

Applied Microbiology and Biotechnology

Supplementary Information

Discovery and biocatalytic characterization of opine dehydrogenases by metagenome mining

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Table S1 - Protein sequences used as templates for metagenome search

<i>UniprotID</i>	<i>Name</i>	<i>Organism</i>	<i>Sequence</i>
Q44297	Opine dehydrogenase (N-(1-D-carboxyethyl)-L-norvaline dehydrogenase)	<i>Arthrobacter</i> sp. (strain 1C)	MIESKTYAVLGLGNGGHAFAYLALKGQSVLAWDIDAQR IKEIQDRGAIIAEGPGLAGTAHPDLLTSDIGLAVKDADVILI VVPAlHHASIAANIASYISEGQLIILNPGATGGALEFRKILR ENGAPEVTIGETSSMLFTCRSERPGQVTVNAIKGAMDFAC LPAAKAGWALEQIGSVLPQYVAVENVLHTSLTNVNAV MH PLPTLLNAARCESGTPFQYYLEGITPSVGLAEKVDAERIA IAKAFDLNVPSVCEWYKESYGQSPATIEAVQGNPAYRGI AGPINLNTRYFFEDVSTGLVPLSELGRAVNVPTPLIDAVLD LISSLIDTDFRKEGRTLEKLGLSGLTAAGIRSAVE
Q9BHM6	Octopine dehydrogenase (OcDH)	<i>Pecten maximus</i> (King scallop) (Pilgrim's clam)	MTVKVCVCVGGGNGAHTLSGLAASRDGVEVRVLTFLFADE AERWTKALGADELTVIVNEKDGQTQTEVKS RPKVITKDPEI AISGADVILTVPAFAHEGYFQAMAPYVQDSALIVGLPSQ AGFEFQCRDILGDKAAAVSMMSFETLPWACRIKEFGRKV EVLGTSVLAASLIKGTAKTV DPLSTLQMLHGAEPVFRL AKHFLEMLIMSYSFVHPAILFGRWGSWDGKPVPEAPLFY QGIDQATADMLTACSNECKDVANAIMAACPGNDLSDVKD IYQWYLEYYHEDIQDDHDLYHAITTNKSYKGLVHPVKAV DGGVAPDFGNRYLTEDIPMG MIVFKGVAIAAGVAIPSN DK LIMWAQEKIGKEYLVDGALTGKDVATTRCPQRYGFNTLD AILTGKK
Q9HUX5	Pseudopaline synthase (Opine dehydrogenase) (ODH) (Pseudopaline dehydrogenase)	<i>Pseudomonas aeruginosa</i>	MNAADESLGNVLLVGLGAVAIQVALDLRRHGAGRLGAL NHPGRRSQRIAEALARGACLQLEGQGQHRWLSGNAALD VFHQDPAELRDDWQTLVLCVPADSYLDVVRGLPWERLG GVRTLLLVSAFIGANLLVRSALPAGCQATVLSLSSYYAATK VIDETQPLRALTKAVKRRVYL GSSRPDCPARETWRRVLAG SGVEVPLATPEAAEGRNVTTYVHSPFFLGEFALARILSE QGPPGFMYKLYPEGPITPGAIGAMRRLWCELS ELLRRMG AEPLNLLRFLNDDNYPVHETMLPRASIDGFAEAGAERQE YLLFVRYAALLVDPFSPADEQGRHFDFSAVPFRRVSRDED GLWRLPRVPLEDYRKLALIVALAAHFDLAMPQARSLLAS YENAVSRFIDCQGASQCHPSLYPIDS RPAADAIYRQWCST C
A0A0H3JT80	Staphylopine synthase (Opine synthase)	<i>Staphylococcus aureus</i>	MSKLLMIGTG PVAIQLANICYLKSDYEIDMVGRASTSEKS KRLYQAYKKEKQFEVKIQNEAHQHLEGKF EFNRLYKDVK NVKGEYETVVMAC TADAYYDTLQQLSLET LQSVKHVILI

	dehydrogenase) (ODH) (Staphylopine dehydrogenase)		SPTFGSQMIVEQFMSKFSQDIEVISFSTYLGDRIVDK EAPNHVLTGTVKKKLYMGSTHSNSTMCQRISALAEQLKIQLE VVESPLHAETRNSSLYVHPPLFMNDFSLKAIFEGTDVPVY VYKLFPEGPITMTLIREMRLMWKEMMAILQAFRVPSVNL LQFMVKENYPVRPETLDEGDIEHFEILPDILQEYLLYVRYT AILIDPFSQPDENGHYFDFSAVPFKQVYKNEQDVVQIPRM PSEDYYRTAMIQHIGKMLGIKTPMIDQFLTRYEASCQAYK DMHQDQQLSSQFNTNLFEGDKALVTKFLEINRTL
Q8CKU7	Yersinopine synthase (Opine dehydrogenase) (ODH) (Yersinopine dehydrogenase)	<i>Yersinia pestis</i>	MHNTLPTLILGAGPAAIQLAVDISATGDARLGLYNRPSTK GERLKQYLALTPTLYLQGTGKAQATQKESVTIDCYIDQL AQAVGDWQRLILAVPADHYAVLQQIPWAALPQLKSVILL SSSMGSGLMVQNLNAAGKRDVEVISLSSYYADTKYIRA ETQDISANTQDINAGTQDIGAIQPYRAYTKAFKQRIYLAN QWGNAGSAEMSWLTAVLARHHIDTLPCSNLLAERFSIT NYVHPPALADTTLQALFYPEQRSQYLYKTQPEGVPCPAV IADLAGLADDYKRLLNRLGVEEINLLRFLNDDNYPVPAS MVSRRWIDFEPQLPPLEQQYALFVRYTALLVDPYSTPDEQ GRFYDFS AVKVATVYQDANALWHLPRVPLEDVHKLRTLL LLAGALDVVMPTAQRLLRQFQQALKAFIDRVGEEHCHPS LLGDDCDRQAAIEQQWRSQT
M1FK93	D-octopine dehydrogenase	<i>Mytilus galloprovincialis</i> (Mediterranean mussel)	MSEKCRILVCGGGNGAHCLSALAASKDNLDVHVLTLYQD EAERWAKHVENENMKLTITKQDGTQYDTFSKPSLITKDA AKAMSGVEIVFLVVPFAHAQYFTAIPHLQPNTLIVGLP GQAGFELQCRHIIGDKASSCTIAASESLPACRIVEFGRHA RILGLKDSLGM SALKGKACQLSFPVVETVQGILGEYPKLE LLKNYIAINLMADANVHPPMMYGRWGNWDGKPLKEEPL FYQGVD DRQADLLSRVSEELLAAKAIEQKRKDVDMSE VIHLFDWYKIHYPDQITDKSSLKMAMRTNKAYDGLVHPM VKTDEGYVPNFNYRYTSEDVPFGMVVMKGIADLAGVPT PAMDEILAWGQQKLGKEYIVGSKLIGKDIGLARAPQSFG MTSVDELFDI
B5D5P2	Alanopine dehydrogenase	<i>Arenicola marina</i> (Lugworm) (Lumbricus marinus)	MGDSITTRLLICGGGNGAHAYAGIASSKKNVEVRVLTLY SDEAERWTNAMENHDFVNFDCGGKHNGEIRAKPSMVS KDPAEVALGCNVIVVVPFAHEQYLRAL EPIYQPGTHIVG MPGQAGFEFACWGILGAKGKQCTLSFESLPWACRIEEFG RTAEVKGTQQLVGALLYGDP RPVREPSAVLQETLGTHP VLHIHGHLLGMTLMGVNAYIHPAILYGRWWNWDGRPVA

			EPPLFYHGMDEFTADTLTAMGDEVMAIAATLSKHPAGPD FSNVCHVFDWYKRCYSDEMSDTSCLLKAIRSNKAYIGLT HPCRPAEAAGDNNGGCNWEPNFGYRYITEDIPFGLVVL GVAQIIGVATPHMDKVVLWSQKVSQKEYLVDSTLSGRDM PDTRSPQKYGLTTIDQILGYD
A0A9I9	Strombine/ alanopine dehydrogenase	<i>Marphysa sanguinea</i> (Polychaete worm) (Nereis sanguinea)	MVVLVICGGGNGAHVLAGIASSNPADVRVLTLYADEAE RWTKAMEGNDFFVTNNQDKSQTLKNKPRLVTKDPAL AANKADMIVFTVPAFAHRQYLDALKPHIRPGTVLVGLPG QSGFEFEVWTAWGDLAKQCSIMSFESLPWACRFTEFGRA ATVIGTKENLAGAAWYGSVPPKADPTLVLGCLGPHPV LTRGALLGITLMATNGYIHPSILFGRWHKWDGNPVNEPPL FYNGLDEFSAQTMSSVSDEILAVASSLMKQRPQVDLTNVA HIWQWYLRVYADDIGDKTSLFTTIRTNAAYSGLTHPTTKT DDGKFVPDFKHRYLMEDIPFGLLVSKGIAEVAGVPTPTIDS VISWAQQKMKEYIVNGKLAGKDVSSSTRCPQRYGLTTVD AILGL
A0A9J0	Alanopine dehydrogenase	<i>Marphysa sanguinea</i> (Polychaete worm) (Nereis sanguinea)	MVVVVVCGGGNGAHTLAGVAASRPNTVVRVLTIFADEA ERWTKAMESSDFVTNIHNHDKSITKLTNKPRLVTKDPGK AAIGAEIIILAVPAFVHAQYLEALKPHVTPGTILVGLPGQPG FEFDVYTIWGPLASKCSIMAFESLPWACRIAEFGQCVEILG TKESLVGAVHIGSVPSKFDPTLLQGLGPHPSLLTKGHLL GITLMSVNSYIHSSILYGRWHDWNGTPLDEAPLFYQGIDQ DTADLMSGMSDEILVIAKEIMKKNPKVDLHNVDHVLQW YLRVYEDDIQDKSNLYTAIRSNEAYIGLTHPMSKTEDGKY LPNFKHRYLSEDIPYGLAVSKGVAEVLGVNTPLIDKVLIW CQEKLGKEYLVDGKMIGKDVTFTRCPQRYSMTTVDAILG K
P0A395	Protein ocs; D- octopine dehydrogenase (Octopine synthase)	<i>Agrobacterium tumefaciens</i> (strain 15955)	MAKVAILGAGNVALTLAGDLARRLGQVSSIWAPISNRNSF NSVRSLSLELVGPDYGGDFQPQLEDDLETAISGAAFIFLT VPTMGQQGILCELANFNLSSSVLVALPGSATSLACKQTLTP AFAPIAVIEATTSPYACRRVNAQVLMLSVKRTFEVASTQAL SEEVRRGGFEILFPNRLQWYQNPASIFFSNTNPVAHPAGILA AKDTIEQGISPFPKFKYRKFVPQAITRVTAIDEERLTIVNALG LESETDFAYCKKWWYGGHASNAREFYETFEGYADIETPRN MNHRYLSESVKHILVLWVEIAEVIGVQVPEMKSVVQEAS DVLNEDLSHTGRGLSSLNLEGSNANAIVRALNGV

Table S2 – Full length ODH protein sequences extracted from the Sativali hot spring metagenome and their synthetic nucleotide sequences.

Sequences that were not expressed due to the lack of a consensus cofactor binding sequence are labelled orange. Sequences are labelled yellow, where the expressed enzyme was not soluble. Sequences are labelled green, where the soluble expression allowed detailed characterization of the enzyme.

>mODH-43 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063522) Closest homologue in UniProt: A0A7X9EIT5 (99%)
MAGHLAIMGFDVKLYNRSEERLRAVKMEGGIDLTGEVTGFGKVQMATSNPPEEAVADVIMIVVPATAHKNLAKIFAPYLKSGQ IVILNPGRTFGALEFSQILKNEGASEDITVGETQTFLYASRVTPGPQAKIFRIKNSVPLATVRAFKIPQTLVIRTAFFPHFVSGDSIFKT SFDNIGCVFHPSLVVLNAGWIEDPSDFEFYFQGTTLSTSKILEQLDKERLSVASALGFRAMSAREWLYAYDVSGKTLYEAIHNSP GYRGIMAPHNTNMRYITEDVPCSLVPMSSIGKKFENVQTPLDISIIRIASAMHETDYYKLGRTVESLGIENMDLKQLRLIAIGEHPN ATGGCGGGTCACCTGGCGATCATGGGCTTTGACGTGAAGCTGTACAACCGTAGCGAGGAACGTCTGCGTGCGGTTAAAATG GAGGGTGGCATTGATCTGACCGGTGAAGTGACCGGTTTCGGCAAGGTTTCAGATGGCGACCAGCAACCCGGAGGAAGCGGT GGCGGATGTGGATGTTATCATGATTGTGGTTCGGCGACCGCGCACAAAGAACCTGGCGAAAATCTTTGCGCCGTACCTGAAG AGCGGCCAGATCGTTATTCTGAACCCGGGTCGTACCTTTGGCGCGCTGGAGTTCAGCCAAATCTGAAAAACGAGGGTGGC AGCGAAGACATTACCGTGGGCGAAACCCAGACCTTCCTGTATGCGAGCCGTGTTACCGGTCCGGGTCAAGCGAAGATCTTT CGTATTAACAGCGTGCCGCTGGCGACCGTTCGTGCGTTCAAGATCCCGCAAACCTGAGCGTGATTCTGACCGCGTTTC CGCACTTCGTTAGCGGTGACAGCATCTTTAAACAGCTTCGATAACATTGGCTGCGTGTTTACCCGAGCCTGGTGGTTCT GAACGCGGGTTGGATCGAGGACCCGAGCGATTTCGAATTTATTTCAGGGCACCACCTGAGCACCAGCAAGATTCTGGA GCAACTGGACAAAGAAGCTCTGAGCGTGGCGAGCGCGCTGGGTTTCCGTGCGATGAGCGCGCGTGAGTGGCTGTACTATGC GTACGATGTTAGCGGCAAAACCTGTATGAAGCGATCCACAGCAACCCGGGTTACCGTGGCATTATGGCGCCGCACAACAC CAACATGCGTTATATCACCGAGGACGTGCCGTGCAGCCTGGTTCGATGAGCAGCATTGGTAAGAAATTTAACGTGCAGACC CCGCTGATCGACAGCATCATTCGTATTGCGAGCGCGATGCACGAGACCGATTACTATAAGCTGGGTCTGACCGTTGAGAGCC TGGGCATCGAAAACATGGATCTGAAACAACCTGCGTCTGATCGCGATTGGTGAACACCCGAACATA
>mODH-44 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063523) Closest homologue in UniProt: A0A1F9ZJZ8 (83%)
MGLGHKKSRRKERVPTFAVLGAGHGLAMAGHLALMGFPTRIWNRSRERIESVQDRGGIDVEGIVDGVGRVDIATSDMGEALDG ADVVMVVVPASGHRYVAERAAPHLKSNQIVVLNPGRTFGALEFLQVLKEQKTKTKPIISEAQTFLYVSRHSEPARARIQIKNSVP LAAIPAHKTPEVLEEVRKAFFQFVAASNVLETSLDNIGAIFHPGLTILNAGRIESTHGDFFEYYLEGVSPSTAKILEADAERVAIGSA LGIHLHTAREWLYLAYDSPGKTLYEAIQATPGYKGVRAATLTHRYLLEDVPMMSLVPMVSVGQQIGVKPTLAALHLASTFHSR DFWAEGRTERVGLKGKSVKDIRLLAVRGISR ATGGGTCTGGGCCACAAGAAAAGCCGTAAAGAGCGTGTTCCGACCTTTGCGGTTCTGGGTGCGGGTCATGGTGGCCTGGCG ATGGCGGGTCACCTGGCGCTGATGGGCTTTCCGACCCGTATCTGGAACCGTAGCCGTGAGCGTATTGAAAGCGTGCAGGAC CGTGGTGGCATCGATGTTGAGGGTATTGTTGACGGTGTGGGCCGTGTTGACATCGCGACCAGCGATATGGGTGAAGCGCTGG ACGCGCGGATGTGGTTATGGTGGTTGTTCCGGCGAGCGGTACCGTTATGTGGCGAACGTGCGGCGCCGCACCTGAAAA GCAACCAAATTGTTGTGCTGAACCCGGGTCGTACCTTCGGCGCGCTGGAGTTTCTGCAGGTTCTGAAGGAACAAAAGACCA

AAACCAAGCCGATCATTAGCGAGGCGCAGACCTTCCTGTATGTTAGCCGTCACAGCGAACCGGCGCGTGCGCGTATCATTC AATCAAAAACAGCGTTCGCTGGCGGCGATTCCGGCGCACAAAACCCCGGAAGTGCTGGAGGAAGTTCGTAAGGCGTTCC CGCAGTTTGTGGCGGCGAGCAACGTTCTGGAAACCAGCCTGGATAACATCGGTGCGATTTTTCATCCGGGTCTGACCATCCT GAACGCGGGTCTGATTGAGAGCACCCACGGCGACTTTGAGTACTATCTGGAAGGTGTGAGCCCGAGCACCGCGAAGATCCT GGAGGCGGCGGATGCGGAACGTGTTGCGATCGGTAGCGCGCTGGGCATTACCTGCACACCGCGCGTGAGTGGCTGTACCT GGCGTATGACAGCCCGGGTAAAACCCTGTACGAAGCGATCCAAGCGACCCCGGGTTATAAGGGCGTGCGTGCGCCGGCGAC CCTGACCCACCGTTACCTGCTGGAAGATGTCCGATGAGCCTGGTTCGATGGTTAGCGTGGGTGAGCAAATCGGCGTTAA ACCCGACCCCTGGCGGCGCTGATTACCTGGCGAGCACCTTCCACAGCCGTGACTTTTGGGCGGAGGGTCTGACCGTGGAA CGTGTGGTCTGAAAGCAAGAGCGTGAAGGATATCCGTCTGCTGGCGGTTCTGTCGATTAGCCGTAA
>mODH-45 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063524) Closest homologue in UniProt: E8MYZ6 (93%)
MTQTLSTIVIGAGHGGKAMAAHLALMGHRVKLYNRTAERIYAIQQRGGIDLESQEFGRGFGKLECATSNIEEALKGSQIMV PSSAHADIARAAPYLQDGQVVLHPGRTCGAIEFTVMRRSGCQADVIVAEAEETFIYASRSDGPAQARIFRIKEAVPLAALPAK RTREALELVNIVYPQFIDGQVNLQTLNMGAIHFHALLNAGWIEATHGDYQFYIDGVTPSVARVLEALDRERITVAASLGIRA RSALWLMAYDAVGNLREAIHNQPGYYGIKAPTTLNHRYIFEDVPMSLVPIASLGMQYGVSVRGMDSIIRLACIVHNTDYWR RGRTVENLGLRGLSVEELTHFVMEGELAV
ATGACCCAGACCCTGAGCATCACCGTTATTGGTGCGGGTCATGGTGCGAAAGCGATGGCGGCGCACCTGGCGCTGATGGGT CACCGTGTGAAACTGTACAACCGTACCGCGGAGCGTATCTATGCGATTAGCAACGTGGTGGCATCGACCTGGAGAGCCAG GAATTCGGTCCGCGTGGTTTTGGCAAGCTGGAATGCGCGACCAGCAACATCGAGGAAGCGCTGAAAGGCAGCCAACTGAT TATGGTGGTTGTGCCGAGCAGCGCGCATGCGGACATTGCGCGTGCGGCGGCGCCGTACCTGCAGGATGGCCAAAGTTGTTGT TCTGCACCCGGGTCTGACCTGCGGTGCGATTGAGTTCACCATGGTTATGCGTCGTAGCGGTTGCCAAGCGGATGTGATTGTT GCGGAGGCGGAAACCTTCATTATGCGAGCCGTAGCGATGGTCCGGCGCAAGCGCGTATCTTCGTATTAAGGAAGCGGTTCT CGCTGGCGGCGCTGCCGCGGAAACGTACCCGTGAGGCGCTGGAACGGTGAACATCGTTTATCCGAGTTCATTGACGGTG GCAACGTGCTGCAAACCGGTCTGAACAACATGGGCGCGATCTTTCATCCGGCGCTGACCCTGCTGAACGCGGGTTGGATTG AGGCGACCCATGGTGACTIONACCAATTTTATATTGATGGTGTACCCCGAGCGTGGCGCGTGTCTGGAGGCGCTGGATCGTGA ACGTATACCGTGGCGGCGAGCCTGGGTATTCTGCGCGTAGCGCGCTGGAGTGGCTGAAGATGGCGTACGACGCGGTGGG CAACGATCTGCGTGAAGCGATCCACAACCAGCCGGGTACTATGGCATTAAAGCGCCGACCACCCTGAACCACCGTTACATC TTCGAGGATGTGCCGATGAGCCTGGTTCCGATTGCGAGCCTGGGTATGCAATATGGCGTGAGCGTTCTGTTGATGGACAGCA TCATTCTGTGCGGTGCATCGTTACACAACACCGATTATTGGCGTCTGGCCGTACCGTGGAAAACCTGGGTCTGCGTGGCCT GAGCGTGGAGGAAGTACCCACTTTGTTATGGAGGGTGAACGGCGGTGTAA
>mODH-47 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063525) Closest homologue in UniProt: A0A7C7RQQ9 (80%)
MSKSDIRFTVIGAGHGGKMAAHLALMGFPVTLNRTPDNIAAIKARGGIELKSFEGGPHGFAMLAATSDIEEALKECDVIMV VVPSTAHAEIARSLAPHLKDGQIIVLNPGRTCGALEVKHLEENGCSADVTVAEETLIYASRSDGPAQARIFGIKETVPLAALPAT RTQLVLDTLAPAYPQFIDGGSVLHTGLNMGAIHFHALLNAGRIESTQGEFQFYIDGVSPSVARVLEVLDRERVTVASALGIRA RTALEWLQMAHYHATGNDLYEAIHNQPGYYGIQAPPTLNHRYITEDVPMSLVPIAALGERYGVSVRGMESIIRLACIIHRTDYWRR GRTLEKLGIDWSVSELTKEYVMEGMV

ATGAGCAAGAGCGACATCCGTTTCACCGTGATTGGTGCGGGTCATGGTGGCAAGGGTATGGCGGCGCACCTGGCGCTGATG
GGTTTTCCGGTTACCCTGTACAACCGTACCCCGGATAACATCGCGGCGGATTAAGGCGCGTGGTGGCATCGAGCTGAAAAGCT
TCGAAGGTGGCCCGCACGGTTTTGCGATGCTGGCGAAGGCGACCAGCGACATCGAGGAAGCGCTGAAAGAGTGCATGTG
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GTGGCGGAGGCGGAAACCCTGATCTATGCGAGCCGTAGCGATGGTCCGGCGCAAGCGCGTATCTTCGGCATTAAAGGAAACC
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GTGAACGTGTTACCGTGGCGAGCGCGCTGGGTATCCGTGCGCGTACCGCGCTGGAGTGGCTGCAGATGGCGTACCACGCGA
CCGGCAACGACCTGTATGAAGCGATCCACAACCAGCCGGGTTACTATGGCATTCAAGCTCCGCCGACCCTGAACCACCGTT
ACATCACCGAGGATGTGCCGATGAGCCTGGTTCCGATTGCGGCGCTGGGCGAGCGTTATGGCGTTAGCGTGCGTGGTATGGA
AAGCATCATCTGCTGGCGTGCATCATCACCGTACCGACTACTGGCGTGTGGTGTGATCCCTGGAAAAGCTGGGCATCCAC
GATTGGAGCGTTAGCGAGCTGACCAAATATGTGATGGAAGGCATGGTTTAA

>mODH-48 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063526)

Closest homologue in UniProt: A0A7X7W637 (100%)

MKIAVIGAGNGGQALAAVLAMRGNEVALYNRSQSRIQPILSKKLKVEGESGGTARLKYVGTDMEKAVSDAELIMVVVPAFAH
AEVARKLSKFISEGQIILNPGRGTGGALEFDKILKECGVRNKILAEAQTFLFASRISGPMVRIFRIKNAVPSVLPKDNELLMPV
IGDVIPEFTLADNVLYTSFNNIGAIFHPGAIIMNAGWIESTHGDFQFYLDGISPAVARVLEEVDRECEVTSKLG VQAMGAREWLD
YAYDARGEDLYNAIHNNEG YRGIMAPISMENRYIIEDVPMSLVPMSCFGKKLGVDTKTMDSVINIAGAIMGKDFWKEGRNIERL
GVKDMSVEDLWHYVEKGDGDG

ATGAAGATTGCGGTGATCGGTGCGGGTAACGGTGGCCAGGCGCTGGCGGCGGTGCTGGCGATGCGTGGTAACGAGGTTGCG
CTGTACAACCGTAGCCAGAGCCGTATTCAACCGATCCTGAAGAGCAAGAACTGAAGGTGGAGGGTGAAAGCGGTGGCAC
CGCGCGTCTGAAGTATGTGGGTACCGACATGGAGAAAGCGGTTAGCGATGCGGAACTGATTATGGTGGTTGTTCCGGCGTTT
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TCAACAACATTGGCGCGATCTTTCACCCGGGTGCGATTATCATGAACGCGGGCTGGATTGAGAGCACCCACGGTGACTTCCA
GTTTTATCTGGATGGTATTAGCCCGGCGGTGGCGCGTGTCTGGAGGAAGTTGACCGTGAGCGTTGCGAAGTGACCAGCAA
GCTGGGCGTTCAAGCGATGGGTGCGCGTGAATGGCTGGACTACGCGTATGATGCGCGTGGTGAGGACCTGTACAACGCGAT
CCACAACAACGAAGGCTATCGTGGTATTATGGCGCCGATCAGCATGGAGAACCCTTACATTATCGAAGATGTGCCGATGAGC
CTGGTTCGATGAGCTGCTTCGGCAAGAACTGGGTGTGGACACCAAAACCATGGATAGCGTTATTAACATCGCGGGCGCG
ATTATGGGCAAGGATTTTTGGAAAGAGGGCCGTAACATCGAACGTCTGGGTGTGAAGGACATGAGCGTTGAGGATCTGTGG
CACTATGTTGAAAAAGGTGACGGCGATGGTTAA

>mODH-49 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063527)

Closest homologue in UniProt: A0A533SSB6 (91%)

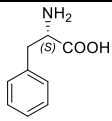
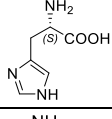
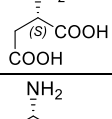
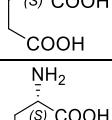
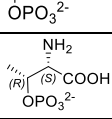
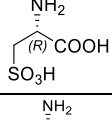
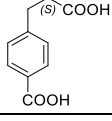
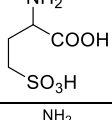
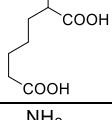
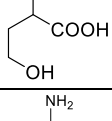
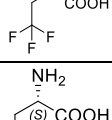
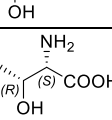
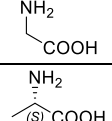
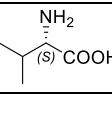
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>mODH-50 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063528) Closest homologue in UniProt: A0A973EU57 (100%)
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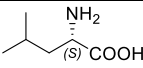
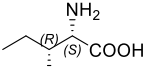
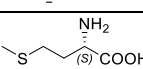
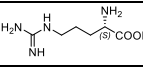
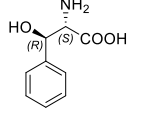
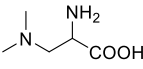
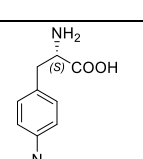
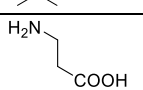
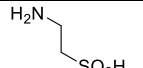
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>mODH-52 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063529) Closest homologue in UniProt: A0A6L9J0X1 (78%)
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>mODH-55 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063530) Closest homologue in UniProt: A0A7C4P2H9 (96%)
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>mODH-581 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063531) Closest homologue in UniProt: A0A937NJY1 (62%)
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>mODH-582 (Nucleotide sequence accession number in the TPA Section of the DDBJ/ENA/GenBank databases: BK063532) Closest homologue in UniProt: A0A7V4ZEF3 (94%)
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Table S3 - Conversion data with all amino acid substrates.^{a,b}

Name	Structure	<i>Ar</i> ODH	mODH-45	mODH-47	mODH-48	mODH-49	mODH-55	mODH-582
L-Phenylalanine (1)		89.9±0.4	6.1±2.6	4.0±1.6	2.7±2.3	8.8±2.0	4.4±3.6	7.2±2.1
L-Histidine (2)		37.8±3.0	3.0±5.6	11.4±4.3	17.8±4.5	26.1±3.5	21.2±5.1	28.6±3.5
L-Aspartate (3)		7.0±3.8	97.4±2.5	94.4±1.0	75.9±1.4	99.6±0.0	37.8±2.4	97.2±0.8
L-Glutamate (4)		5.8±0.9	35.0±11.7	57.2±9.5	2.1±1.2	47.7±1.8	54.0±3.3	96.4±0.6
phospho-L-serine (5)		0.0±2.9	17.9±4.5	13.4±4.2	10.7±2.8	15.4±2.3	5.7±2.9	0.8±5.5
phospho-L-threonine (6)		0.0±0.9	33.6±3.5	12.7±4.3	13.9±5.9	13.7±2.0	2.9±7.3	3.1±1.7
L-cysteic acid (7)		0.0±1.0	97.6±0.2	99.3±1.0	71.1±4.1	99.3±0.2	66.3±0.7	98.2±0.0
4-carboxy-L-phenylalanine (8)		0.9±1.9	9.3±5.8	5.6±2.1	37.2±4.2	0.7±2.9	0.6±4.0	0.0±1.2
Homocysteic acid (9)		0.0±3.9	41.0±2.1	41.2±1.4	41.0±1.5	40.6±1.5	33.5±4.9	39.4±1.7
2-aminopimelic acid (10)		0.0±1.3	43.6±1.0	43.8±1.4	42.7±0.9	42.3±2.6	34.3±3.1	29.8±1.7
Homoserine (11)		53.0±3.3	42.3±2.8	18.3±4.6	31.1±6.1	35.6±3.4	15.0±6.2	6.8±9.0
Trifluoro-homoalanine (12)		84.5±2.4	36.4±3.7	9.6±5.6	8.2±5.7	16.9±5.0	2.4±7.7	12.0±8.0
L-Serine (13)		99.3±0.0	12.3±9.2	5.0±6.0	1.6±2.5	3.5±3.0	9.7±10.3	10.8±12.5
L-Threonine (14)		100.0±0.0	14.6±10.3	4.2±2.7	4.9±3.5	3.3±1.0	16.5±4.7	11.6±2.6
Glycine (15)		100.0±0.0	23.9±4.1	20.2±7.0	41.0±3.6	30.2±2.3	8.4±3.7	15.6±1.5
L-Alanine (16)		100.0±0.0	15.6±3.3	3.5±3.7	18.9±4.1	13.8±3.1	9.0±1.1	6.4±0.9
L-Valine (17)		98.0±0.1	5.7±4.7	1.5±5.8	13.1±6.5	6.3±3.0	1.7±3.1	16.2±3.3

L-Leucine (18)		100.0±0.0	14.8±2.1	13.1±3.9	8.9±6.2	19.3±3.3	7.9±2.9	10.8±4.0
L-Isoleucine (19)		96.5±0.2	0.0±4.4	15.0±4.5	16.8±4.3	13.7±4.5	0.0±5.7	21.5±4.4
L-Methionine (20)		100.0±0.0	15.5±4.6	15.9±5.6	8.5±5.3	20.9±4.1	9.4±4.2	6.2±4.9
L-Arginine (21)		0.0±4.2	5.4±4.8	2.2±4.5	0.0±5.9	12.6±7.5	12.2±4.4	2.3±7.8
L-threo-phenylserine (22)		100.0±0.0	8.1±2.8	6.8±3.7	1.7±4.7	5.1±3.3	0.5±5.6	2.5±6.0
Dimethylamino-alanine (23)		4.8±19.7	19.3±13.5	4.9±22.2	0.0±13.8	14.4±12.2	12.1±10.9	0.0±19.1
Dimethylamino-phenylalanine (24)		46.1±1.6	7.6±3.8	6.0±5.1	5.2±6.9	4.9±3.6	6.4±2.1	0.0±2.0
β-alanine (25)		100.0±0.0	13.1±13.0 ^c	33.7±2.2 ^c	30.7±1.6 ^c	30.5±2.5 ^c	30.2±7.6 ^c	21.3±12.3 ^c
Taurine (26)		0.0±6.4	10.5±6.7	18.7±5.6	11.8±10.8	9.5±10.8	0.7±7.3	0.0±10.8

^a Standard errors are calculated according to the Gaussian error propagation rules: $D \sqrt{\left(\frac{\sigma_A}{A}\right)^2 + \left(\frac{\sigma_{bl}}{A_{bl}}\right)^2}$, where D is the % of amino acid depletion, A is the average area under the chromatographic peak of the amino acid substrate, σ_A is its standard deviation, A_{bl} is the same average area in a blank reaction (without the addition of the enzyme), σ_{bl} is its standard deviation.

^b The conversion was calculated as described in Materials and Methods.

^c No product formation was observed by HPLC-MS despite apparent conversions.

Table S4 - Conversion data with all ketoacid substrates.^{a,b}

		ArODH	mODH-45	mODH-47	mODH-48	mODH-49	mODH-55	mODH-582
Pyruvate (a)		7.0±3.8	97.4±2.5	94.4±1.0	75.9±1.4	99.6±0.0	37.8±2.4	97.2±0.8
Glyoxylate (b)		0.0±8.0	3.3±7.5	11.8±7.1	2.3±6.7	4.4±6.9	0.0±10.6	5.2±8.1
α-Ketobutyrate (c)		1.3±4.5	14.9±1.5	8.4±1.6	13.0±2.8	16.8±1.9	9.0±2.0	21.6±1.3
α-Ketoglutarate (d)		0.3±5.6	8.3±3.1	15.8±5.8	2.1±5.2	9.9±4.6	7.8±3.1	5.7±3.3
α-Ketoglutarate (d) ^c		0.0±14.6	5.2±8.8	2.0±9.6	0.6±10.2	6.1±8.4	0.0±11.3	0.0±11.3
α-Ketovalerate (e)		0.0±4.5	22.9±5.1	6.4±4.3	5.5±5.3	11.0±3.9	11.6±5.9	0.0±8.9

^a Standard errors are calculated according to the Gaussian error propagation rules: $D\sqrt{\left(\frac{\sigma_A}{A}\right)^2 + \left(\frac{\sigma_{bl}}{A_{bl}}\right)^2}$, where D is the % of amino acid depletion, A is the average area under the chromatographic peak of the amino acid substrate, σ_A is its standard deviation, A_{bl} is the same average area in a blank reaction (without the addition of the enzyme), σ_{bl} is its standard deviation.

^b The conversion was calculated as described in Materials and Methods.

^c L-Alanine substrate was used instead of L-aspartate

Table S5 – Comparison of key active site residues of ODHs, that were mutated by Codexis when engineering**ArODH**

ArODH	A111	S136	K156	V197	N198	M201	Y259	Y280	R292	Y293
mODH-582	R	Q	K	I	G	F	L	Y	R	Y
mODH-48	R	Q	K	I	G	F	L	Y	R	Y
mODH-47	R	E	K	M	G	F	L	Y	R	Y
mODH-45	R	E	K	M	G	F	L	Y	R	Y
mODH-49	R	E	K	M	G	F	L	Y	R	Y
mODH-55	R	E	K	M	G	F	L	Y	R	Y

Table S6 – Primers used for mutagenesis*ArODH A111R*

Name	Oligo (Uppercase = target-specific primer)	Len	%GC	Tm	Ta *
pET14b-ArODH_A111R_fw	GAACCCGGGTcgtACCGGTGGCG	23	74	62°C	62°C
pET14b-ArODH_A111R_rev	AGAATGATCAGCTGACCC	18	50	61°C	

* Ta (recommended annealing temperature)

mODH-582 R110A

Name	Oligo (Uppercase = target-specific primer)	Len	%GC	Tm	Ta *
pET14b-mODH-582_R110A_fw	GAACCCGGGTgcgACCGGCGGTG	23	78	63°C	64°C
pET14b-mODH-582_R110A_rev	AGCAGCAGAACCTGACCA	18	56	66°C	

* Ta (recommended annealing temperature)

ArODH A111R N198G

Name	Oligo (Uppercase = target-specific primer)	Len	%GC	Tm	Ta *
pET14b-ArODH_N198G_fw	GACCAACGTGGGCGCGGTTATGC	23	65	64°C	63°C
pET14b-ArODH_N198G_rev	AGGCTGGTGTGCAG	14	64	62°C	

* Ta (recommended annealing temperature)

mODH-582 R110A G198N

Name	Oligo (Uppercase = target-specific primer)	Len	%GC	Tm	Ta *
pET14b-mODH-582_R110A_fw	GGATAACATCAACGCGGTGTTCCAC	25	52	58°C	58°C
pET14b-mODH-582_R110A_rev	AGGCTGGTTTCCAG	14	57	57°C	

* Ta (recommended annealing temperature)

Table S7 – Conversions obtained with mutant and wild type enzymes. Reaction conditions are the same as described for the screening.

	<i>Ar</i> ODH wt	<i>Ar</i> ODH A111R	mODH-582 wt	mODH-582 R110A
L-Aspartate	<5%	<5%	90%	<5%
L-Phenylalanine	>99%	<1%	<1%	<1%

Table S8 – Conversion data for cofactor preference study of mODHs with L-aspartate.^{a,b}

Cofactor	mODH-45	mODH-47	mODH-48	mODH-49	mODH-55	mODH-582
NADH (2h)	16.0±8.4	n.d.	56.7±3.5	70.2±3.2	45.6±33.3	73.1±3.4
NADPH (2h)	28.6±2.1	n.d.	15.1±3.7	17.4±2.5	4.5±2.5	49.7±5.2
NADH (24h)	54.9±1.8	4.2±2.1	99.5±0.1	99.1±0.0	54.5±7.9	99.1±0.3
NADPH (24h)	94.7±1.4	76.7±4.6	75.1±10.3	99.6±0.3	39.0±5.5	99.4±0.5

^a Standard errors are calculated according to the Gaussian error propagation rules: $D \sqrt{\left(\frac{\sigma_A}{A}\right)^2 + \left(\frac{\sigma_{bl}}{A_{bl}}\right)^2}$, where D is the % of amino acid depletion, A is the average area under the chromatographic peak of the amino acid substrate, σ_A is its standard deviation, A_{bl} is the same average area in a blank reaction (without the addition of the enzyme), σ_{bl} is its standard deviation.

^b The conversion was calculated as described in Materials and Methods.

Table S9 – Kinetic constants of ODHs with L-aspartate. The corresponding Michaelis-Menten curves can be seen on Figure S15.

Enzyme	c [μ M]	cofactor	V _{max} [μ mol/min]	K _M [mM]	k _{cat} [1/s]
ArODH	0.015	NADH	no reaction observed		
ArODH A111R N198G	1.5	NADH	0.025 $\pm$ 0.006	58.3 $\pm$ 19.6	2.80 $\pm$ 0.69
mODH-45	0.71	NADPH	0.0032 $\pm$ 0.0005	21.5 $\pm$ 5.3	0.76 $\pm$ 0.11
mODH-47	0.72	NADPH	0.0005 $\pm$ 0.00003	1.2 $\pm$ 0.3	0.11 $\pm$ 0.01
mODH-48	0.72	NADPH	0.0030 $\pm$ 0.00009	0.5 $\pm$ 0.1	0.70 $\pm$ 0.02
mODH-49	0.70	NADPH	0.0039 $\pm$ 0.0003	15.0 $\pm$ 2.3	0.93 $\pm$ 0.07
mODH-55	0.71	NADPH	0.0066 $\pm$ 0.0002	1.5 $\pm$ 0.2	1.55 $\pm$ 0.05
mODH-582	0.73	NADH	0.058 $\pm$ 0.006	1.0 $\pm$ 0.5	13.1 $\pm$ 1.43
mODH-582	0.73	NADPH	0.0039 $\pm$ 0.0004	24.8 $\pm$ 4.6	0.89 $\pm$ 0.10

Table S10 – Kinetic constants of ODHs with L-phenylalanine. The corresponding Michaelis-Menten curves can be seen on Figure S16.

Enzyme	c [μ M]	cofactor	V _{max} [μ mol/min]	K _M [mM]	k _{cat} [1/s]
ArODH	0.015	NADH	0.077 $\pm$ 0.006	1.4 $\pm$ 0.5	861 $\pm$ 70
ArODH	0.015	NADPH	0.001 $\pm$ 0.0001	18.1 $\pm$ 4.5	11.1 $\pm$ 1.5
mODH-582	0.73	NADPH	no reaction observed		
mODH-582 R110A G198N	1.5	NADH	0.041 $\pm$ 0.009	n.d.	4.6 $\pm$ 1.0
mODH-582 R110A G198N	1.5	NADPH	0.015 $\pm$ 0.001	32.7 $\pm$ 4.7	1.7 $\pm$ 0.1

Table S11 – Kinetic constants of ODHs with NADPH. The corresponding Michaelis-Menten curves can be seen on Figure S17.

Enzyme	c [μ M]	substrate	V _{max} [μ mol/min]	K _M [μ M]	k _{cat} [1/s]
ArODH	0.015	Phe	Non-Michaelis-Menten kinetics		
ArODH A111R N198G	1.5	Asp	0.0007 $\pm$ 0.0004	890 $\pm$ 636	0.08 $\pm$ 0.04
mODH-45	0.71	Asp	0.0042 $\pm$ 0.0003	118 $\pm$ 24	1.00 $\pm$ 0.08
mODH-47	0.72	Asp	0.0014 $\pm$ 0.0001	93 $\pm$ 21	0.33 $\pm$ 0.03
mODH-48	0.72	Asp	0.0033 $\pm$ 0.0002	17 $\pm$ 7	0.76 $\pm$ 0.04
mODH-49	0.70	Asp	0.0052 $\pm$ 0.0004	85 $\pm$ 17	1.23 $\pm$ 0.09
mODH-55	0.71	Asp	0.0077 $\pm$ 0.0005	68 $\pm$ 13	1.80 $\pm$ 0.11
mODH-582	0.73	Asp	0.0027 $\pm$ 0.0006	318 $\pm$ 112	0.62 $\pm$ 0.14
mODH-582 R110A G198N	1.5	Phe	Non-Michaelis-Menten kinetics		

Table S12 – Kinetic constants of ODHs with NADH. The corresponding Michaelis-Menten curves can be seen on Figure S18.

Enzyme	c [μM]	substrate	V_{max} [$\mu\text{mol}/\text{min}$]	K_{M} [μM]	k_{cat} [1/s]
ArODH	0.015	Phe	0.11 ± 0.02	292 ± 77	1240 ± 174
ArODH A111R N198G	1.5	Asp	0.0067 ± 0.0003	220 ± 18	0.75 ± 0.03
mODH-45	0.71	Asp	0.0038 ± 0.0003	447 ± 64	0.90 ± 0.08
mODH-47	0.72	Asp	no reaction observed		
mODH-48	0.72	Asp	0.019 ± 0.001	93 ± 15	4.36 ± 0.25
mODH-49	0.70	Asp	0.021 ± 0.004	1828 ± 423	4.95 ± 0.95
mODH-55	0.71	Asp	0.0021 ± 0.0002	442 ± 63	0.50 ± 0.04
mODH-582	0.73	Asp	0.016 ± 0.001	29 ± 11	18.1 ± 1.4

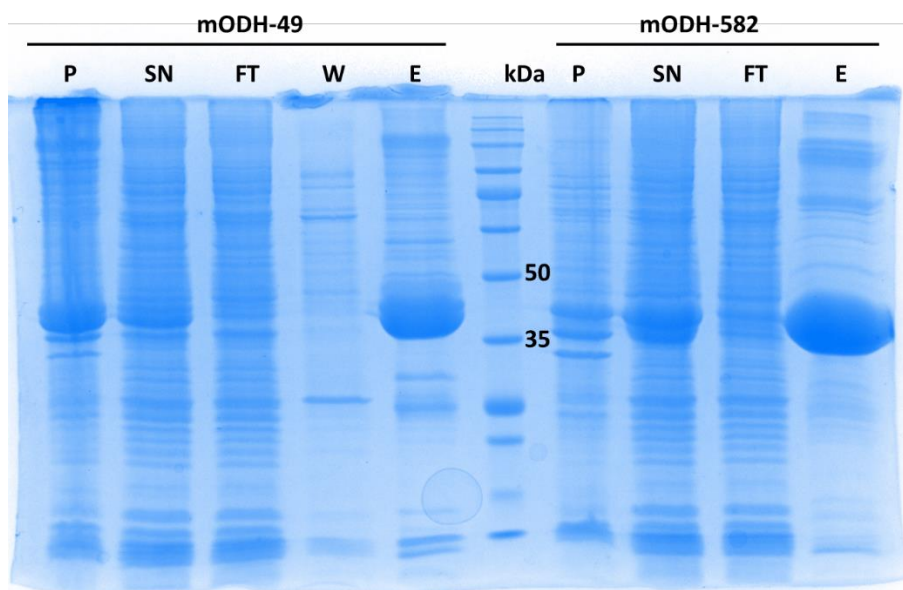


Figure S1 – Representative SDS-PAGE analysis of the Ni-affinity purification of mODHs.
P=pellet, SN=supernatant, FT=flowthrough, W=wash, E=elution; 5 mL sample was loaded for qualitative analysis.

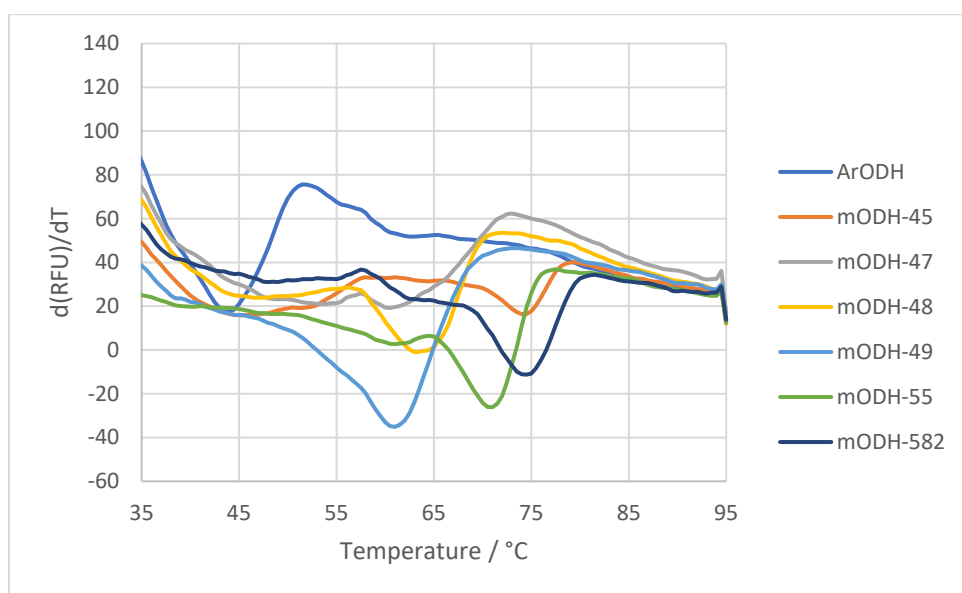
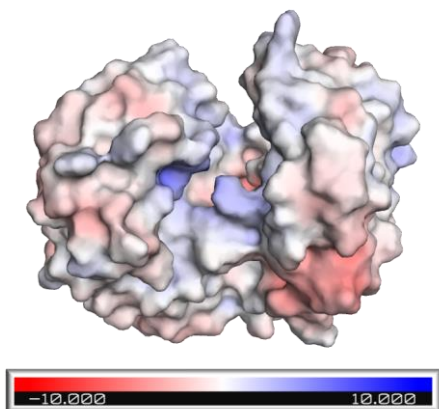
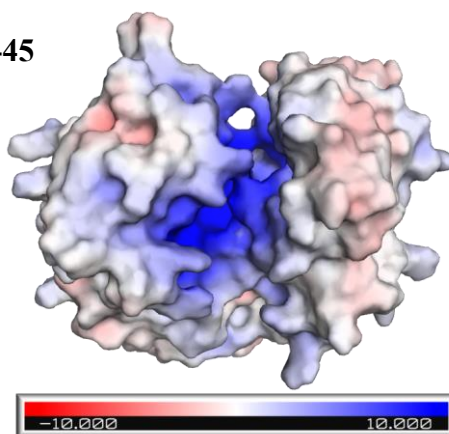


Figure S2 – Melting curves of ODHs measured by differential scanning fluorimetry.

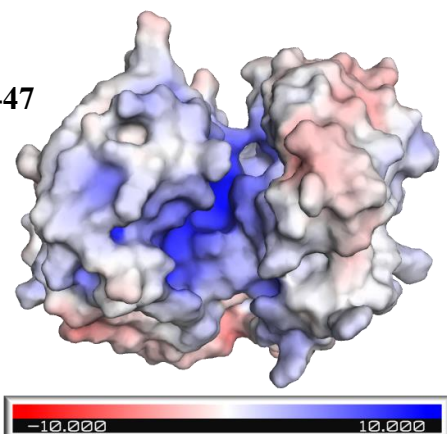
ArODH



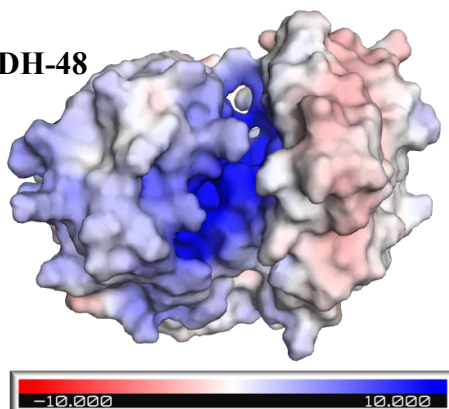
mODH-45



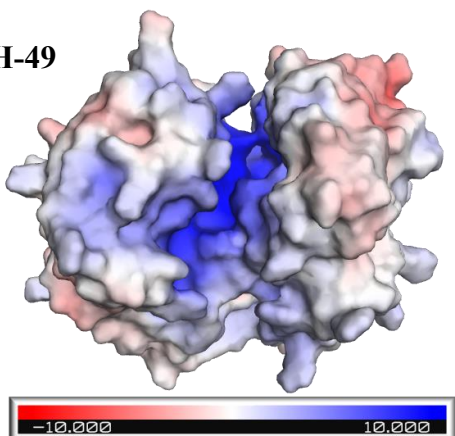
mODH-47



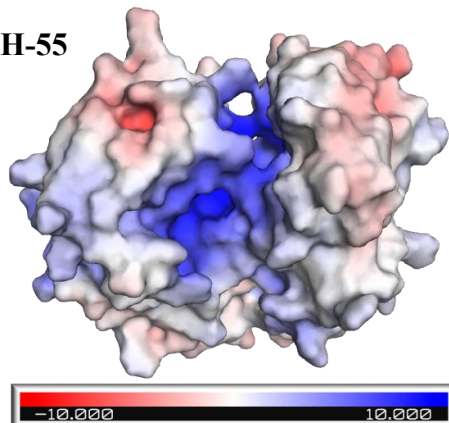
mODH-48



mODH-49



mODH-55



mODH-582

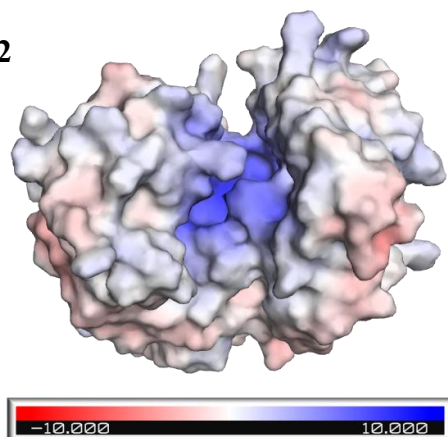


Figure S3 – Surface potential of ArODH and all mODHs

Surface potential is given between -10 and 10 in units $K_b T/E_c$, where K_b is the Boltzmann constant, T is the temperature and E_c is the charge of an electron.

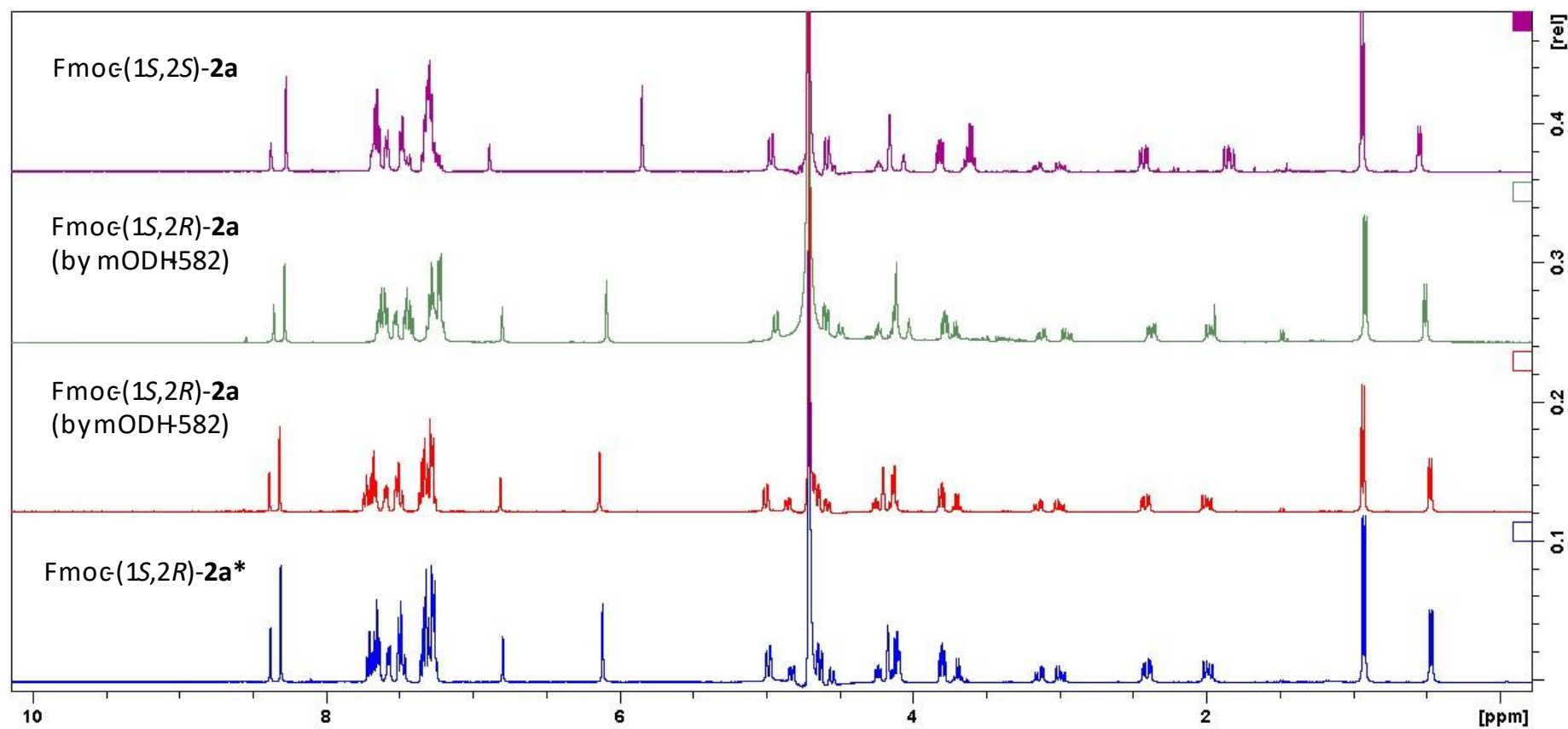
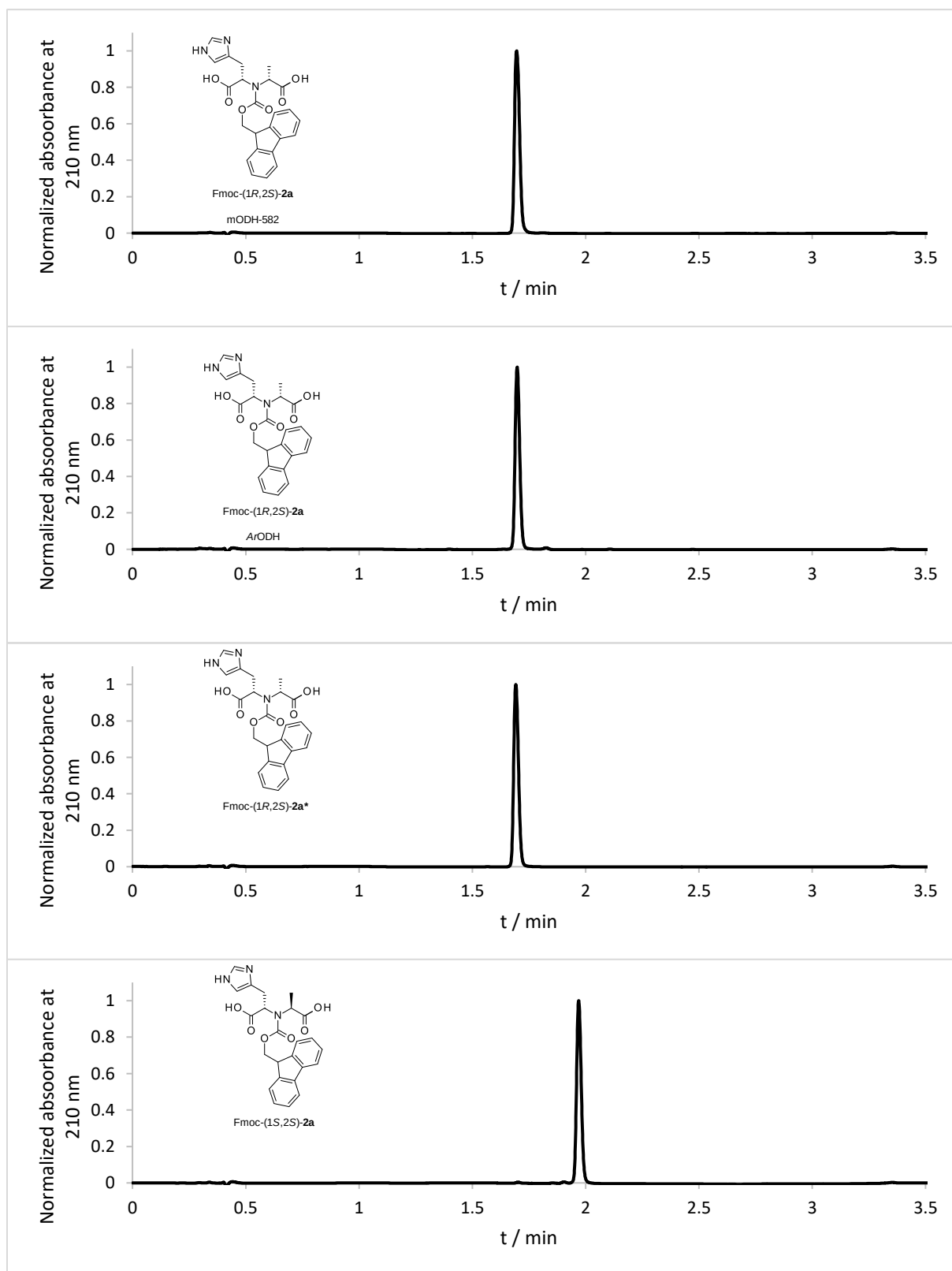


Figure S4 – Comparison of NMR spectra of enzymatic products with chemically synthesized reference compounds

Figure S5 – Comparison of HPLC/UV chromatograms of enzymatic products with chemically synthesized



reference compounds

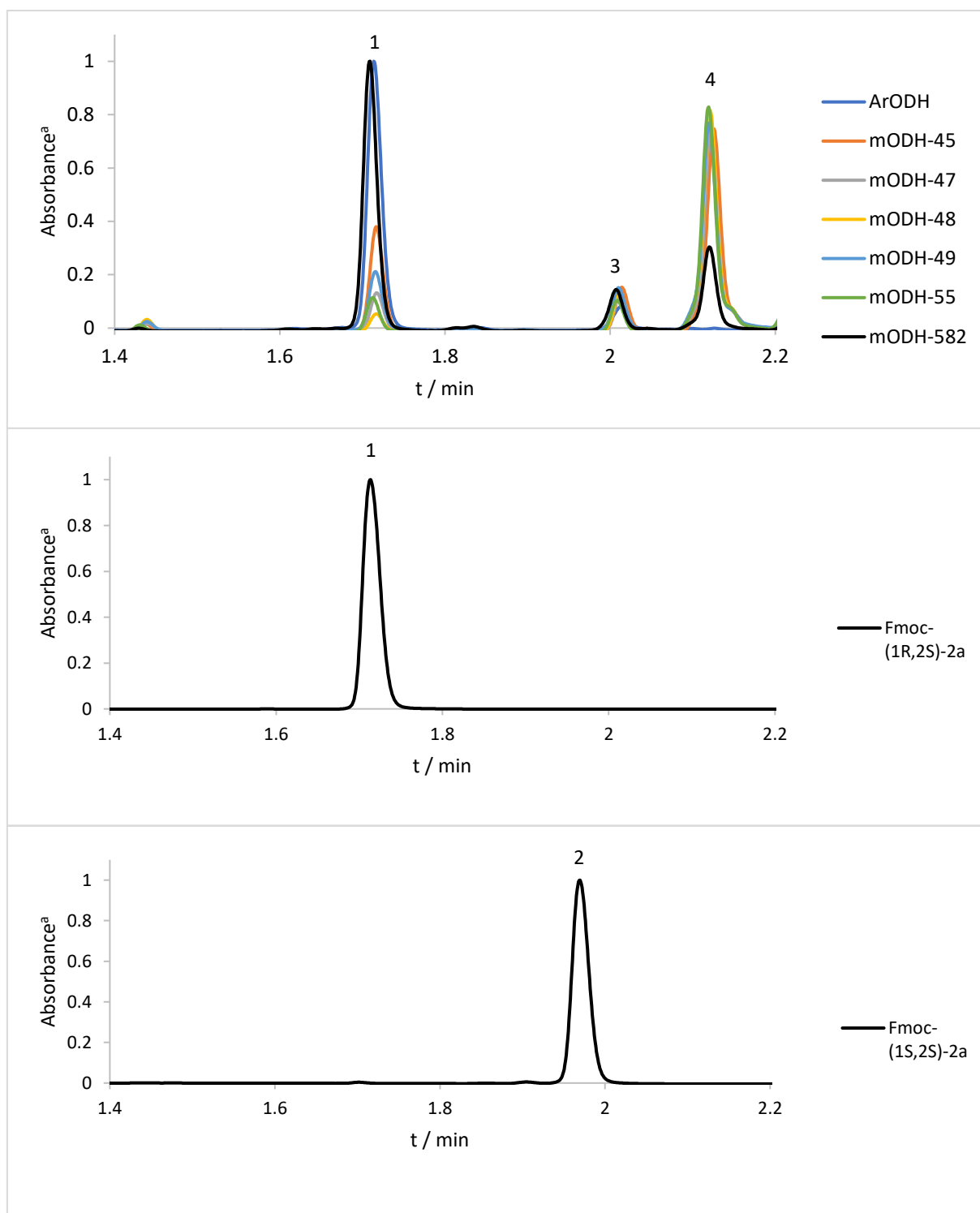


Figure S6 – Comparison of HPLC/UV chromatograms of *ArODH* and *mODHs* in enzymatic reaction with *L*-histidine (2) and pyruvate (a) with chemically synthesized reference compounds

a: Normalized absorbance at 210 nm; 1: Fmoc-(1*R*,2*S*)-**2a**; 2: Fmoc-(1*S*,2*S*)-**2a**; 3: uncharacterized side product (ESI⁺ *m/z*=436) 4: Fmoc-*L*-histidine

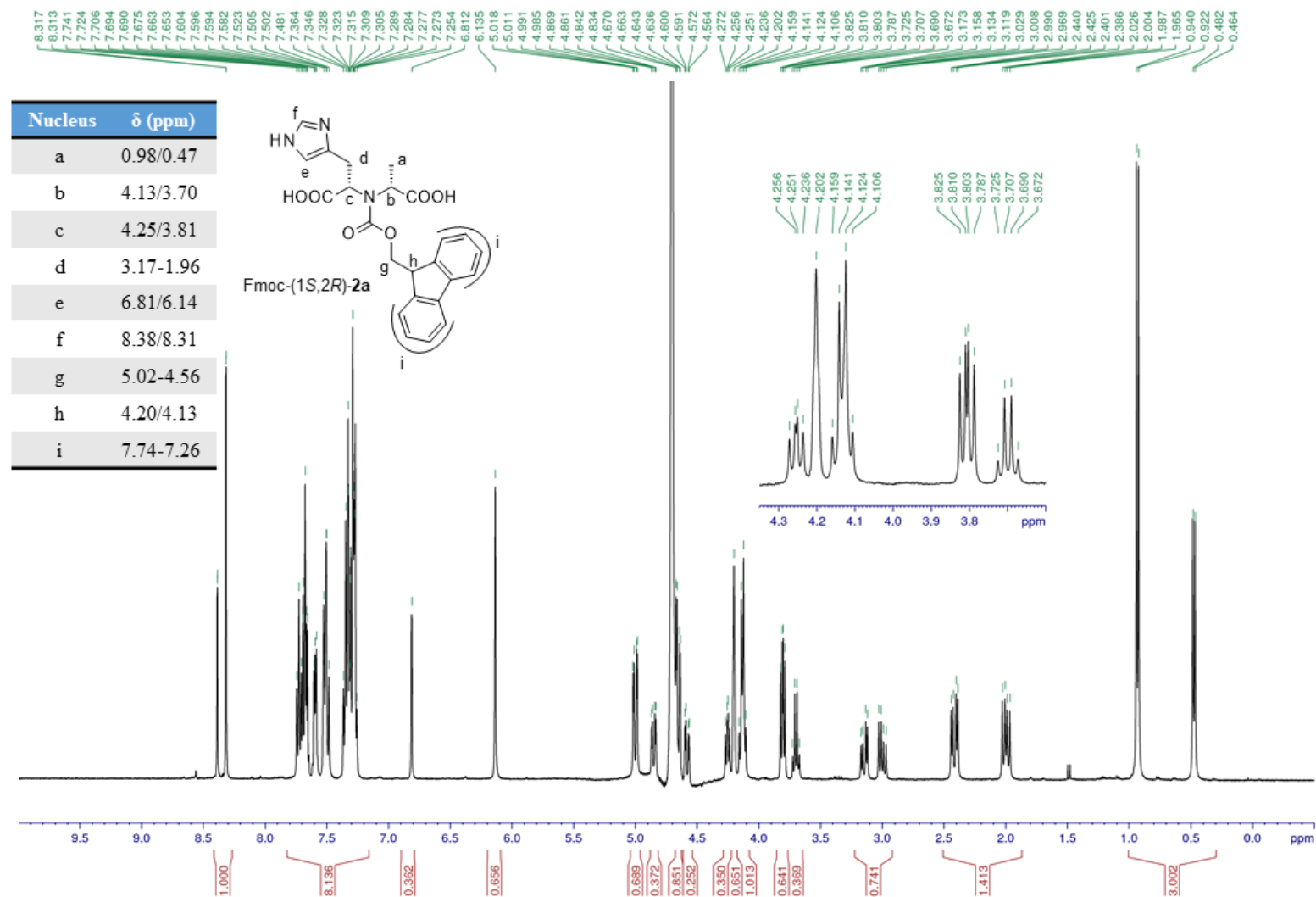


Figure S7 - ¹H-NMR spectrum of Fmoc-(1*R*,2*S*)-2a (prepared by mODH-582)

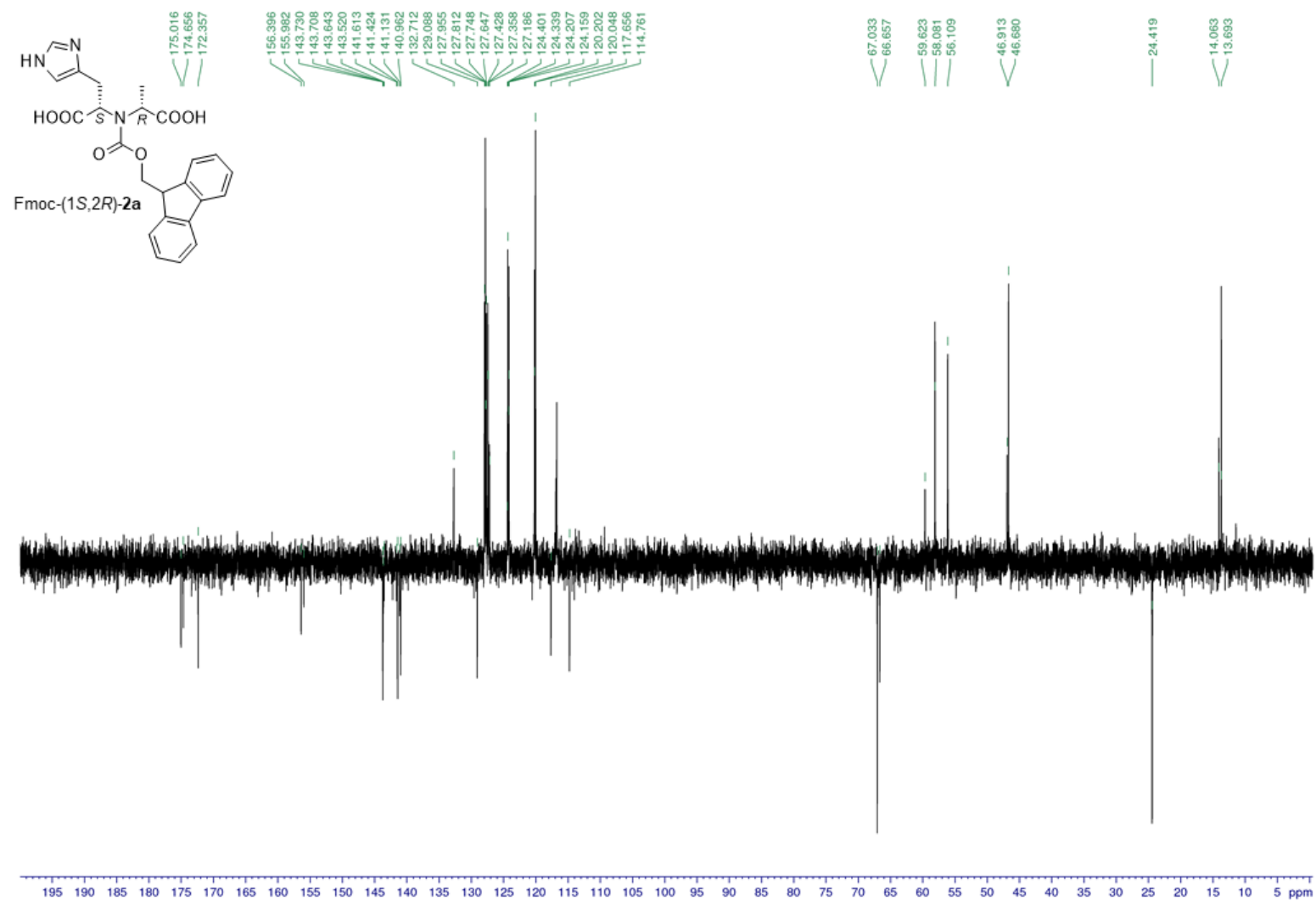


Figure S8 - ^{13}C -NMR spectrum of Fmoc-(1*R*,2*S*)-2a (prepared by mODH-582)

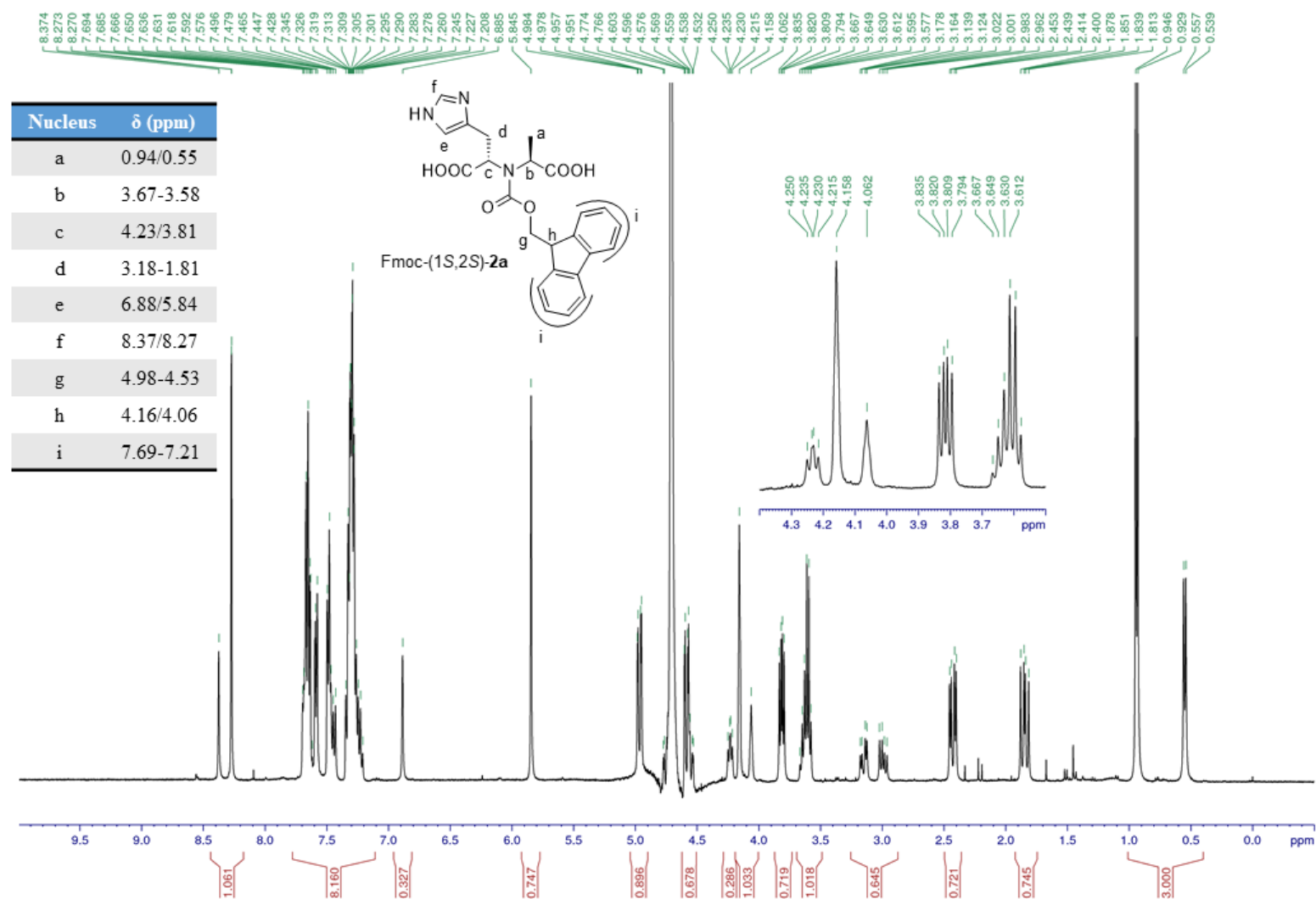


Figure S9 - ^1H -NMR spectrum of Fmoc-(1S,2S)-2a

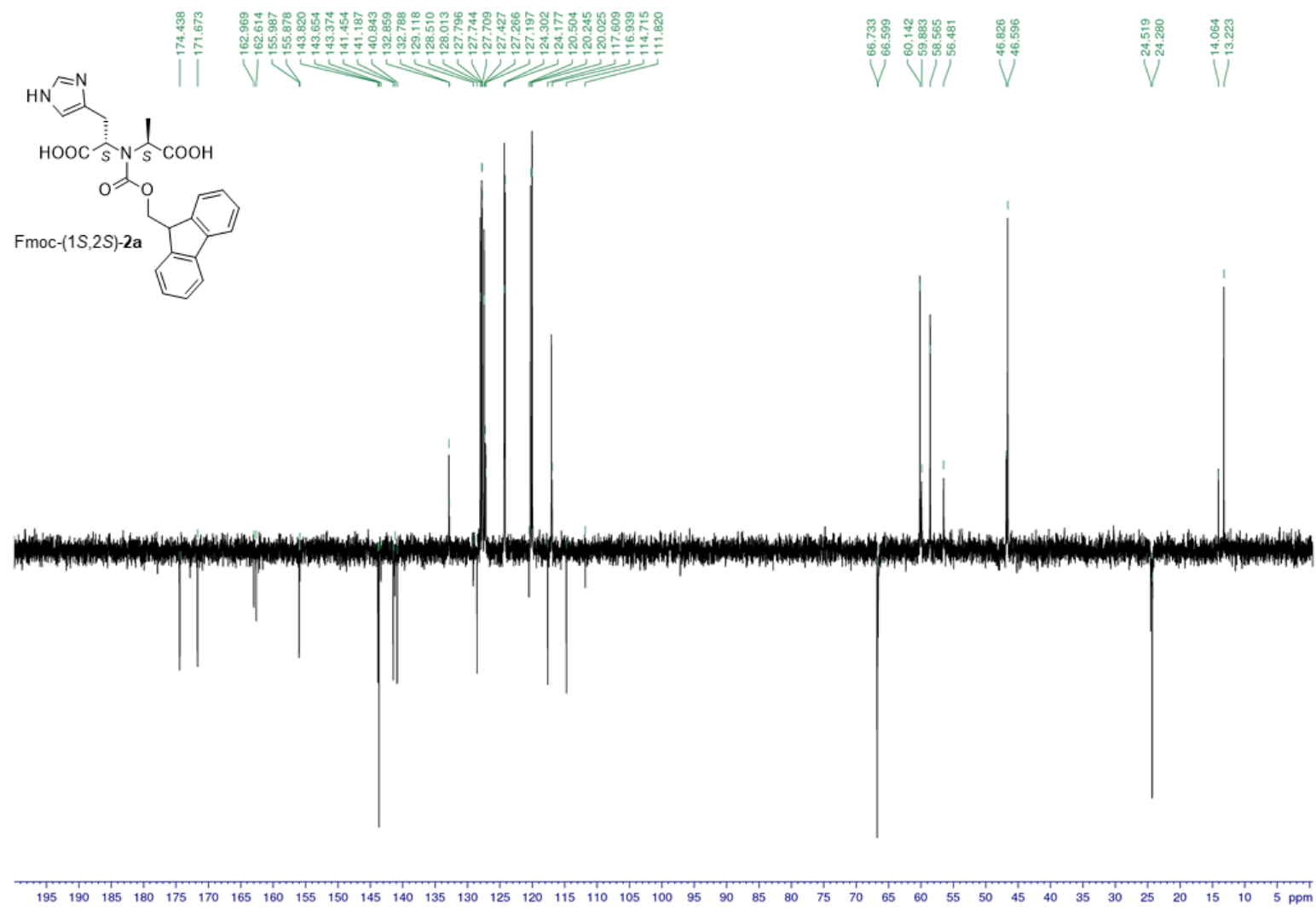


Figure S10 - ^{13}C -NMR spectrum of Fmoc-(1S,2S)-2a

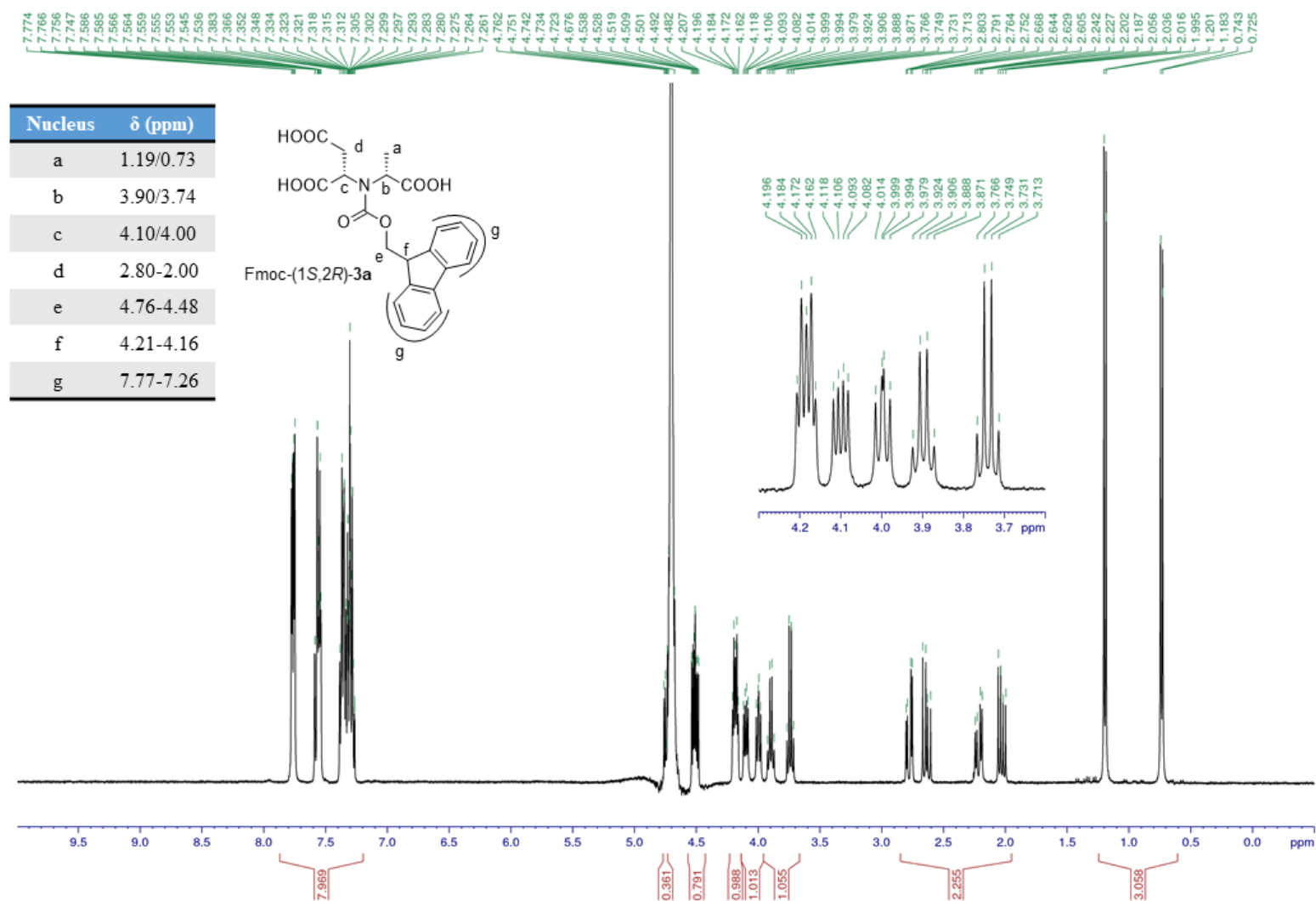


Figure S11 - ^1H -NMR spectrum of Fmoc-(1R,2S)-3a

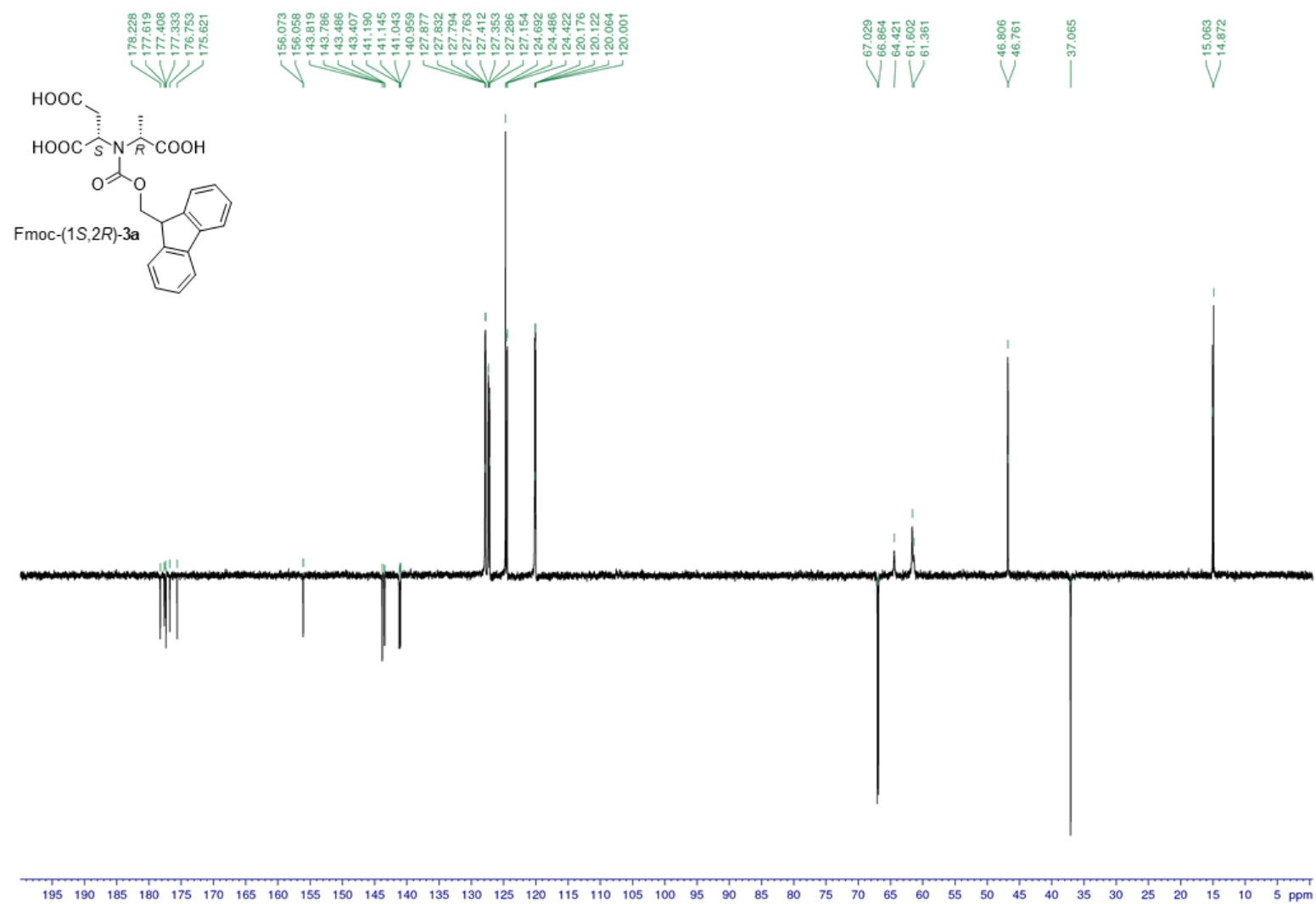


Figure S12 - ^{13}C -NMR spectrum of Fmoc-(1R,2S)-3a

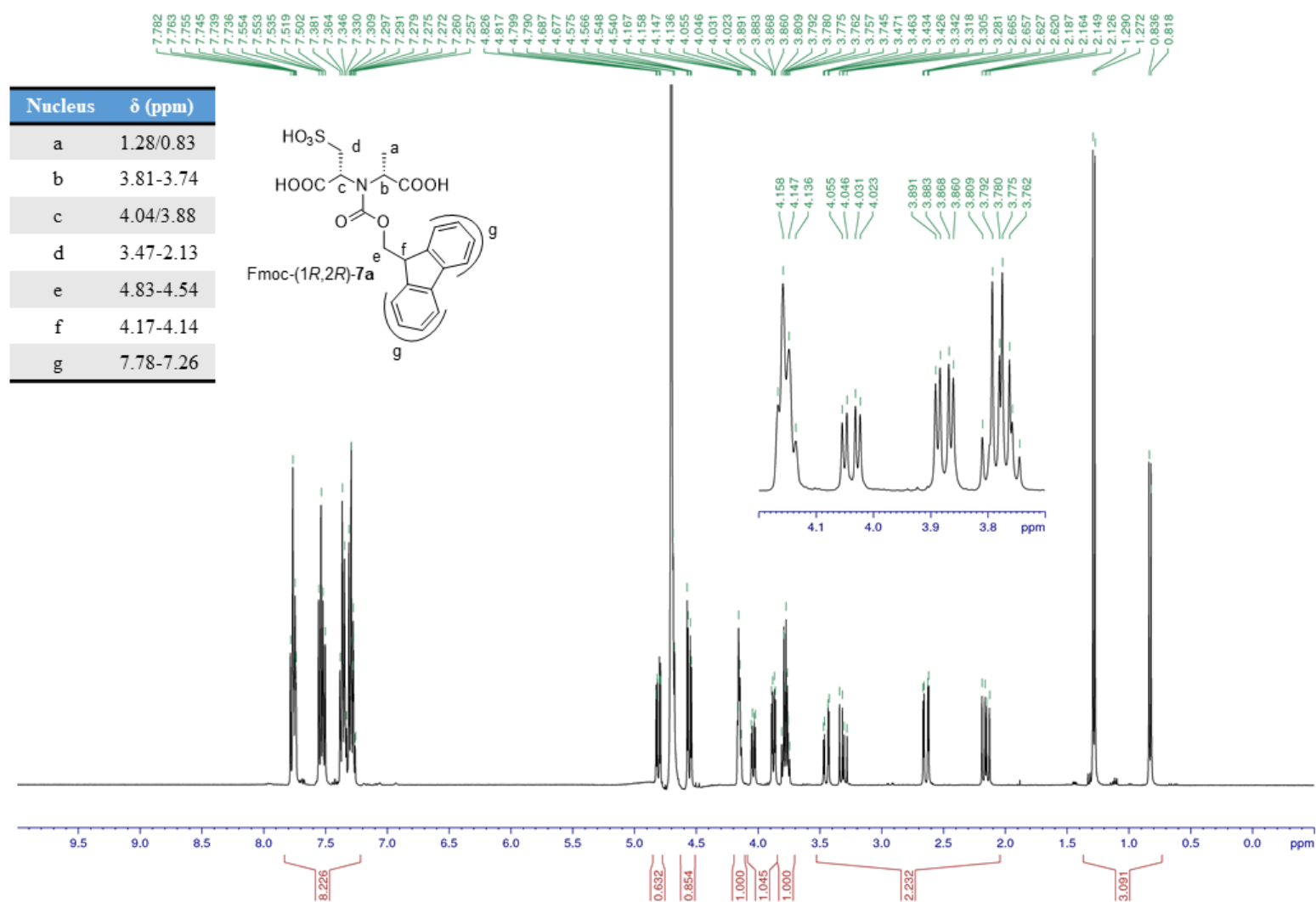


Figure S13 - ¹H-NMR spectrum of Fmoc-(1*R*,2*R*)-7a

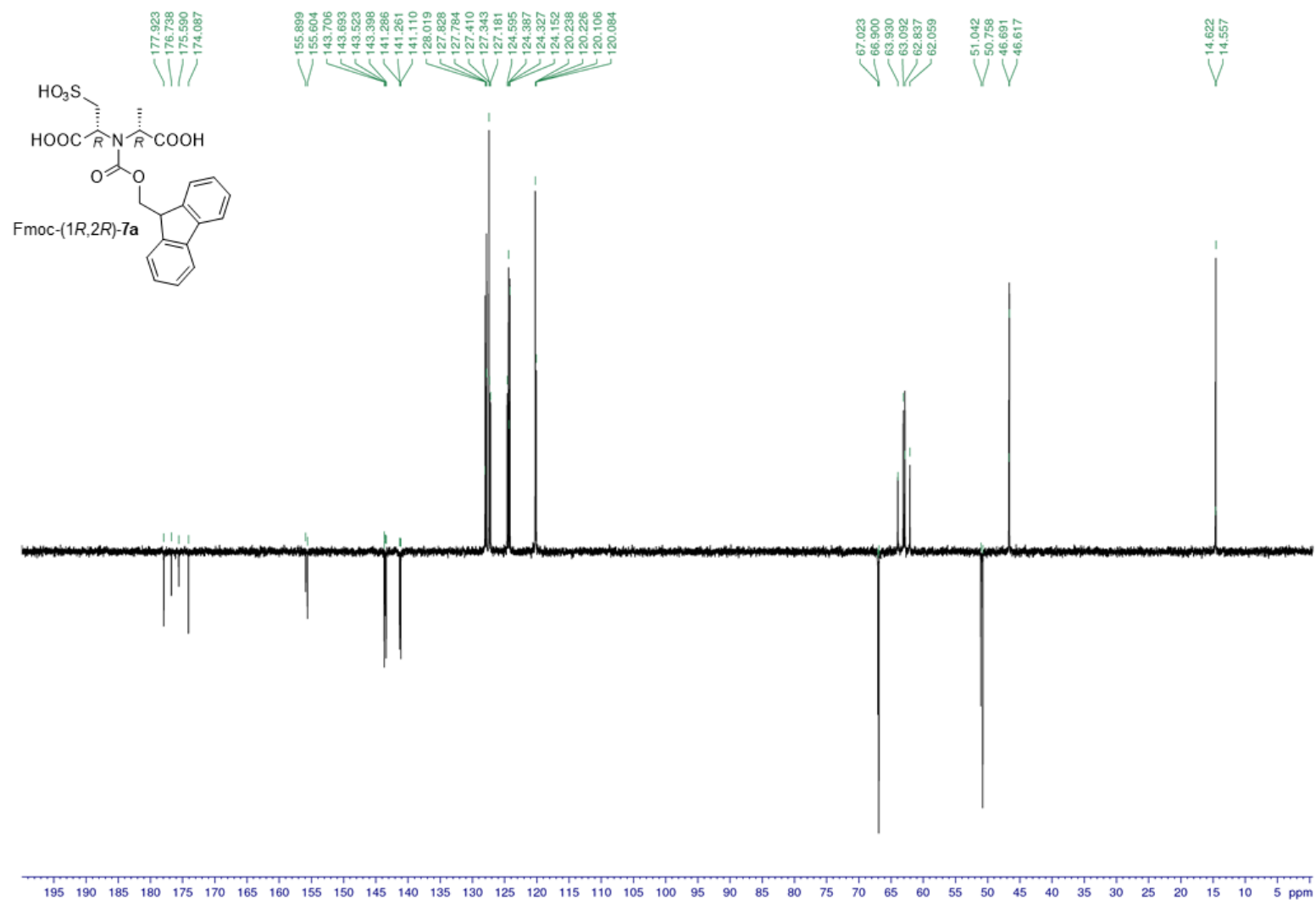


Figure S14 - ¹³C-NMR spectrum of Fmoc-(1R,2R)-7a

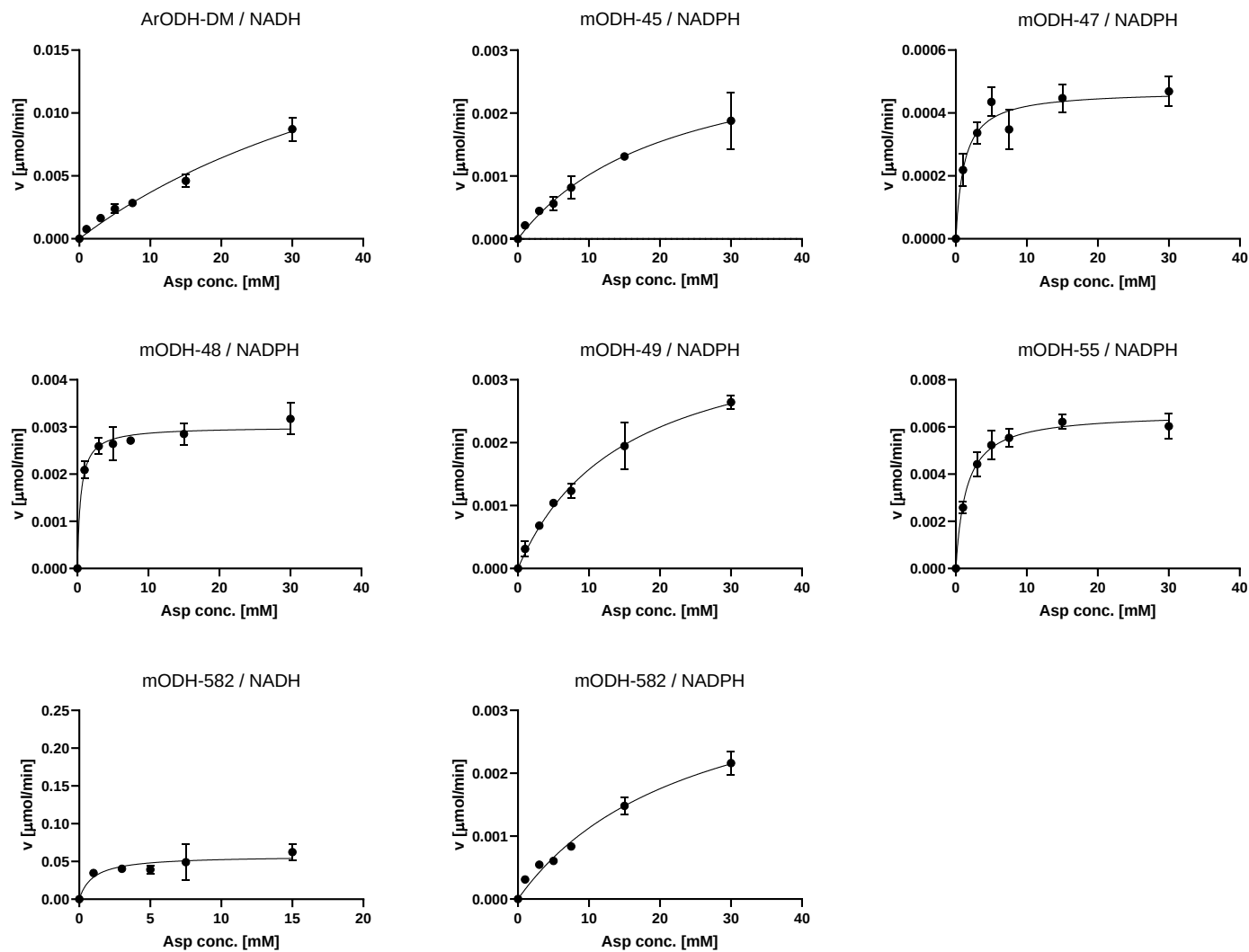


Figure S15 – Measured Michaelis-Menten curves of ODHs for l-aspartate.

mODH-582-DM corresponds to mODH-582 R110A G198N double mutant, while ArODH-DM corresponds to ArODH A111R N198G.

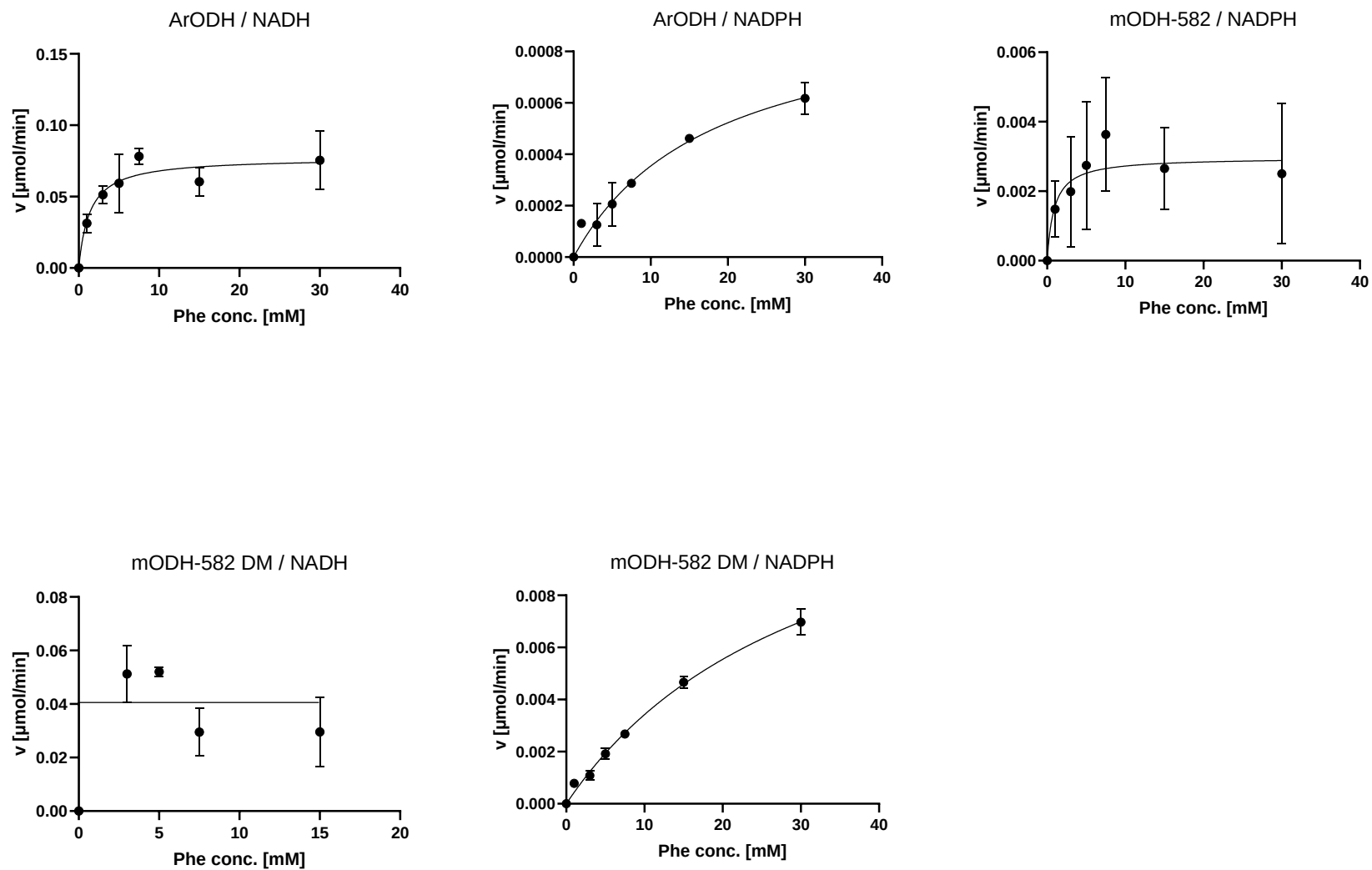


Figure S16 – Measured Michaelis-Menten curves of ODHs for L-phenylalanine.
mODH-582 DM corresponds to mODH-582 R110A G198N double mutant.

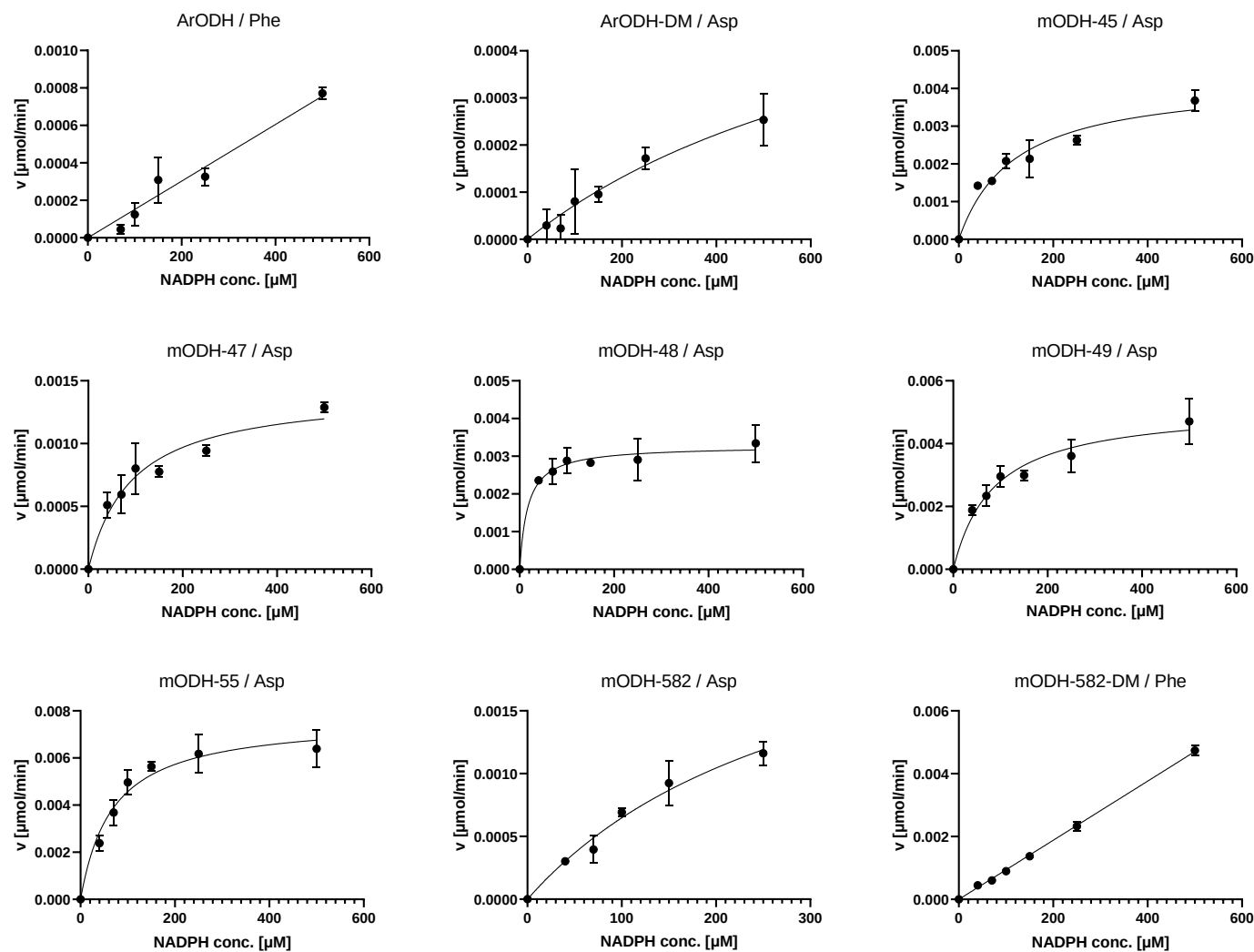


Figure S17 – Measured Michaelis-Menten curves of ODHs for NADPH.

mODH-582-DM corresponds to mODH-582 R110A G198N double mutant, while ArODH-DM corresponds to ArODH A111R N198G.

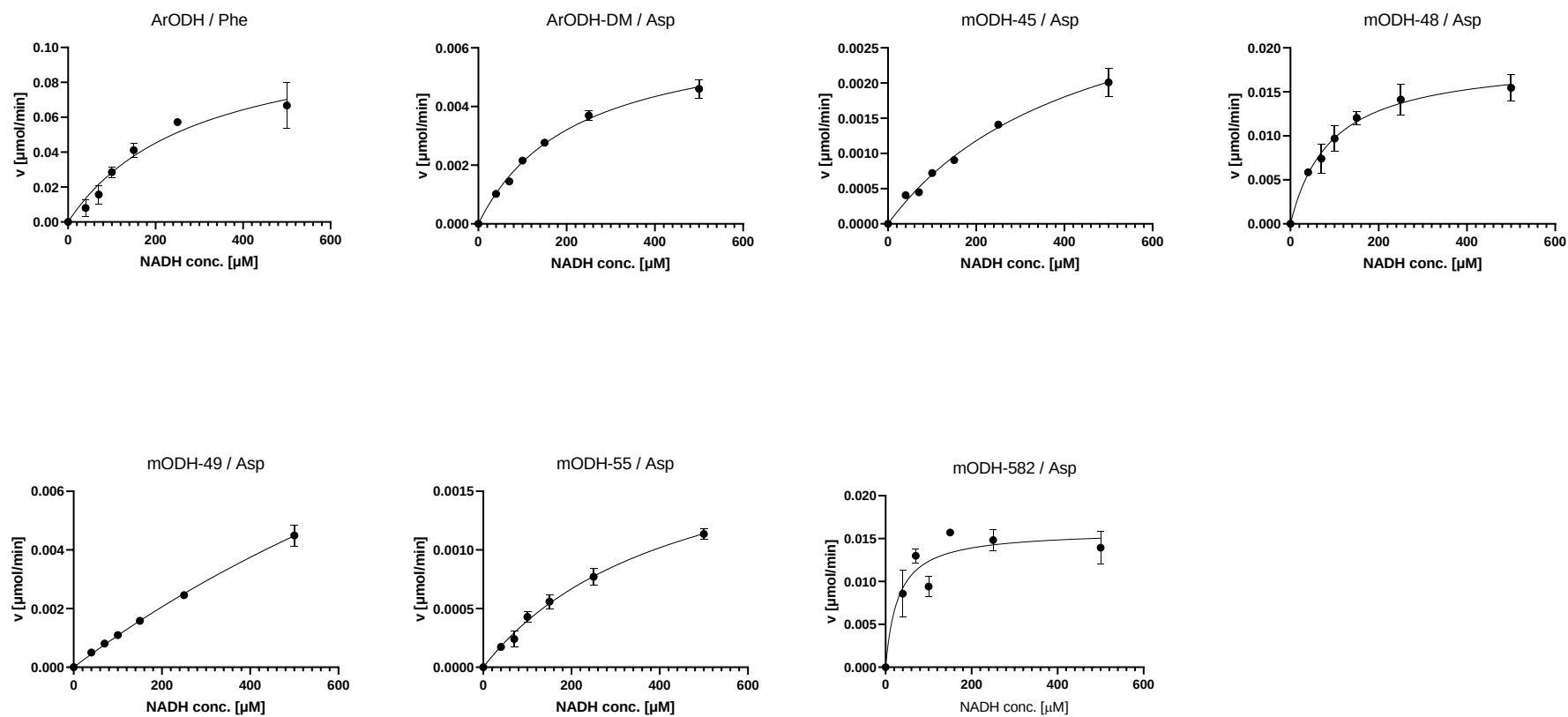


Figure S18 – Measured Michaelis-Menten curves of ODHs for NADH.

ArODH-DM corresponds to ArODH A111R N198G.