.....PTSHVSVVSEAGEVLAKFTAM..RFSEIE...
ATX STLFFD...QPRLR..MPAHIHGVQVYTTQPPLKVGYLYVEKAGDRD.....LAVHVSVCDELGTVLARFESM..RFSEIEG...
ChlB1 PTAIPG...PPALR..MVASFEEIVTEG.APPAGPATIQVAADPVHE.....NTVDVRIADTDGQAVAWVRGL..RYDGMDQGG
CurF SY.AIP...HTET..DKIYLPVGVEKFKLYRQTISQVWAIAEIRQTN.....LTANIFLVDNQGTVLVELEGL..RVKVTEP..
CurH PDLFDFS.....SDSGVFWAPWRVKEMTLSHPTPSRFYAYVEEPSRVNEQLQTRSYDIQLLDETGQAFGRINGF..TVKRAPSQL
CurJ IALVLD....QSGNKNETFVPFSIDKFTFYNSSDNDLLWCYTCGSKDKQSGEKFKADIQLFDQHGQLVAQVIGF..EGRKANPKI
CurK GH.AIG...NTETD.DKAYLPVGIDKLKQYRQTITQVWAIVEIPENT.....LKGSIKLVDNQGSLLAEIEGL..RVTATTADA
RifDH10 MVSAAA...DTESYGDEVRLPFAWNGLRLLHAAGASVLRVRVAKPERD.....SLS.LEAVDESGGLVVTLDSL..VGRPVSNDQ
EryDH4 SLGALG...EPGGG....KLPFAWNTVTLHASGATSVRVVATPAGAD.....AMA.LRVTDPAGHLVATVDSL..VVRSTGEKW
LPFXW
R

Fig. S3. Multiple sequence alignment of DH domains from several reducing PKSs Similar conserved residues are colored in red. The catalytic dyads are marked by stars and the underlined residues are common motifs found in DH domains. Highly reducing PKSs: Hpm8 from *Hypomyces subiculosus* (B3FWT3), Rdc5 from *Pochonia chlamydosporia* (B3FWU0), ResS1 from *Sarocladium zeae* (AHV78252), Zea2 from *Gibberella zeae* PH-1 (A0A098D8A0), LasS1 from *Lasiodiplodia theobromae* (AHV78245), CurS1 from *Aspergillus terreus* (L7X8J4), Dhc3 from *Alternaria cinerariae* (A0A0N7D4P6), LovB from *Aspergillus terreus* (Q9Y8A5), LovF from *Aspergillus terreus* (Q9Y7D5); partially reducing PKSs: 6-MSAS from *Penicillium griseofulvum* (P22367), ATX from *Aspergillus terreus* NIH2624 (Q0CJ59), ChlB1 from *Streptomyces antibioticus* (AAZ77673); modular PKSs: CurF (AAT70101), CurH (AAT70103), CurJ (AAT70105), CurK (AAT70106) from *Lyngbya majuscula* , RifDH10 from *Amycolatopsis mediterranei* (AEK39124), EryDH4 from *Saccharopolyspora erythraea* NRRL 2338 (CAM00064). GenPept accession numbers are given in brackets.

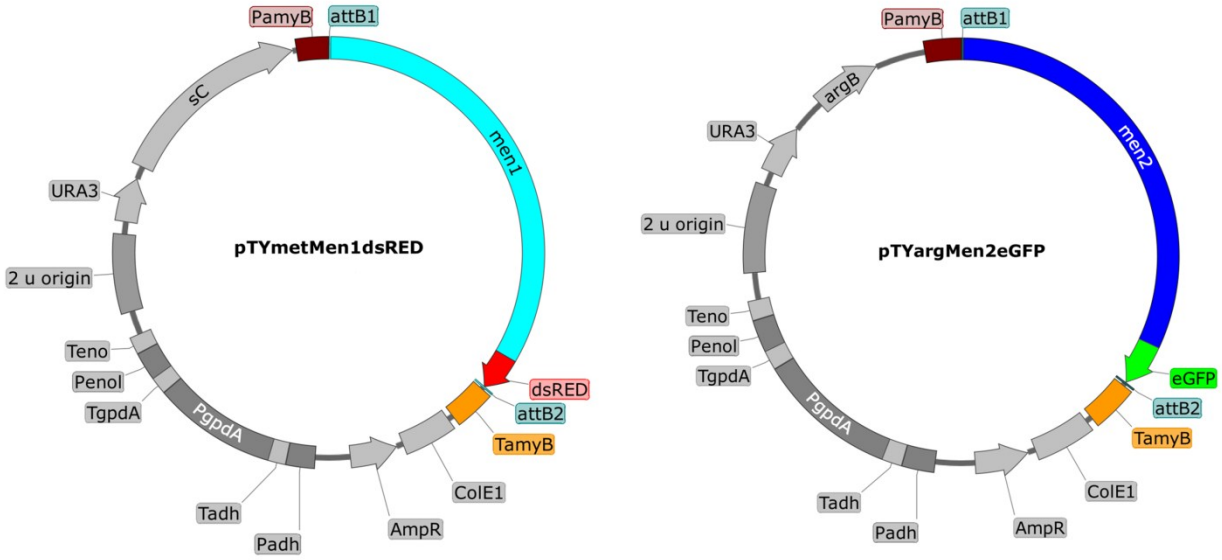
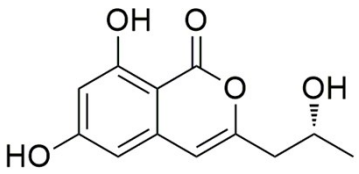


Fig. S4: Plasmid maps of pTYmetMen1dsRED and pTYargMen2eGFP generated using SnapGene software (from GSL Biotech; available at snapgene.com)

The ^{1}H and ^{13}C chemical shifts of compounds **(1)** – **(3)** are listed below.



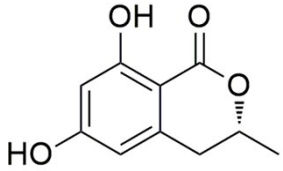
(-)-orthosporin **(1)**

^{1}H NMR (400 MHz, CD_3COCD_3): δ 11.16 (s, 1H), 6.43 (s, 1H), 6.41 (d, J = 2.2 Hz, 1H), 6.37 (d, J = 2.2 Hz, 1H), 4.16 (m, 1H), 2.59 (m, 2H), 1.23 (d, J = 6.2 Hz, 3H).

^{13}C NMR (100 MHz, CD_3COCD_3): δ 167.2, 166.5, 164.6, 156.6, 141.0, 106.5, 103.4, 102.3, 100.0, 65.6, 44.0, 23.7.

ESITOFMS *m/z* calcd for $C_{12}H_{12}O_5Na$ $[M+Na]^+$ = 259.0577, found = 259.0595

Both ^{1}H and ^{13}C NMR data and optical activity are in agreement with those reported in the literatures.[1, 2]



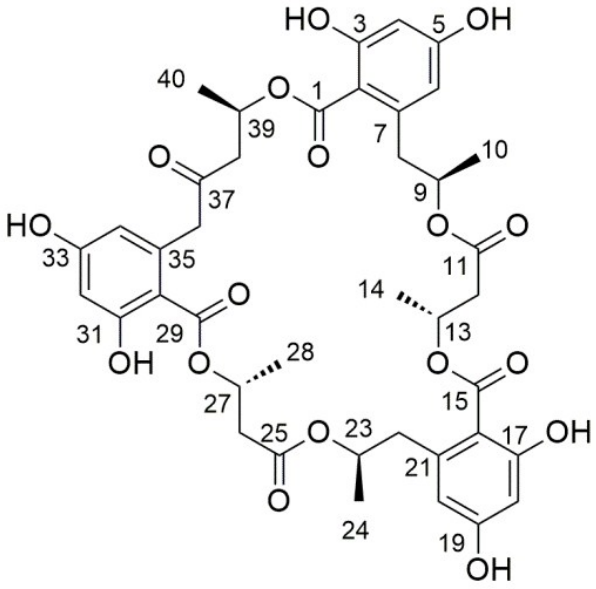
(-)-6-hydroxymellein **(2)**

^{1}H NMR (400 MHz, CD_3COCD_3): δ 11.28 (s, 1H), 6.29 (br, 1H), 6.26 (d, J = 1.9 Hz, 1H), 4.69 (m, 1H), 2.81-2.97 (m, 2H), 1.45 (d, J = 6.3 Hz, 3H).

^{13}C NMR (100 MHz, CD_3COCD_3): δ 170.8, 165.5, 165.4, 143.3, 107.6, 102.0, 101.7, 76.4, 35.1, 20.9.

ESITOFMS *m/z* calcd for $C_{10}H_{10}O_4Na$ $[M+Na]^+$ = 217.0471, found = 217.0471

Both ^{1}H and ^{13}C NMR Data and optical activity are in agreement with those reported in the literature.[3]



Compound **(3)** was identified as ascotrichalactone A and NMR data in acetone-d_6 are shown in the Table S2 and Figure S1 and S2. This was also confirmed when NMR data of compound **(3)** were collected in DMSO-d_6 and data are in accordance with those reported in the literature.[2]

ESITOFMS *m/z* calcd for $C_{40}H_{44}O_{17}Na$ $[M+Na]^+$ = 819.2476, found = 819.2472

Table S2: NMR data for compound **(3)**, ascotrichalactone A, in acetone-d_6:

Position	δ_C (ppm)	δ_H (ppm)
2,4-dihydroxy-6-(2-hydroxy-*n*-propyl)benzoic acid		
1, 15	171.4, 171.3	
2, 16	106.3, 106.2	
3, 17	166.0, 165.9	
4, 18	102.8, 102.7	6.28 (d, *J* = 2.4 Hz, 1H), 6.24 (d, *J* = 2.7 Hz, 1H)
5, 19	163.2, 163.3	
6, 20	113.1, 112.4	6.36 (d, *J* = 2.4 Hz, 1H), 6.33 (d, *J* = 2.4 Hz, 1H)
7, 21	143.3, 143.6	
8, 22	42.0, 41.7	3.58 (dd, *J* = 6.9, 3.3 Hz, 1H-8a), 2.93 (dd, *J* = 6.8, 12.3 Hz, 1H-8b) 3.55 (dd, *J* = 7.6, 4.7 Hz, 1H-22a), 2.96 (dd, *J* = 13.6, 7.2 Hz, 1H-22b)
9, 23	72.8, 72.7	5.15-5.24 (m, 2H)
10, 24	19.9, 20.1	1.21 (d, *J* = 6.2 Hz, 3H), 1.22 (d, *J* = 6.2 Hz, 3H)
3- hydroxybutyric acid		
11, 25	170.4, 170.4	
12, 26	41.4, 41.0	2.94 (dd, *J* = 15.8, 7.0 Hz, 1H-12a), 2.82 (dd, *J* = 15.8, 6.3 Hz, 1H-12b) 2.76 (br d, *J* = 6.3 Hz, 2H)
13, 27	70.1, 70.1	5.56 (m, 1H), 5.45 (m, 1H)
14, 28	20.2, 19.8	1.43 (d, *J* = 6.3 Hz, 3H), 1.34 (d, *J* = 6.4 Hz, 3H)
2,4-dihydroxy-6-(4-hydroxy-2-oxo-*n*-pentyl)benzoic acid		
29	171.1	
30	105.9	
31	166.4	
32	102.9	6.29 (d, *J* = 2.4 Hz, 1H)
33	163.5	
34	113.9	6.24 (d, *J* = 2.8 Hz, 1H)
35	140.5	
36	51.5	4.25 (d, *J* = 17.8 Hz, 1H-36a), 3.94 (d, *J* = 17.8, 1H-36b)
37	204.7	
38	48.1	3.29 (dd, *J* = 17.3, 5.2 Hz, 1H-38a), 3.03 (dd, *J* = 17.3, 7.8 Hz, 1H-38b)
39	69.9	5.58-5.65 (m, 1H)
40	20.7	1.47 (d, *J* = 6.2 Hz, 3H)
OH		11.49 (br), 11.41 (br), 11.29 (br), 9.29 (br)

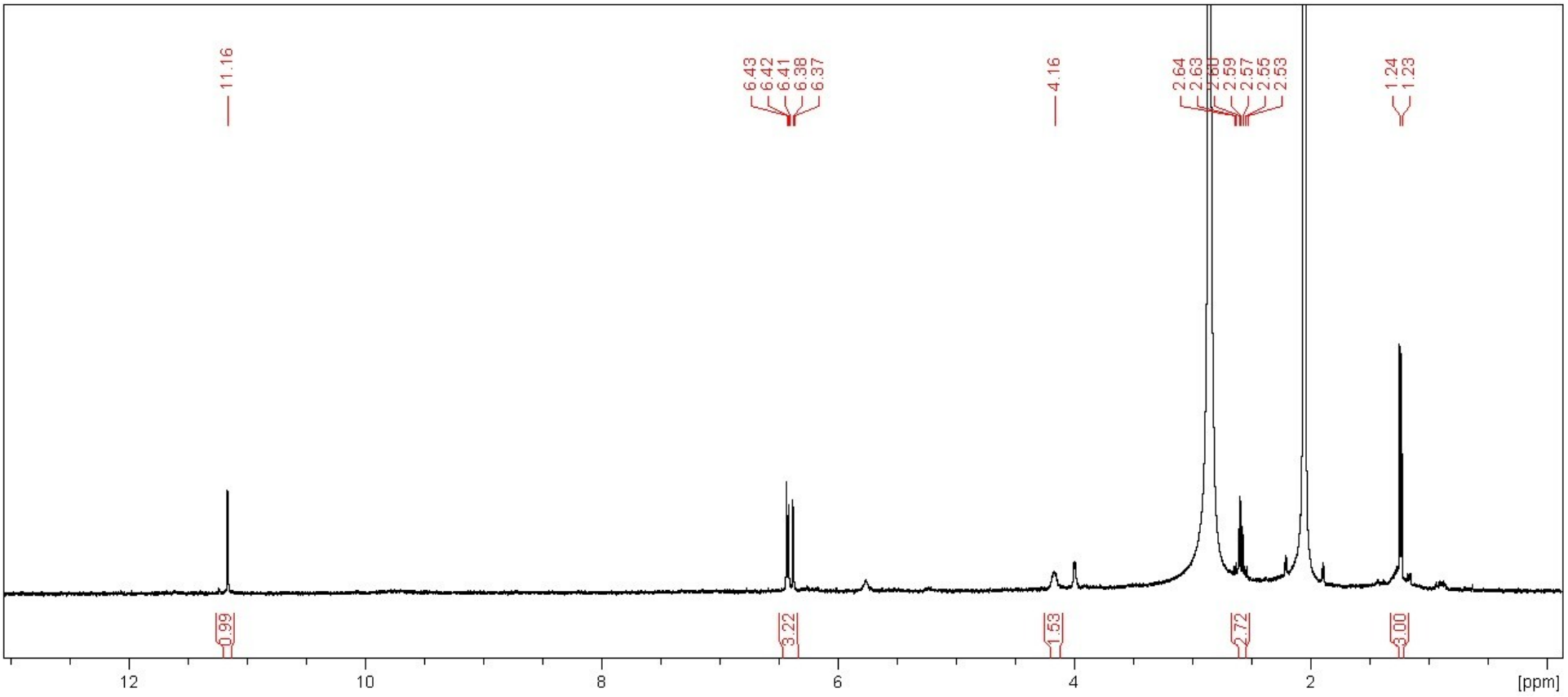


Fig. S5: ^{1}H-NMR spectrum of compound **(1)**, (-)-orthosporin, in acetone-d_6

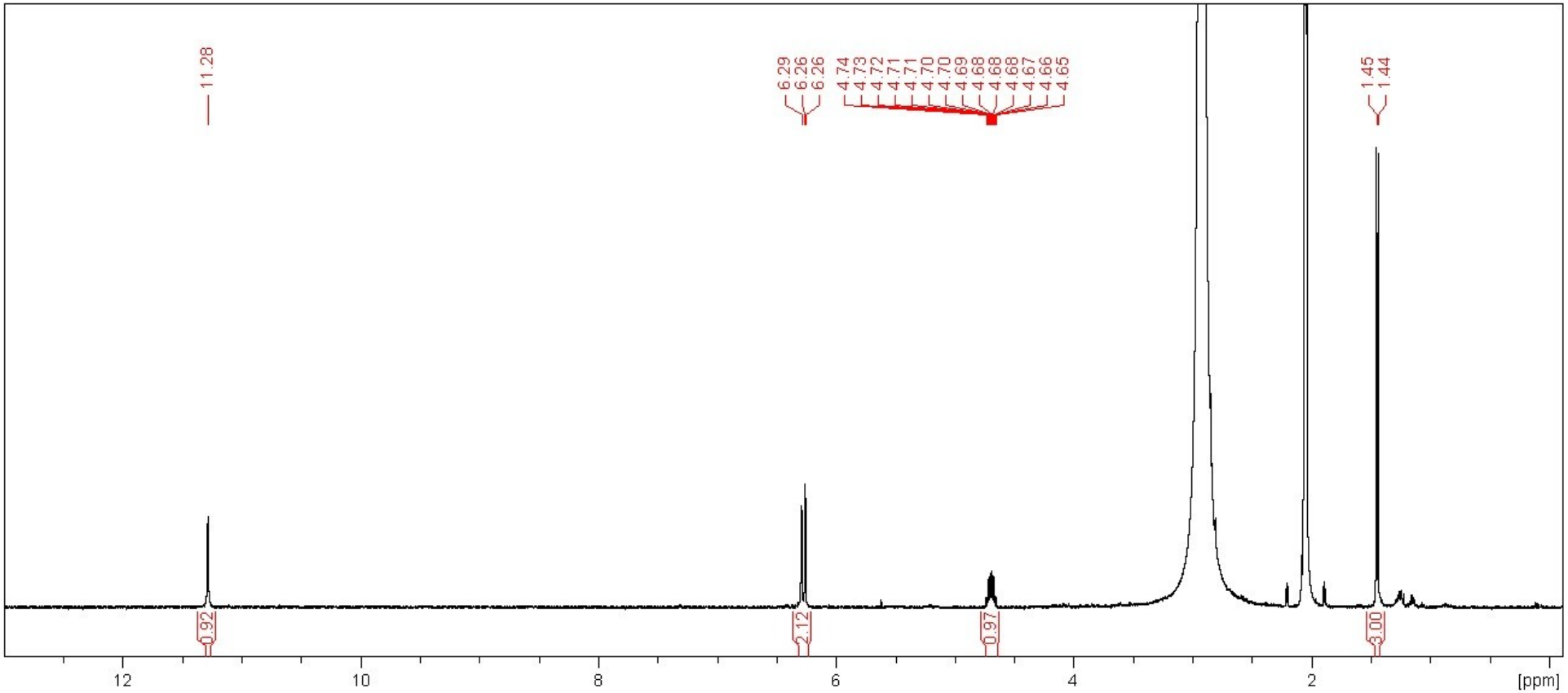


Fig. S6: ^{1}H-NMR spectrum of compound **(2)**, (-)-6-hydroxymellein, in acetone-d_6

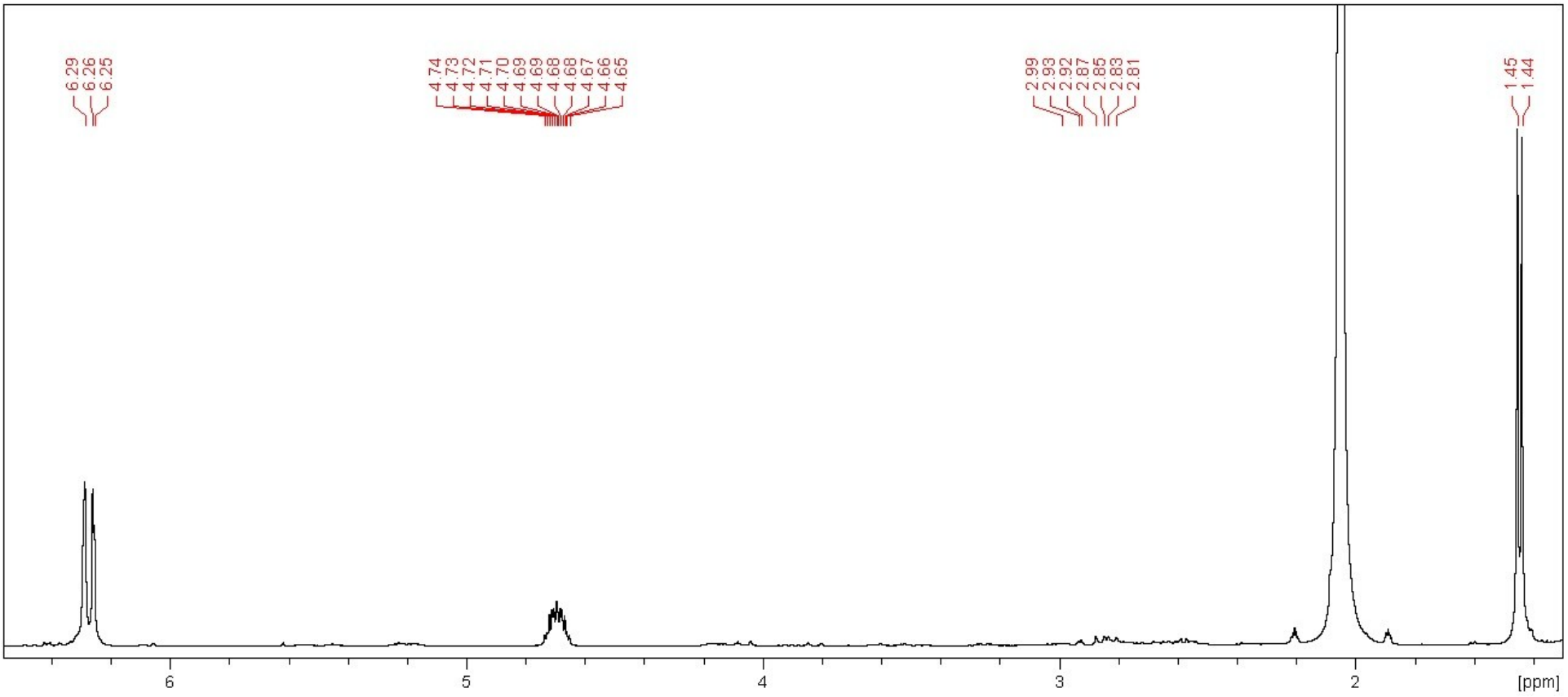


Fig. S7: ^{1}H-NMR spectrum with water suppression of compound **(2)**, (-)-6-hydroxymellein, in acetone-d_6

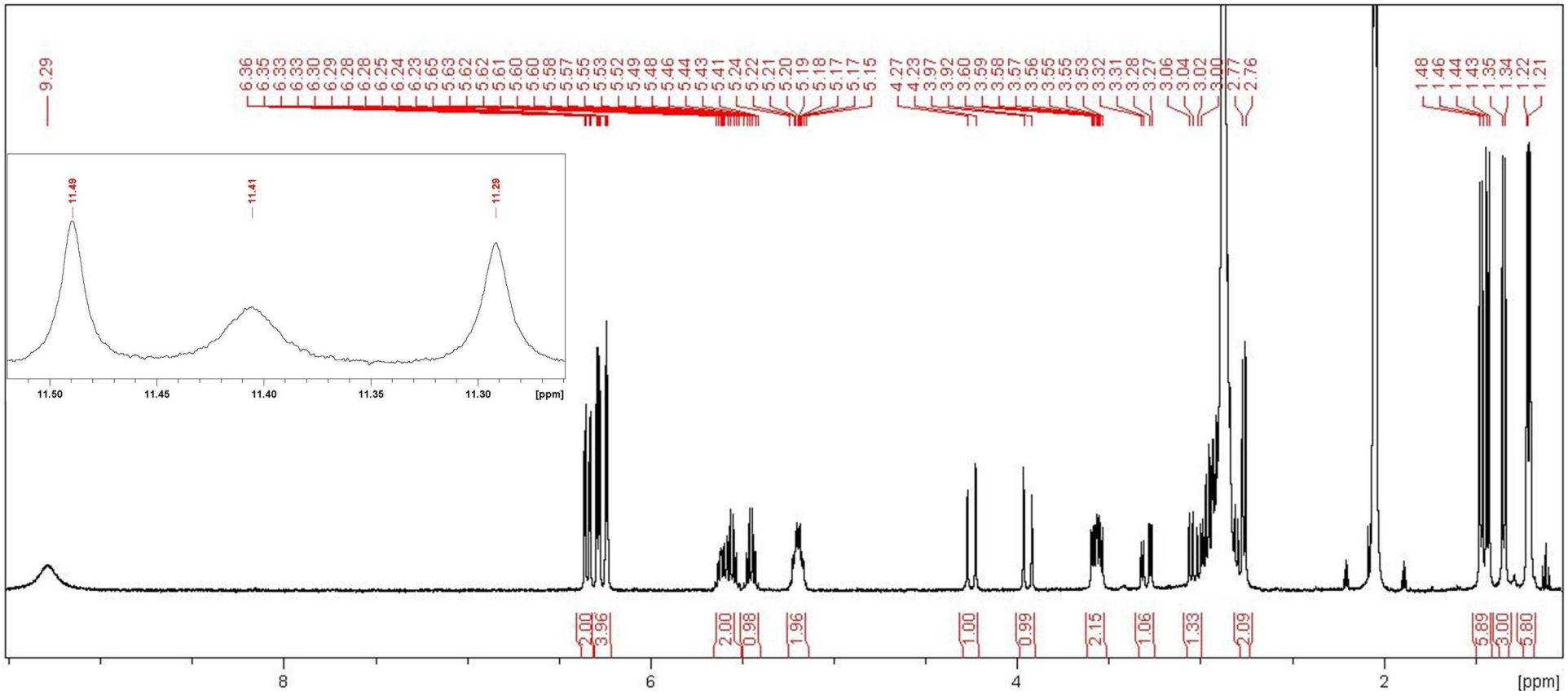


Fig. S8: ^{1}H-NMR spectrum of compound **(3)**, ascotrichalactone A, in acetone-d_6

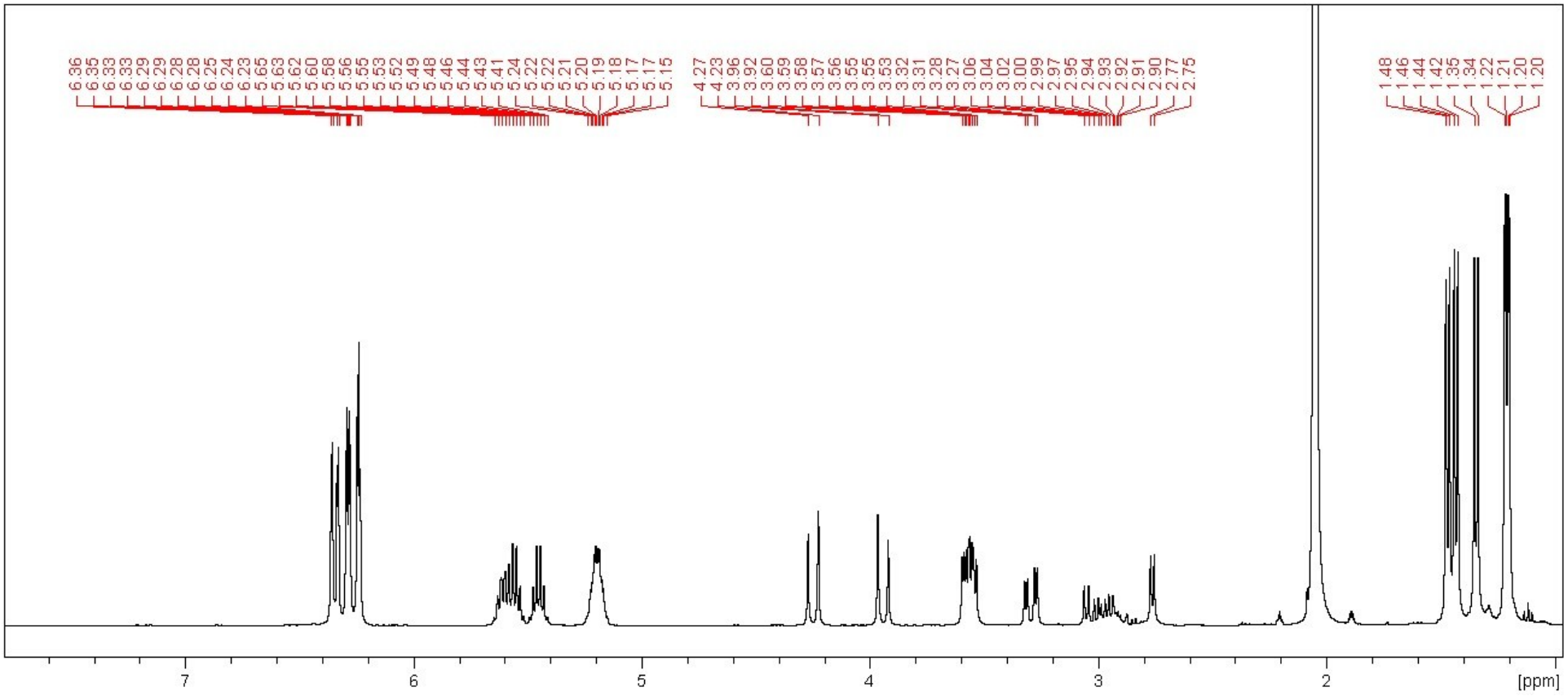


Fig. S9: ^{1}H-NMR spectrum with water suppression of compound **(3)**, ascotrichalactone A, in acetone-d_6

References

1. Y. F. Hallock, J. Clardy, D. S. Kenfield and G. Strobel, *Phytochemistry*, 1988, **27**, 3123-3125.
2. J.-L. Zhang, W.-J. Wang, X.-M. Xu, D.-Y. Li, H.-M. Hua, E.-L. Ma and Z.-L. Li, *Tetrahedron*, 2016, **72**, 4895-4901.
3. M. S. Islam, K. Ishigami and H. Watanabe, *Tetrahedron*, 2007, **63**, 1074-1079.