Supplementary Information

Pathway discovery and engineering for cleavage of a ß-1 lignin-derived biaryl compound

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Supplemental Tables.

Table S1. DNA sequences of oligonucleotides and gBlocks used in this study.

Name	Sequence (5' → 3')	Purpose/Desc.
NaRS14230	GGTCGACCTCCTCAAGCTGGGCACTTTCATCGGCACGCCTATGCGCGAGGGCGTGGCCGACCCGATGG GACTGACAGCCACCGATCTCAGGATCGTCCTTGCCCTCGGCGGCGAGGGCGAACTGGCCGGCCACGA ACTATCGGATATCATGGGCCTTCCGCCGATGAACGTCAGCCGCGCCATCGCCGCGCTGACGCAGCGGG GGTTGGTCGAGCCGGGCCTCGACCGCTCGAACCGCCGCCGCAAGCCCGTCCGTCTCAGCGCGGAAGG CCAGCGCCTCTTCGCGCAGACGATCCCGGCCATGGCGCATGTCGGCGCGGACCTGTTCAAGGGCGTGC CCCAGCGCGACCGCGAGGCCTTCCGCCGCGTCGCGGCATCCGTCCTCGCCCGCATCGGACGCTGGGGC GGCTAGGCCCGCGCCTCAGGCGCTCAGGGCCACTTCTTCTGAAGGGCGTTCACATCGGTCATCGTCAG ACTCTCCCAAGTCGGTAATTTGTAAGCAACACTAACAAATATCACGCCGAACGCGAGAGGAAAAATG AGGCCTCGGGACACCTTGCCTGCGGATCGCCGCGCTGTACTCTCTTTGCACACAGGCCAGACAGGGAA CGCTCCCCATGTGCGATGAATTCACCGAAGCCGAAAACGACCAGTGGGCCCTGAACCGCCGCGAATTT GCCGCGATCGGCGCCGGAGTGGCCGCGCTCGCCGCCTTGCCCGGCTGCGCGACGGCGGGCGAATCGG CCACCGCCAGCCGCGACGTGGTGATCGACACGCCGCACGGAAAGGCCGACGCCTTCTTCGTCTACCCC AGGAAGGGCAGACATGCGGCCGTCATCATGTGGCCCGACATCGCCGGCCTGCGCGATGCCTACAAGA CCATGGGCACCCGCCTTGCCG	Construct for deletion of RS14230 from *N. aromaticivorans* DSM12444. Upstream and downstream homology arms are shown in blue and red, respectively. Synthesized by Genescript.
NaRS14250	TCCACGCGCAACCCGAGGCCTTCATCAGGGCTTCGGTCTCGATATGATCGTGCCGCACTTCGGCGGGG TTCGCCGGATCGTCGATACCGATGAAGCTGGTGTAGACCAGGTGCGCCACGCCCGCCGCTGCCGCCGC GTCGATCGCCGCCTTGTGCTGAACCACCCGCGCGCCGACCCGCGTGCCCGAGATCAGCAGCATCCGCT CCGCCCCCTGCACCGCCTCCGCCAGCGTTTCCGGCTTGTCGAAATCGCCATAGCGCACGGTGCAGCCC TGTGCTGCCCGGTCGGCCAGCTTCTCGGGCTTGCGCGTGATGAGGATCAGGTCTTCGGCGCGCCCCTG CGCGATCAGGCGGTCCGTCACGCCCCGGCCATAGTTGCCCGAGGCTCCGGTAATGACGATGCGGCTCA CGCTGCGGAGCCTTCAAGCGCGTCCAGTTGCGCCTGGGGCACCTGCGGAAAAGCGCCCATGGCAAGA ACCTCACCCTATCAATTGTTATCGTTCTTGTAGCGTTTCCCTCCCCGAGATCAAGCCCACCAGTAGGCC GTAGCGCGCATTTCGATGCTGACGCGCGGGGGGGCATCGGCCGGGCAATCGGGCAGGTCGAAGGCCA CGTGCGGCACATGGTGCGCGCGGGCCGGATCGCTGTCGTTGGTCTTGAACAGCACCGCCTCGTCGATC CGCATATCCGGAAAGAAGTACCAGCGGTGCGCGGGATTGTGGGCCACGACCAGCCCTTCGAACGACC ATTCCGGCTTGCCCGGTTCGTCGAACACCGCGTCCGCCTCGATCAGGTCTCCGGGCGCAAGGCTGCGC GCGTCGCAGAACGCCAGCGGCACGTCCTGCGGGGGCGGAGAGATTGCGCGCCACACGTTGTAGGCGG CGCAGCGCACGAAAGGCCG	Construct for deletion of RS14250 from *N. aromaticivorans* DSM12444. Upstream and downstream homology arms are shown in blue and red, respectively. Synthesized by Genescript.
EcRS14230	CATATGACCGACGTGAACGCGAGCAGCGAGAGCCGTCTGGCGGCGCTGGAAGCGCGTGTTACCGAGC TGGAAGATCTGAACGCGATCCGTCGTCTGCAGTGGGCGTACGGTTACTATATTGACTATAACCGTCCG GAGGAAGTGGCGGGTCTGTTCGCGAAGGATGGCGCGGTGGTTTTTCTGAGCGGCGAGTACGTGGGTTA TGAAGGCGTTATGCGTCTGTACGGCACCTGGTTCCAGAACCTGTTTACCGGTGGCCGTCGTGGTCCGG TGCATGGTCTGCTGCTGGACCACTTCCAGCTGCAAGATGTTATCACCATTGCGCCGGACGGTCAAACC GCGAAAGGTCGTTTTCGTGGTATCCTGGCGGGTGGCTGGCATGACGATATTGTGAAGGATAAACCGGA GGGTATGCCGCAGCAATTCTGGGAGAGCGGCATCTACGAAAACGACTATGTGAAGGAAGATGGTGTT TGGAAGATTAAACGTCTGGACTACATGATGCAGTGGCAAGCGGATTATGAGACCGGCTGGAGCAAGA CCATTGCGCACCTGCAGCCGGCGGCGGTTTGCTTCCCGGAAAACCCGATTGGTCCGGACCGTCTGCTG CCGGAGACCGAAGTGCGTCAAACCTGGCCGCACCGTGCGGAAGTGCCGATGAGCTTTGCGCATCCGG TGCTGGCGAAGGCGTTTGCGGTTGGCGAATTTACCAAACTGCAAAAGAAATGGCCGtaaGTCGAC	Codon optimized (OptimumGene™) *RS14230* DNA sequence for expression in *E. coli.* The open reading frame is shown in dotted underline. Fragment ordered from Genscript.
EcRS14250	CATATGGGTGCGTTCCCGCAGACCATCTACTTTACCGGTGCGAACGCGCCGGTGGGTGAAGAACCGGA CCTGCGTGGTCTGAAAGTGGAGGGCGATCTGCCGGCGGAAGTTCGTGGTAGCTTCTATCGTGCGATTC CGGACCCGGCGTTCCCGCCGCGTTTTGAGAACGACCACACCCTGAGCGGTGATGGCATGGTTAGCCGT CTGAGCTTCAACGGTGACGGCACCGCGGATTTTATTCAGAAGTACGTTGAGACCGCGCGTTATAAGGC GGAAAAAGCGGCGGGTAAAGCGCTGTTCGGCAAGTACCGTAACCCGTTTACCGACGATCCGGAAGTG CAAGGTGTTGACCGTACCGTTGCGAACACCACCCCGGTTTGGCATGCGGGTCGTATGCTGATGGCGAA AGAAGACGGCCGTCCGTACCGTGTGGATCCGCGTACCCTGGCGACCATCGGTAGCTATGATTTCGGTG GCGCGCTGAAGAGCGAGACCATGACCGCGCACGTTCGTATTGACGCGGGTACCGGCGAGCTGTTCTTT TACGGTTATGAAGCGGATGGCCAGGCGAGCACCAAAGTGGCGTACTGCATTGTTGGTCCGGACGGCG AGCTGAAGCGTGAACAATGGTTTGACGCGCCGTATTGCGCGATGATGCACGATTTCACCATCAGCGAA AACTACGCGCTGTTTCCGATTTATCCGACCACCGCGGACCTGGATCGTCTGAAAGCGGGTGGCGAGCA CTGGCACCACCAGCCGGAACTGGACAGCTGGCTGGGTGTGATGCCGCGTTACGGCGATGTTAGCGAG ATCAAGTGGTTCAAAGGTCCGAAGGGCTGCCACAGCTATCACATGATGAACGCGTGGGAAGACGCGG ATGGTATGCTGCACTTTGACGCGTGCCTGAACAACACCAACGCGTTCGCGTTTATCCGTGAGCCGAGC GGTATTCACATGGGCCCGCAAGACATTAAAGGTGCGCTGACCCGTTGGACCGTGGATCCGCGTGCGGA TGGTGGCGATGTGGTTGAAACCGTGATTGGTCCGCCGGGTGATTTCCCGGTTATTCCGGCGAAACTGC AGGGTCGTCCGTACAAGACCGGCTGGATGCTGAGCATGAACCCGGAGCTGCAAGGTCCGCCGCTGTTT GCGGGTCCGGTGGGCGTTAGCTTTAACCTGCTGCTGCGTCTGGATGGTATGGATACCCCGGCGCCGCA GGTTACCGGTGCGCTGGCGCTGCCGCCGATGGCGGGCTTTAACGAACCGGTGCATGTTCCGGCGGCGG ACCCGGCGAAAGATGGTTGGCTGGTGTTCCTGGTTGACCAGCAAGTGGGCGATAACCAGTTTGTTCAC GAAGCGTGGGTGGTTGACGCGGGTAACATTGGTGCGGGTGCGGTGGCGAAGGTTCACATTCCGACCC GTCTGCGTCCGCAAGTGCACGGTTGGTGGGTTCCGCAGGCGCAACTGGATGCGCTGGAAGGCAGCGC GGCGtaaGTCGAC	Codon optimized (OptimumGene™) *RS14250* DNA sequence for expression in *E. coli.* The open reading frame is shown in dotted underline. Fragment ordered from Genscript.
gAW002	tatataTCTAGAacaattaataggctaaggccataaggacaacttATGACTGACGTCAACGCCAGTAGCGAATCCCGCCTGGC CGCTCTGGAAGCGCGCGTCACCGAACTGGAGGACTTGAACGCGATCCGCCGTCTGCAGTGGGCATAC GGGTACTACATCGACTACAACCGTCCAGAGGAAGTCGCCGGCCTGTTCGCCAAGGACGGCGCCGTAG TATTCCTGTCCGGTGAGTACGTTGGCTACGAGGGGGTAATGCGCCTGTACGGCACCTGGTTCCAGAAC CTCTTCACCGGCGGTCGCCGCGGCCCAGTGCACGGCCTCCTGCTCGACCACTTCCAGCTGCAGGACGT GATCACCATCGCCCCAGACGGCCAGACTGCTAAGGGCCGTTTCCGTGGTATCCTGGCCGGTGGTTGGC ACGACGACATCGTTAAAGACAAGCCTGAGGGTATGCCGCAGCAGTTCTGGGAGAGCGGTATCTATGA AAACGATTACGTTAAGGAAGATGGCGTCTGGAAGATCAAGCGCCTGGATTACATGATGCAGTGGCAG GCCGACTACGAGACCGGCTGGTCGAAGACCATCGCTCACCTGCAGCCGGCTGCGGTTTGCTTCCCGGA AAACCCGATCGGTCCAGACCGCCTCCTGCCAGAAACCGAAGTGCGCCAGACCTGGCCGCACCGCGCG GAGGTCCCGATGTCCTTCGCTCATCCGGTTCTGGCCAAGGCTTTCGCAGTTGGCGAATTCACCAAACTG CAGAAGAAGTGGCCGACTAGTtatata	Codon optimized (Optimizer) *RS14230* DNA sequence for expression in *P. putida* (CAI=0.72). The open reading frame is shown in dotted underline. RBS was designed in RBSCalculator (double underlined, AU=10070). XbaI and SpeI cut-sites (underlined) and six random base pairs inserted flanking. gBlock ordered from IDT.

gAW004	tatataTCTAGAtcaaaaaccaaccctaagcaggaattctATGGGTGCCTTTCCCCAAACCATCTATTTTACCGGTGCTAAC GCACCGGTGGGCGAAGAGCCAGACCTGCGTGGCTTGAAAGTCGAGGGCGATTTGCCGGCAGAAGTGC GGGGTAGCTTTTACCGCGCTATTCCGGACCCCGCTTTTCCTCCACGCTTCGAAAATGATCACACGTTGA GCGGTGACGGCATGGTGAGCCGTCTGTCGTTCAACGGCGATGGCACCGCGGACTTCATCCAGAAATAC GTCGAGACGGCGCGCTACAAAGCCGAGAAGGCCGCCGGCAAAGCTTTGTTTGGCAAATACCGCAATC CATTTACCGACGATCCCGAGGTGCAGGGCGTGGACCGTACGGTCGCAAACACCACGCCAGTCTGGCA CGCAGGCCGTATGTTGATGGCGAAAGAAGATGGCCGGCCGTACCGCGTGGACCCACGCACGCTCGCC ACGATTGGGAGCTACGACTTCGGTGGTGCATTGAAGTCGGAAACCATGACGGCACATGTCCGTATTGA CGCCGGTACGGGGGAGCTGTTTTTTTACGGCTATGAAGCAGATGGTCAGGCCAGCACCAAGGTGGCCT ATTGTATCGTGGGGCCTGACGGCGAGCTCAAGCGTGAACAATGGTTCGACGCACCTTATTGTGCGATG ATGCATGATTTCACCATTTCGGAGAACTACGCTCTCTTCCCTATTTACCCTACCACGGCTGATTTGGAC CGCCTCAAAGCCGGGGGTGAGCATTGGCACCATCAACCCGAATTGGATTCGTGGTTGGGGGTGATGCC ACGTTACGGTGACGTCTCGGAAATCAAATGGTTCAAAGGTCCTAAAGGTTGTCATTCGTACCATATGA TGAACGCATGGGAGGATGCTGATGGGATGTTGCACTTCGATGCATGCTTGAACAACACGAATGCCTTC GCCTTCATCCGGGAACCATCCGGGATCCACATGGGCCCTCAAGACATTAAAGGTGCGCTGACGCGCTG GACGGTCGATCCCCGCGCCGACGGTGGTGACGTGGTGGAGACCGTCATCGGCCCTCCTGGGGATTTCC CAGTCATTCCGGCGAAGCTGCAAGGGCGTCCTTATAAAACCGGGTGGATGTTGTCGATGAATCCGGAG CTGCAGGGTCCCCCGCTGTTCGCAGGCCCCGTCGGGGTGTCGTTCAATCTGCTGCTGCGCCTGGACGG TATGGACACGCCTGCCCCCCAAGTGACCGGTGCGCTCGCTCTCCCTCCGATGGCAGGGTTTAACGAGC CCGTCCATGTGCCTGCGGCGGACCCGGCGAAAGATGGGTGGTTGGTGTTCTTGGTCGACCAGCAGGTC GGGGACAACCAGTTCGTCCACGAGGCATGGGTCGTCGATGCTGGTAACATCGGCGCTGGTGCTGTCGC CAAAGTCCATATTCCCACCCGGTTGCGGCCGCAAGTGCACGGGTGGTGGGTCCCACAAGCCCAACTGG ACGCATTGGAAGGGTCGGCTGCCACTAGTtatata	Codon optimized (IDT) *RS14250* DNA sequence for expression in *P. putida* in IDT's codon optimization tool. The open reading frame is shown in dotted underline. RBS was designed in RBSCalculator (double underlined, AU=10522). XbaI and SpeI cut-sites (underlined) and six random base pairs inserted flanking. gBlock ordered from IDT.
RS14230 SeqF	GTGATGCAGTTTGACCACACC	Primer set for sequence confirmation of RS14230 deletion in JMN18
RS14230 SeqR	ATCATCGGTTTGAGCTTCTTCTGC	
RS14250 SeqF	CAGGATCATCGCATCGGCATAG	Primer set for sequence confirmation of RS14230 deletion in JMN19
RS14250 SeqR	TTTGCCCACATCGACATTTCCG	
oAW046	ggaattgtgagcggataacaatttcacacTCTAGAACAATTAATAGGCTAAGGCCATAAGGACAACTTTATGA	Primer set for amplification of *RS14230* (gAW002) with 5' overlap (oAW046, underlined) to *RS14230* and 3' overlap (oAW060, underlined) to *fpvA* region for HiFi Assembly of pAW001.
oAW060	cctgcttagggttggtttttgagcggccgcCGGCCACTTCTTCTGCAGTTTGG	
oAW061	caaactgcagaagaagtggccggcggccgcTCAAAAACCAACCCTAAGCAGGAATTCTATGG	Primer set for amplification of *RS14250* (gAW004) with 5' overlap (oAW061, underlined) to *RS14230* and 3' overlap (oAW059, underlined) to *fpvA* region for HiFi Assembly of pAW001.
oAW059	atgagtaaaaagcctccggtcggaggcttttgactACTAGTGGCAGCCGACCC	
oAW027	GTACTACCAGGGCATTGCC	pAW001 sequencing
oAW029	GCCGTTTCCGTGGTATC	pAW001 sequencing
oAW031	GACGGCACATGTCCG	pAW001 and pAW008 sequencing
oAW062	GACGGCATGGTGAGC	pAW001 sequencing
oAW063	GGGTGGATGTTGTCGATG	pAW001 sequencing
oAW103	catacacgcacgagatttatggggccatttGCGGCCGCGAGCTGTTGACAATTAATCATCGGCTCGTATAATG	Primer set for amplification of *RS14230-RS14250* expression cassette (from pAW001) with 5' overlap (oAW103, underlined) to *crc* HR_up and 3' overlap (oAW104, underlined) to *crc* HR_dn region for HiFi Assembly of pAW008.
oAW104	ggggctgcaatgcagccccaatggcttGCGGCCGCGGCAGCCGACCCTTCCAATGCGTCCAG	
oAW077	GAACTGGAGGACTTGAACGC	pAW008 sequencing
oAW105	CAAGCCGGCATTGAAGAAATACG	pAW008 sequencing
oAW157	CAGACCGCCTCCTGCCAGAAACC	pAW008 sequencing
oAW158	GATCCACATGGGCCCTCAAGAC	pAW008 sequencing
oAW023	ACATCACCTGCTACGAAGC	Colony PCR for AW006

oAW024	GATTCGCATGACACGCTG	(3634 bp, Tm=72°C)
oAW080	CGTGTGATCATTTTCGAAGCG	Colony PCR for AW049 (1251 bp, Tm=65°C)
oAW105	CAAGCCGGCATTGAAGAAATACG	

Table S2. Plasmids and strains used in this study.

PLASMIDS			
Name	**Utility or Genotype**	**Description/Construction Details**	**Reference**
pAK405	To genetically modify the chromosome of *N. aromaticivorans* via homologous recombination	Vector backbone allowing markerless chromosomal modifications in diverse sphingomonads. Uses kanamycin for initial selection and streptomycin for counterselection.	[1]
pJM296	To delete RS14230 from *N. aromaticivorans*	Includes the synthesized sequence NaRS14230 above	This study
pJM297	To delete RS14250 from *N. aromaticivorans*	Includes the synthesized sequence NaRS14250 above	This study
pET-22b	E. coli expression vector	Contains a T7 promoter for gene expression, a pBR322 origin of replication, and an ampicillin selection marker	Novagen
pET22b-lsdA	Heterologous expression of LsdA in *E. coli*	Includes the synthesized sequence EcRS14250 above	This study
pET22b-lsdE	Heterologous expression of LsdE in *E. coli*	Includes the synthesized sequence EcRS14230 above	This study
pK18mobsacB	To genetically modify the chromosome of *P. putida* KT2440 via homologous recombination	"Cloning vector allowing mobilization into a wide range of Gram- and Gram+ bacteria. After mobilization, the plasmid can be maintained by integration into the host chromosome via homologous recombination. Excision of the intervening plasmid sequence by a double crossover event can be facilitated by selection on medium containing 10% sucrose. The *sacB* gene has been modified to eliminate the HindIII and EcoRI sites in the coding region."	ATCC® 87097™
pCJ033	To integrate DNA into the *crc* genomic locus in *P. putida* KT2440	As described in: Johnson, C. W., Abraham, P. E., Linger, J. G., Khanna, P., Hettich, R. L., Beckham, G. T., Eliminating a global regulator of carbon catabolite repression enhances the conversion of aromatic lignin monomers to muconate in Pseudomonas putida KT2440. Metabolic Engineering Communications, Vol. 5, 2017, pp. 19-25.	[2]
pCJ107	To integrate DNA into the *fpvA* genomic locus in *P. putida* KT2440	As described in Salvachúa, D., Johnson, C. W., Singer, C. A., Rohrer, H., Peterson, D. J., Black, B. A., Knapp, A., Beckham, G. T., 2018. Bioprocess development for muconic acid production from aromatic compounds and lignin. Green Chem. 20, 5007-5019.	[3]
pAW001	To integrate a codon optimized *RS14230* and *RS14250* expression cassette into the *P. putida* KT2440 genome at the *fpvA* locus	The RS14230 amino acid sequence was codon optimized for expression in *P. putida* using Optimizer's guided random method; [4] the RS14250 amino acid sequence optimized with Optimizer contained a prohibitively high GC content for synthesis and therefore was optimized using Integrated DNA Technology's codon optimization tool (www.idtdna.com) (Table S1). Ribosome binding sites were designed for each gene using the RBSCalculator and the two-gene operon was driven by the constitutive tac promoter (Ptac). Both genes with their respective RBS were synthesized as gBlocks (IDT Corp.). gBlocks were amplified using Q5® Hot Start High-Fidelity DNA Polymerase (NEB, Inc.) and oligos as listed in Table S1, purified with the QIAquick PCR Purification Kit (ThermoFisher Scientific), assembled with HiFi DNA Assembly (NEB, Inc.) into XbaI/SpeI-digested pCJ107, and transformed into NEB® *E. coli* 5α F'I^q. The plasmid was sequence verified using oAW027, oAW029, oAW031, oAW062, and oAW062 (GENEWIZ.com).	This study
pAW008	To integrate a codon optimized *RS14230* and *RS14250* expression cassette into the *P. putida* KT2440 genome at the *crc* locus with simultaneous *crc* deletion	The *RS14230* and *RS14250* expression cassette was amplified from pAW001 with oAW103 and oAW104, assembled into pCJ033 digested with NotI with HiFi DNA Assembly (NEB, Inc.), and transformed into NEB® *E. coli* 5α F'I^q. Construction was confirmed via colony PCR using oAW105 and oAW106 (2.8 kB, Tm=°56) and sequence verified using oAW031, oAW077, oAW105, oAW157, and oAW158 (GENEWIZ.com).	This study
STRAINS			
Escherichia coli			
BL21 Star (DE3)	F$^-$ *ompT hsd*S_B (r_B^-, m_B^-) *gal dcm rne*131 (DE3)	Host for heterologous protein expression using plasmids pET22b-lsdA and pET22b-lsdE	Invitrogen Cat. #C601003
NEB® 5α F'I^q	F′ *proA*$^+$*B*$^+$ *lacI*^{q} Δ*(lacZ)M15 zzf::Tn10* (Tet^R) / *fhuA2*Δ*(argF-lacZ)U169 phoA glnV44 Φ80Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	Host for pK18mobsacB-based vectors (ATCC® 87097™)	NEB Cat. #C2992I
Novosphingobium aromaticivorans			
DSM12444	*Novosphingobium aromaticivorans* (*N. aromaticivorans*) F199	Wild-type *N. aromaticivorans* DSM12444	
JMN2	Evolved mutant of *N. aromaticivorans* F199	Evolved mutant of DSM12444	This study
JMN18	*N. aromaticivorans* JMN2 Δ*RS14250 (lsdA)*	RS14230 was deleted using pJM296. Deletion was confirmed with RS14230 SeqF/SeqR.	This study
JMN19	*N. aromaticivorans* JMN2 Δ*RS14230 (lsdE)*	RS14250 was deleted using pJM297. Deletion was confirmed with RS14250 SeqF/SeqR.	This study
Pseudomonas putida			
KT2440	*P. putida* KT2440	Wild-type *P. putida* KT2440	ATCC® 47054™
AW006	*P. putida* KT2440 *fpvA:*P_{tac}*:RS14230:RS14250*	pAW001 was transformed into KT2440. Integration was confirmed by colony PCR with oAW023 and oAW024 (3.6 kB product, Tm=72°C).	This study
CJ475	*P. putida* KT2440 Δ*catRBC*::P_{tac}*:catA* Δ*pcaHG*::P_{tac}*:aroY:ecdBD fpvA*:P_{tac}*:vanAB*	As described in: Salvachúa, D., Johnson, C. W., Singer, C. A., Rohrer, H., Peterson, D. J., Black, B. A., Knapp, A., Beckham, G. T., 2018. Bioprocess development for muconic acid production from aromatic compounds and lignin. Green Chem. 20, 5007-5019.	[3]

AW049	*P. putida* KT2440 Δ*catRBC*::P_{tac}:*catA* Δ*pcaHG*::P_{tac}:*aroY*:*ecdBD* Δ*crc*::P_{tac}:*RS14230*:*RS14250* *fpvA*:P_{tac}:*vanAB*	pAW008 was transformed into CJ475. Integration was confirmed by colony PCR with oAW080 and oAW105 (1.3 kB, Tm=65°C).	This study

Table S3. Optimized quantifying and qualifying multiple reaction monitoring (MRM) transitions for liquid chromatography-electrospray ionization tandem mass spectrometry analysis.

Analyte Name	Precursor Ion	Ion	MRM quantifying transition	CE (V)	Fragmentor (V)	MRM qualifying transition	CE (V)
tGG bDAP	319.1	$[M-H]^-$	319.1 → 271	8	95	241	28
eGG bDAP	319.1	$[M-H]^-$	319.1 → 271	8	85	212.9	40
tHH bDAP	259.1	$[M-H]^-$	259.1 → 211	8	95	117	72
eHH bDAP	259.1	$[M-H]^-$	259.1 → 211	8	95	182	44
4-Hydroxybenzoic Acid	137	$[M-H]^-$	137 → 93	16	70	65.1	36
4-Hydroxybenzaldehyde	121	$[M-H]^-$	121 → 120	20	98	92	28
Vanillic Acid	167	$[M-H]^-$	167 → 152	12	89	108	20
Vanillin	151	$[M-H]^-$	151 → 136	12	65	92	20

Supplemental Figures

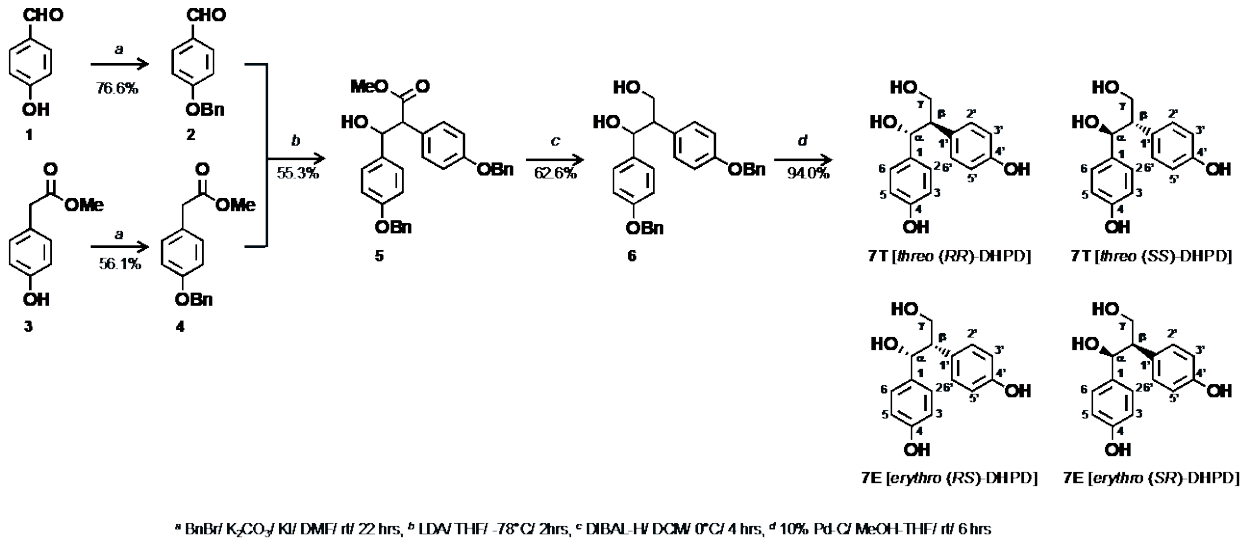

Figure S1. Scheme for the synthesis of the HH β-1 dimer, 1,2-di(p-hydroxyphenyl)propane-1,3-diol (DHPD, compound **7E**/**7T**, **7E**: *erythro*, **7T**: *threo*) was prepared from *p*-hydroxybenzaldehyde (**1**) in 5 steps, similar to a previous synthesis.[5] Benzylated methyl 4-hydroxyphenylacetate (**4**) was condensed with benzylated *p*-hydroxybenzaldehyde (**2**) to obtain γ-methyl ester (**5**) with 55.3 mol% yield, including *erythro* and *threo* isomers. The γ-methyl ester was reduced to γ-hydroxymethyl dimer (**6**) using DIBAL at 0°C with 62.6 mol% yield for the *threo* isomer and 92.1 mol% yield for the *erythro* isomer. The phenolic-OH group in compound **6** was deprotected to obtain DHPD (**7**) at a 94.0 mol% yield for the *threo* isomer (**7T**) and a 56.6 mol% yield for the *erythro* isomer (**7E**). See SI Text 1 for more details of the synthesis.

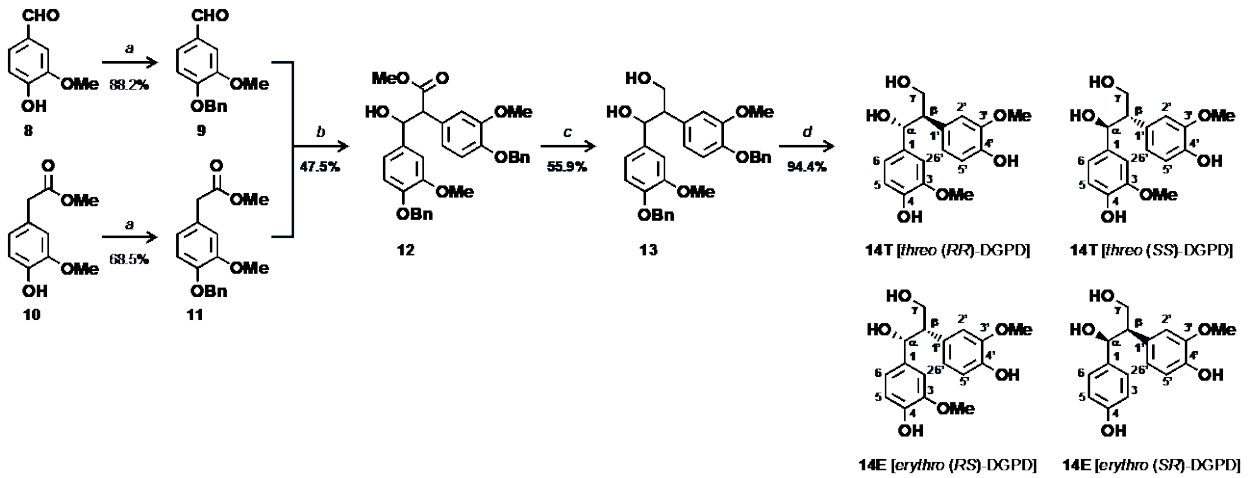

Figure S2. Scheme for the synthesis of the GG β-1 dimer, 1,2-diguaiacylpropane-1,3-diol (DGPD, compound **14E**/**14T**, **14E**: *erythro*, **14T**: *threo*). Benzylated methyl 4-hydroxy-3-methoxyphenylacetate (**11**) was condensed with benzylated vanillin (**9**) to obtain γ-methyl ester (**12**) with a 47.5 mol% yield, including *erythro* and *threo* isomers. The γ-methyl ester was reduced to γ-hydroxymethyl dimer (**13**) using DIBAL at 0°C at a 55.9 mol% yield for the *threo* isomer and a 27.0 mol% yield for the *erythro* isomer. The phenolic-OH group in compound **13** was deprotected to obtain DGPD (**14**) with 94.4 mol% yield of the *threo* isomer (**14T**) and 64.0 mol% yield for the *erythro* isomer (**14E**). See SI Text 1 for more details of the synthesis.

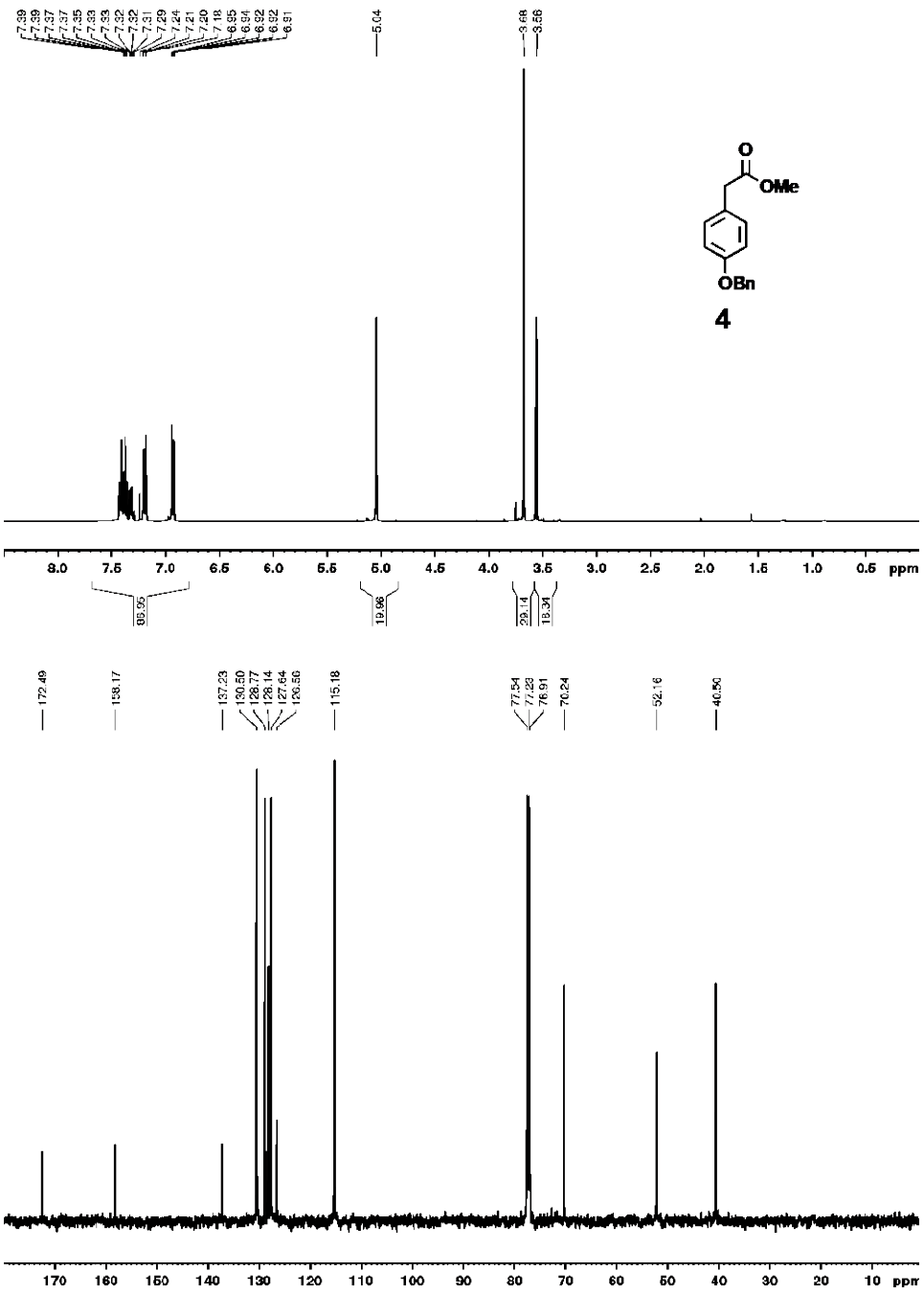

Figure S3. ^{1}H and ^{13}C-NMR ($CDCl_3$) spectra of compound **4**.

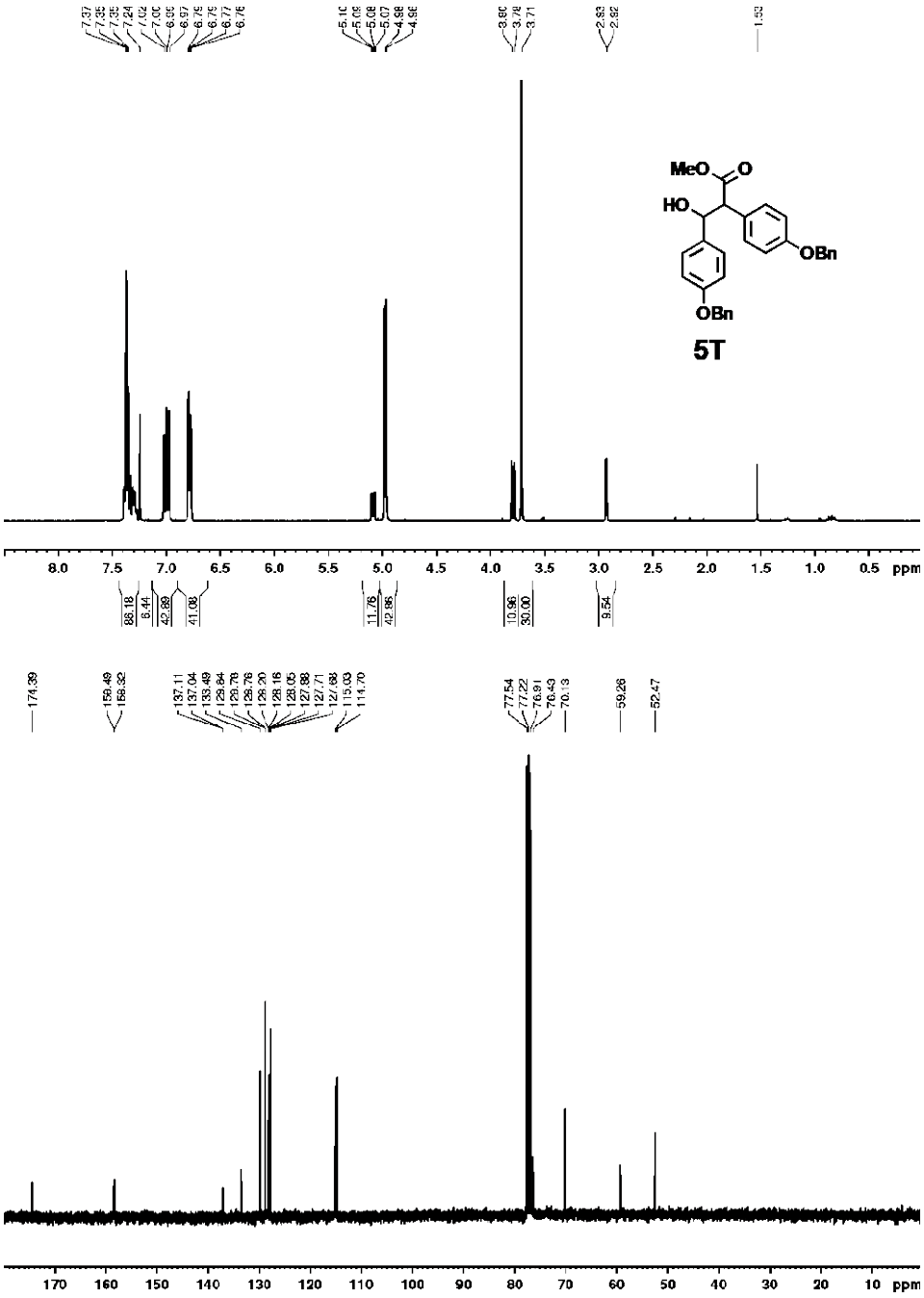

Figure S4. ^{1}H and ^{13}C-NMR ($CDCl_3$) spectra of compound **5T** (*threo* isomer).

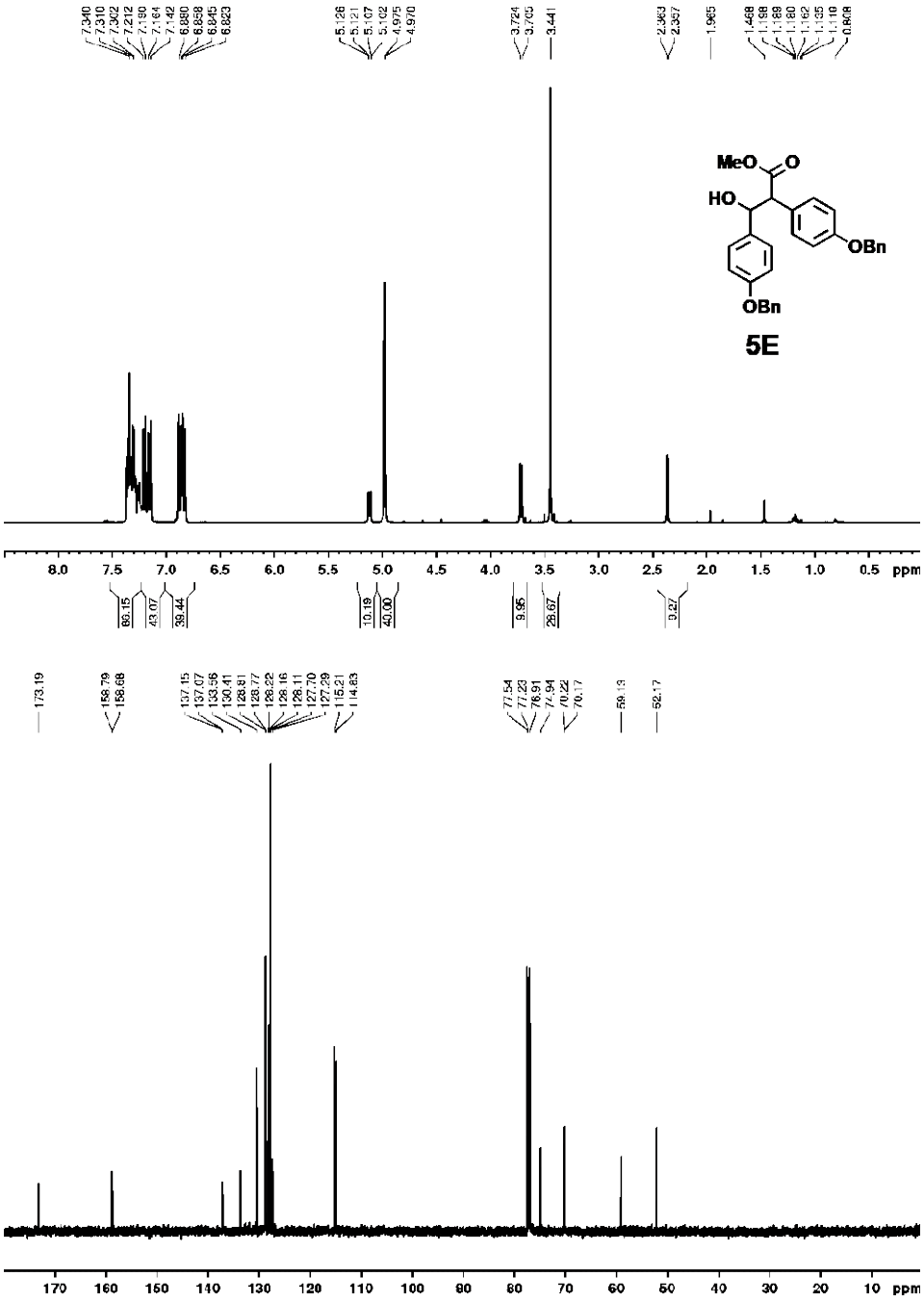

Figure S5. ^{1}H and ^{13}C-NMR ($CDCl_3$) spectra of compound **5E** (*erythro* isomer).

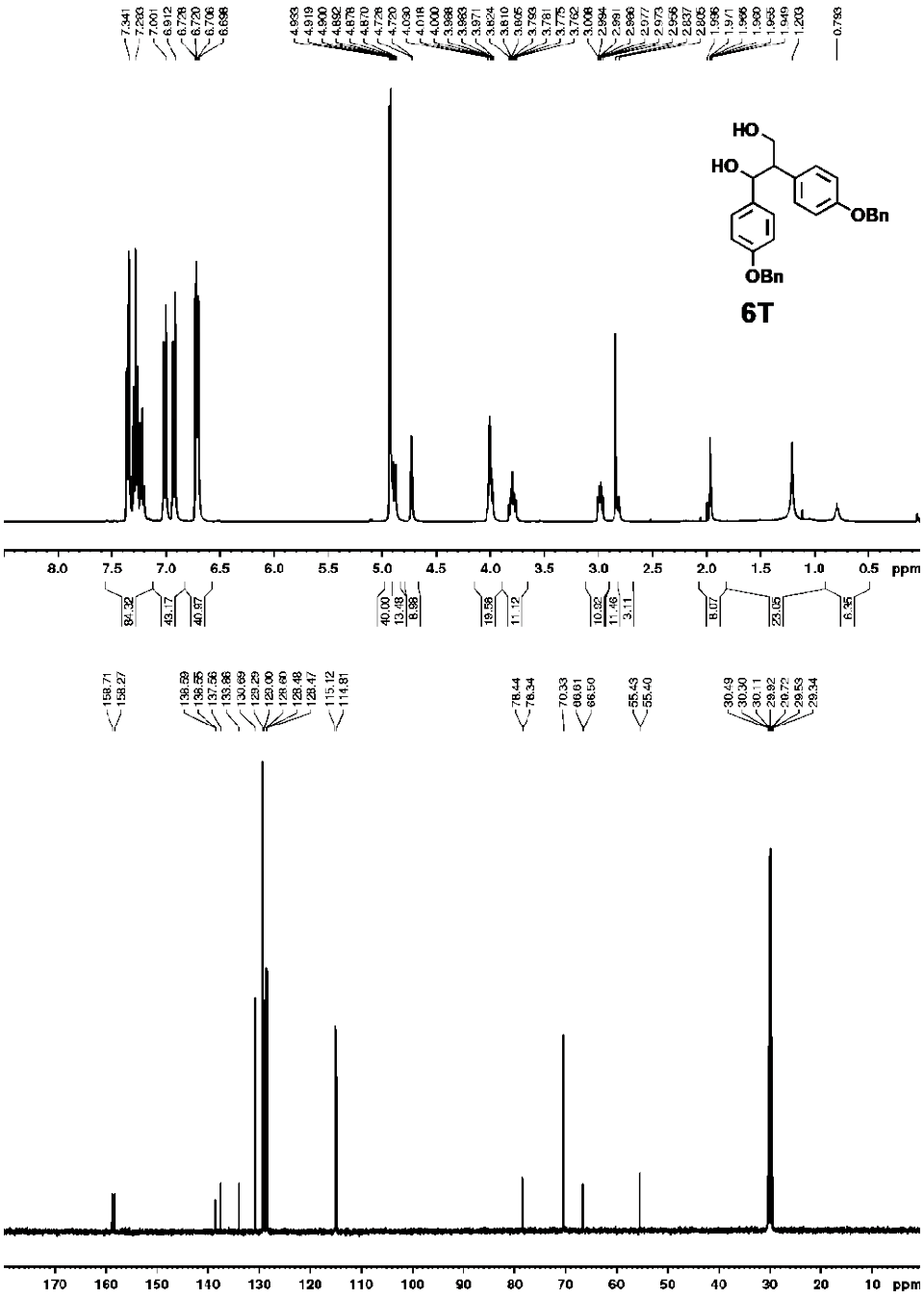

Figure S6. ^{1}H and ^{13}C-NMR ($(CD_3)_2CO$) spectra of compound **6T** (*threo* isomer).

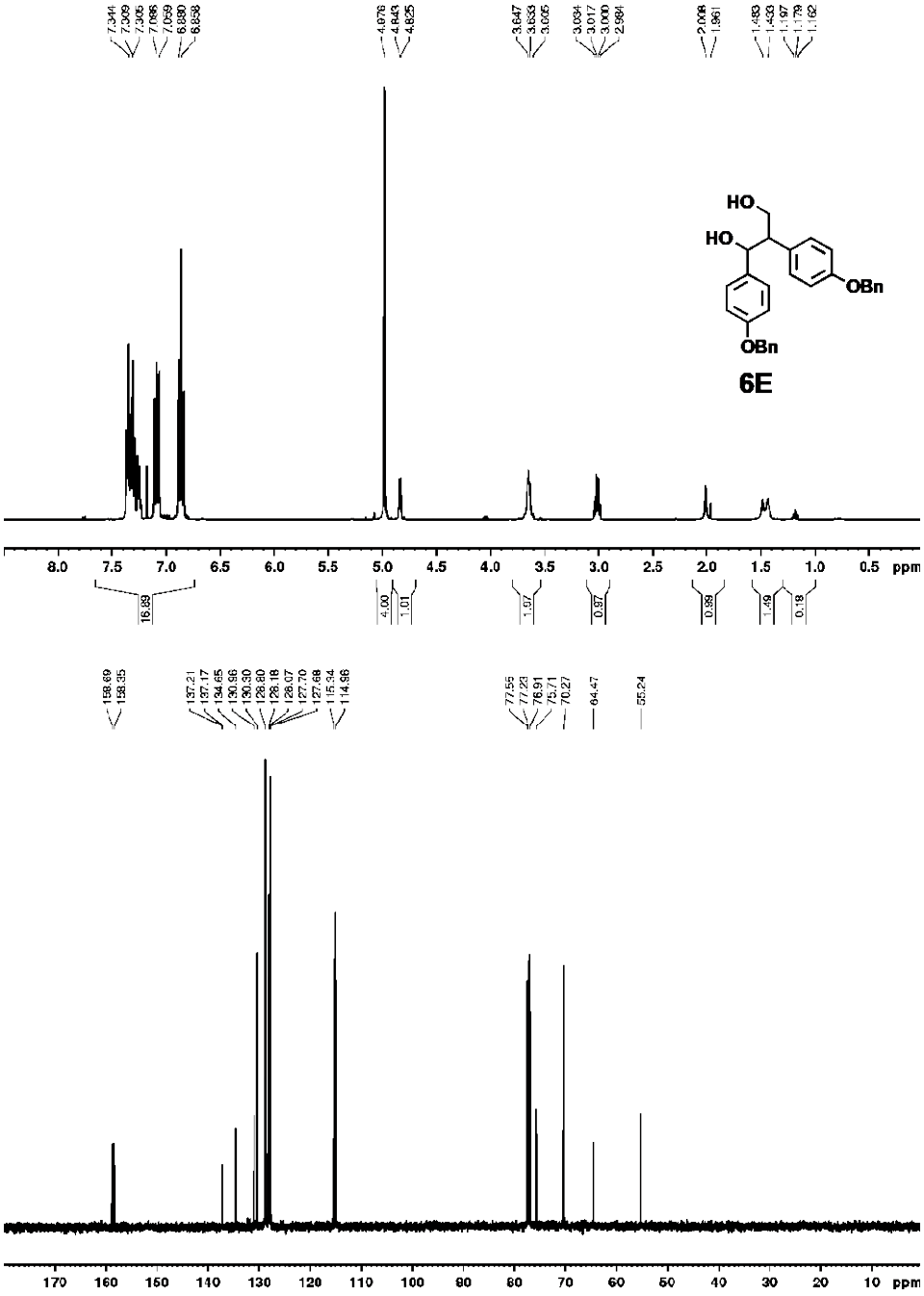

Figure S7. ^{1}H and ^{13}C-NMR ($CDCl_3$) spectra of compound **6E** (*erythro* isomer).

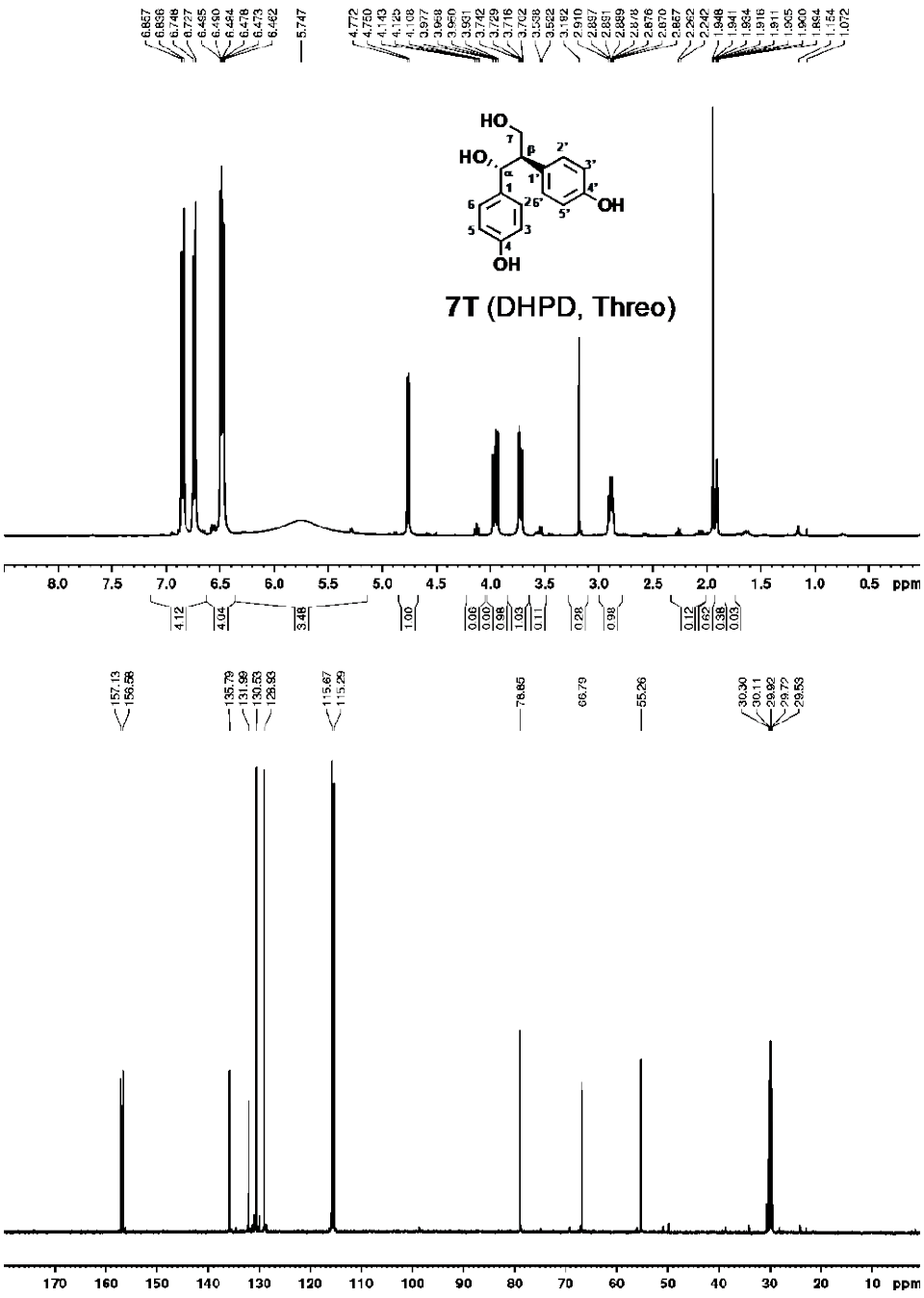

Figure S8. ^{1}H and ^{13}C-NMR ($(CD_3)_2CO$) spectra of compound **7T** (*threo* isomer).

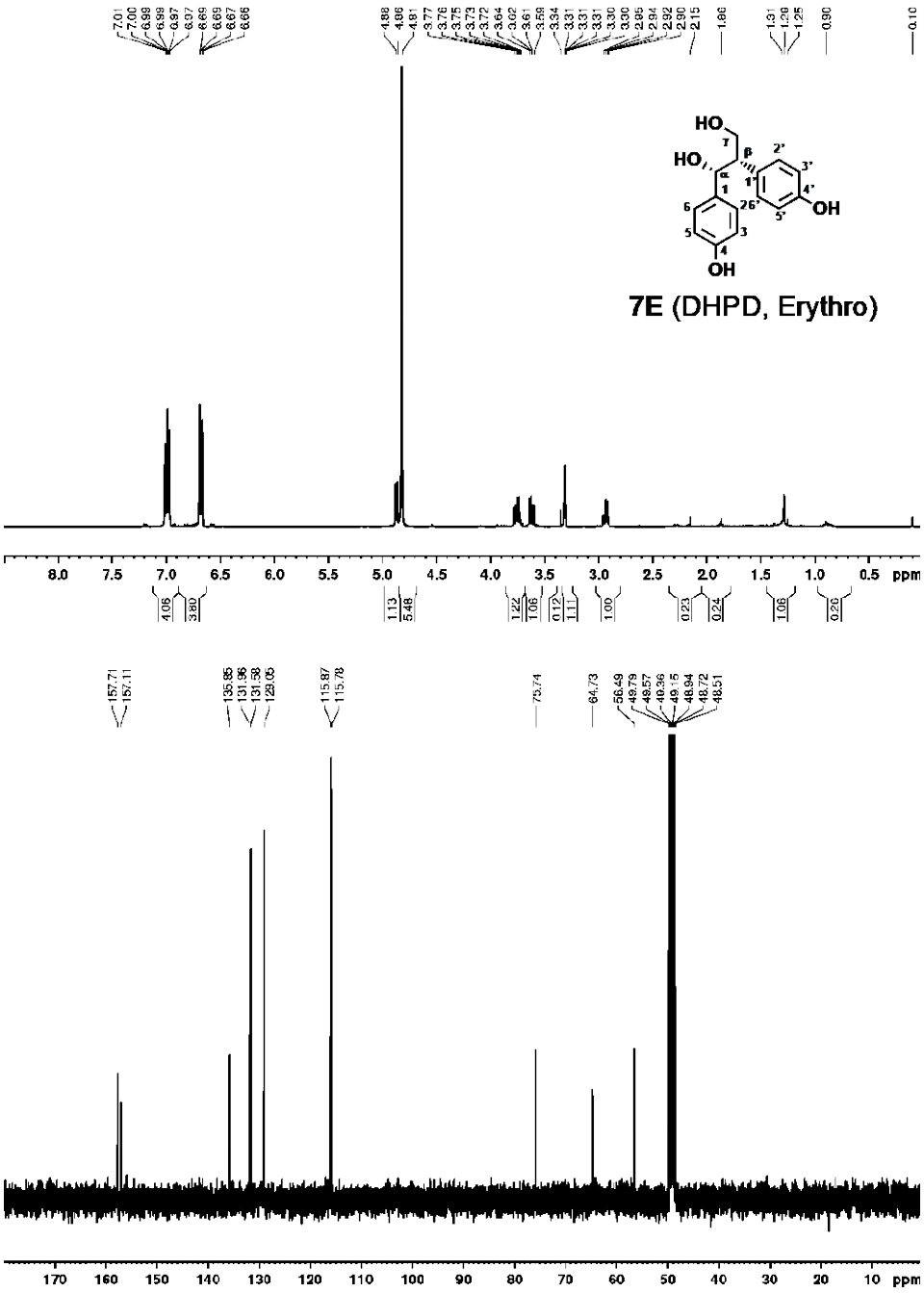

Figure S9. ^{1}H and ^{13}C-NMR (CD_3OD) spectra of compound **7E** (*erythro* isomer).

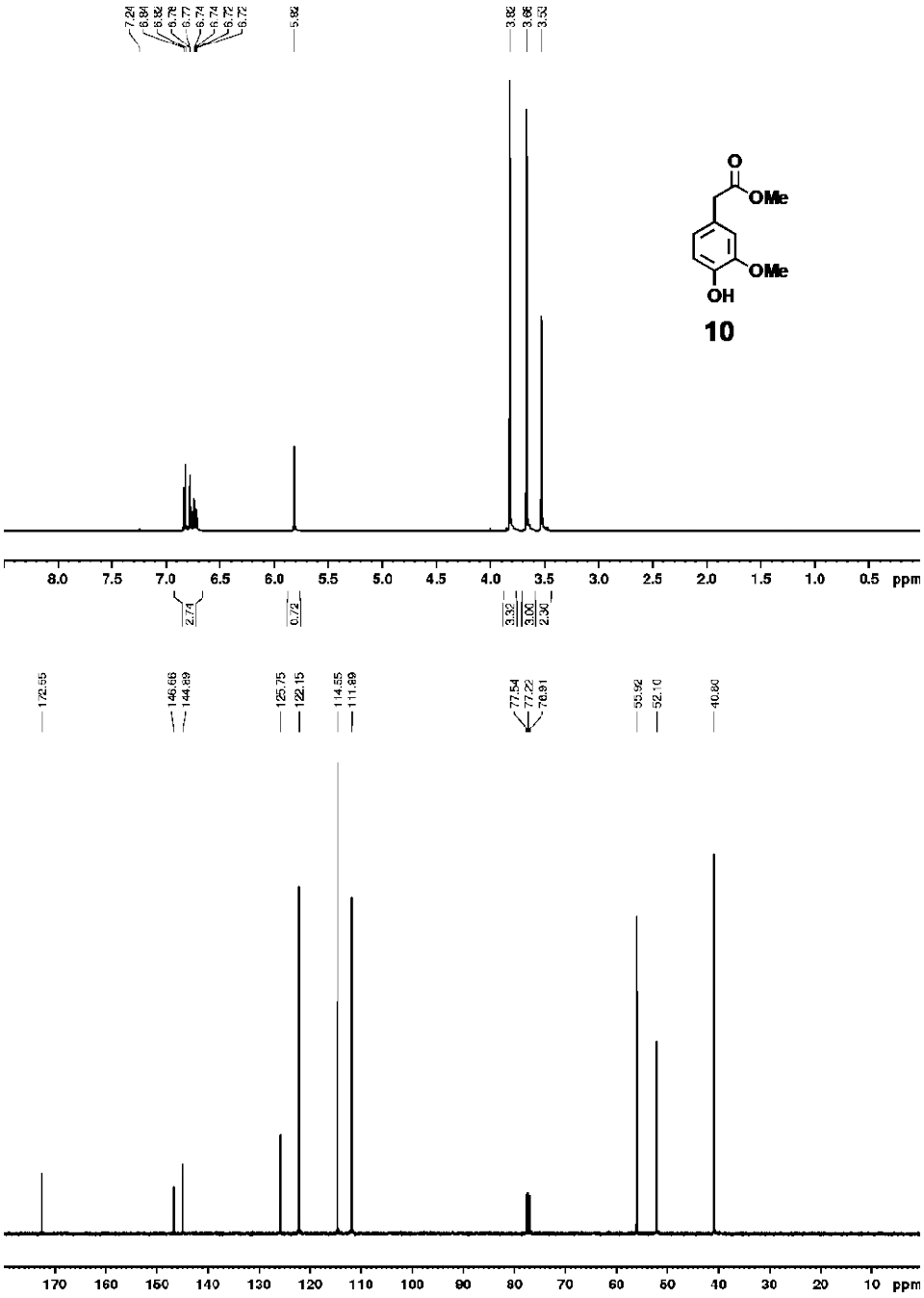

Figure S10. ^{1}H and ^{13}C-NMR ($CDCl_3$) spectra of compound **10**.

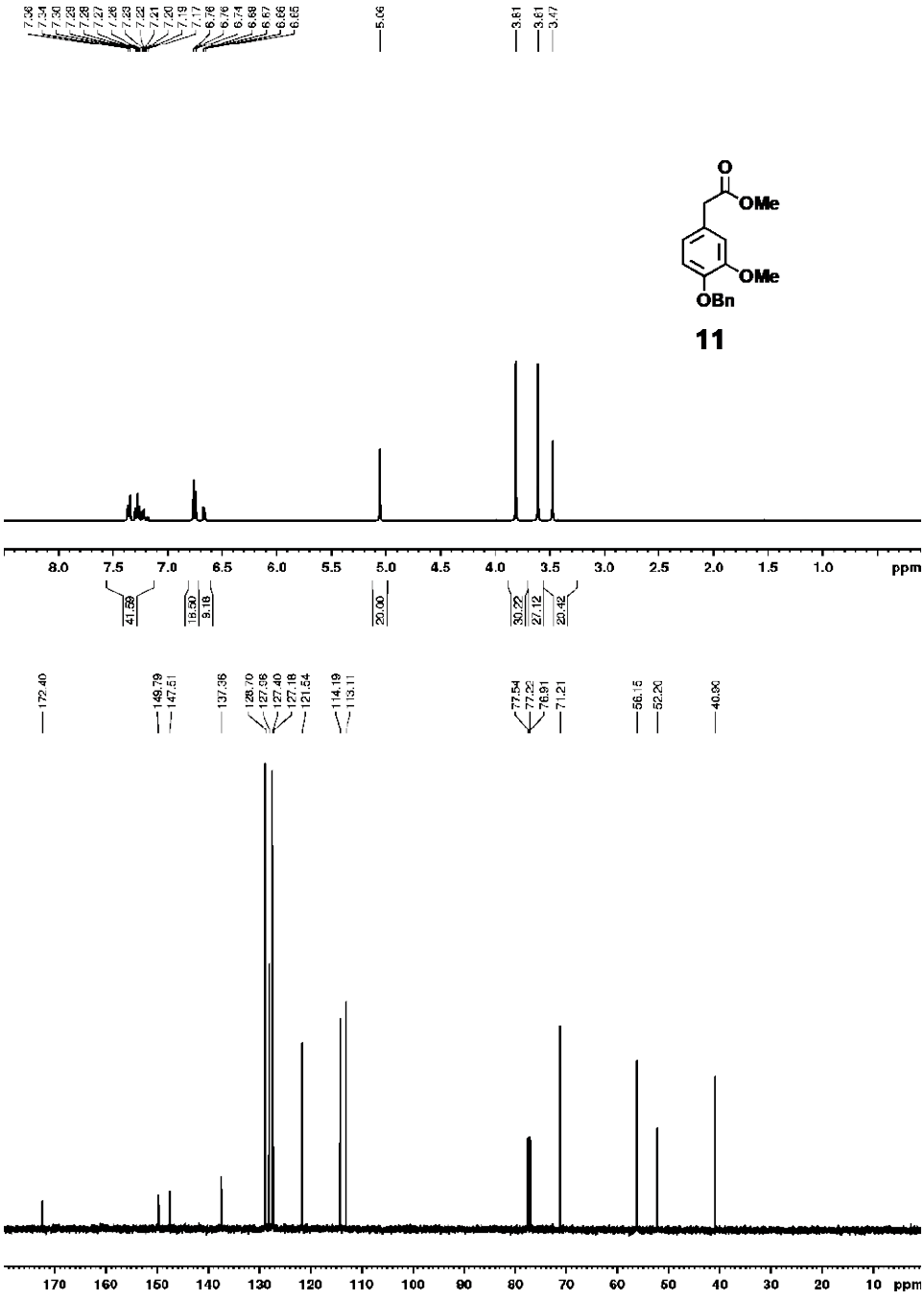

Figure S11. ^{1}H and ^{13}C-NMR ($CDCl_3$) spectra of compound **11**.

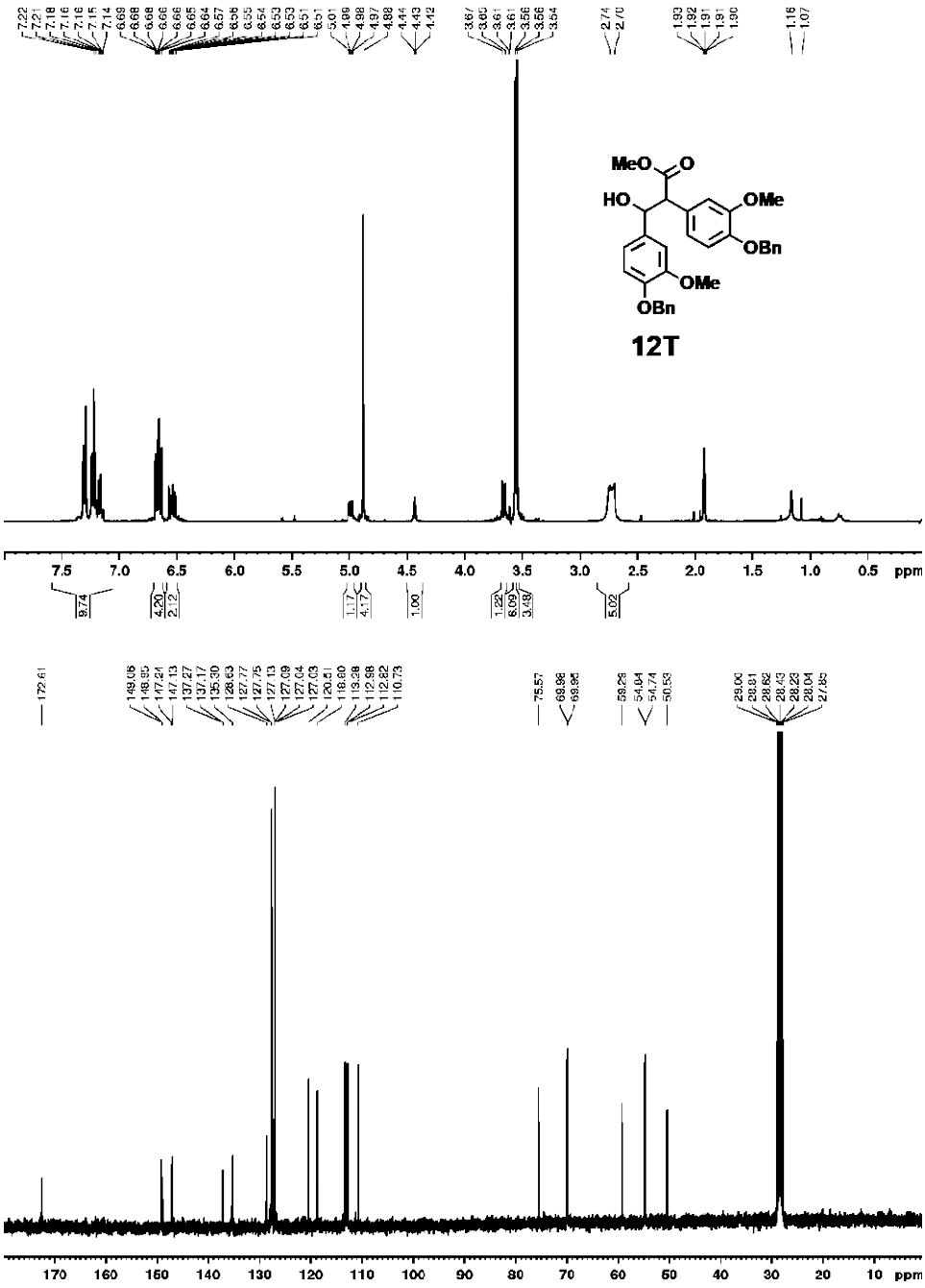

Figure S12. ^{1}H and ^{13}C-NMR ($(CD_3)_2CO$) spectra of compound **12T** (*threo* isomer).

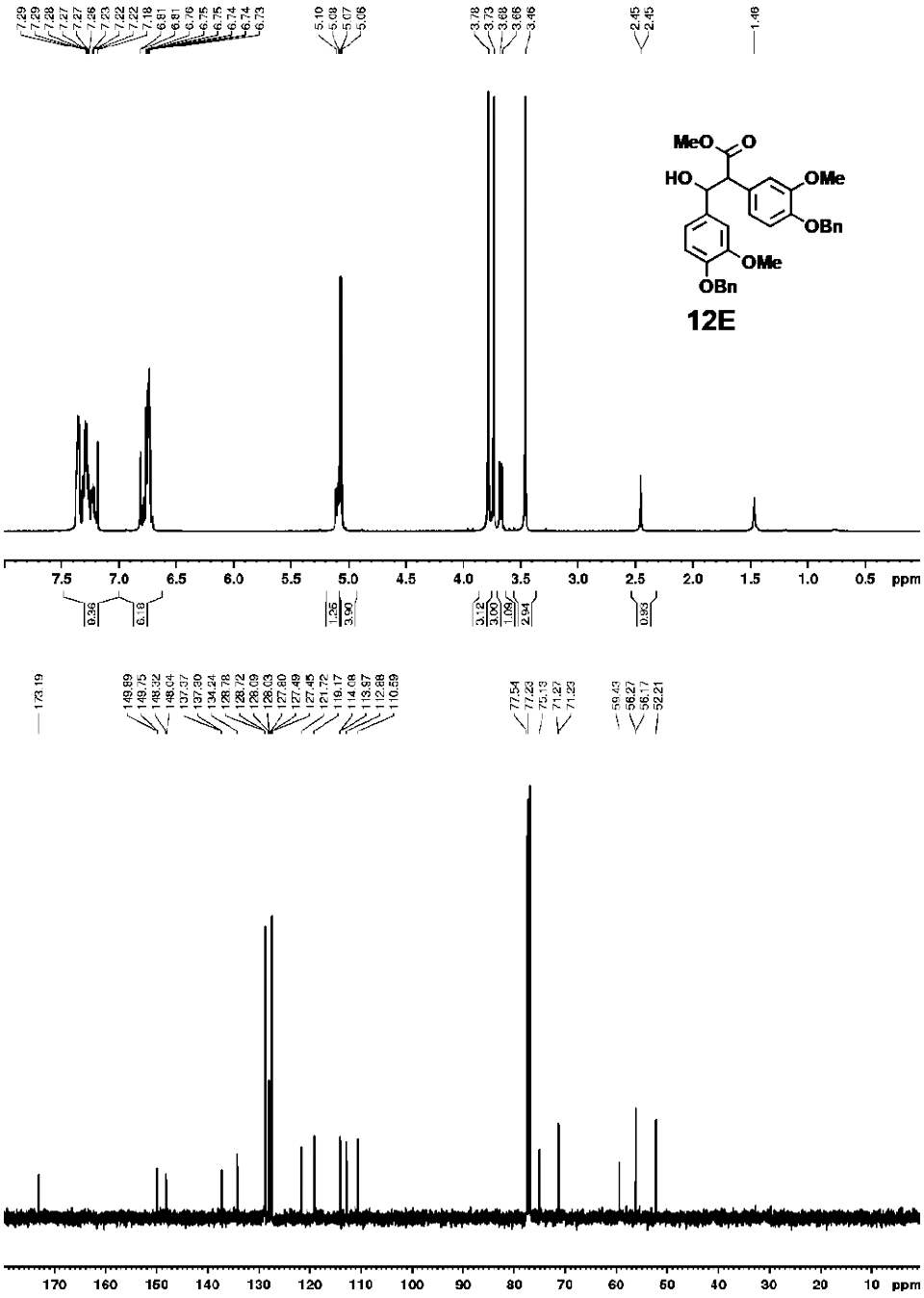

Figure S13. ^{1}H and ^{13}C-NMR ($CDCl_3$) spectra of compound **12E** (*erythro* isomer).

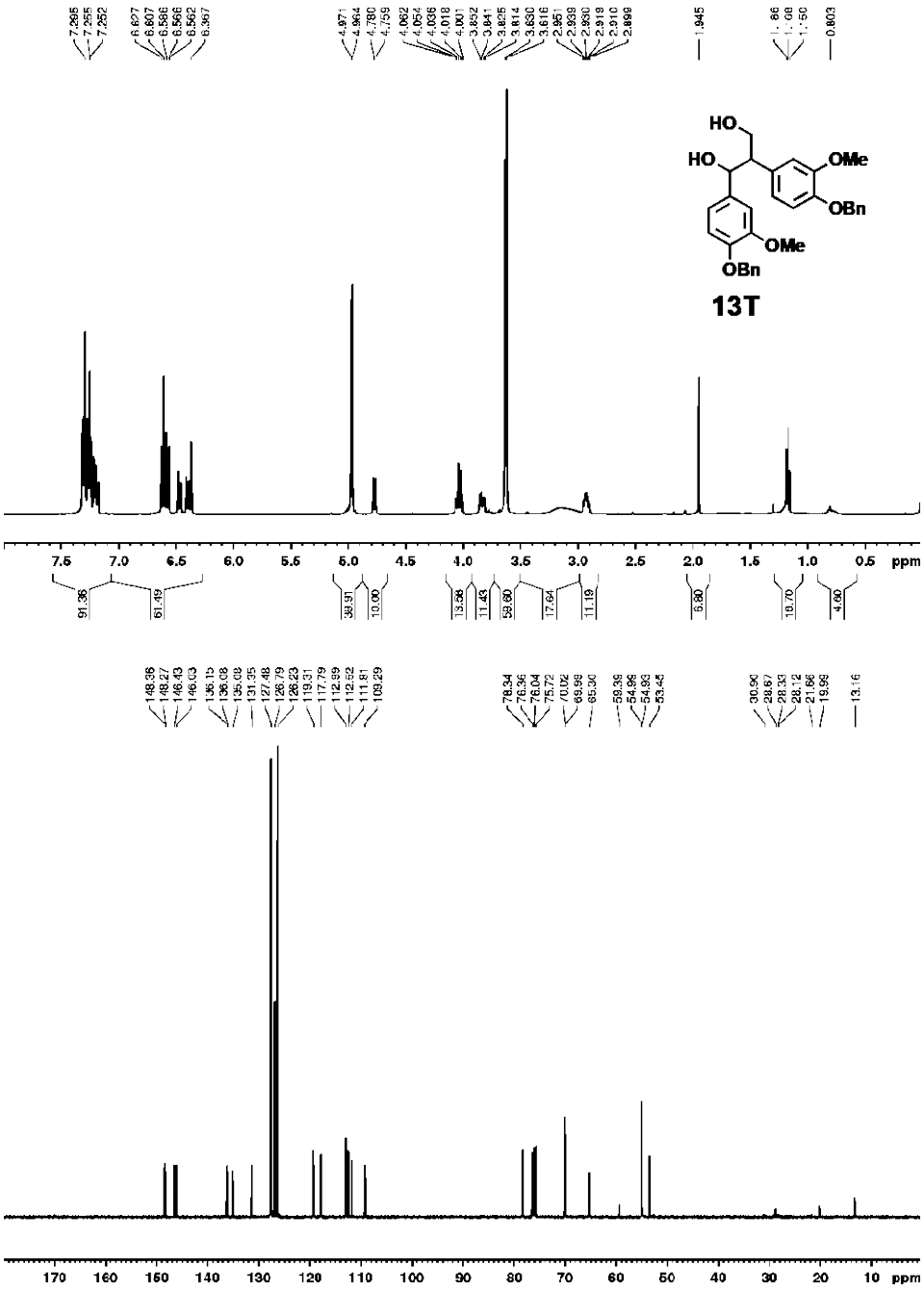

Figure S14. ^{1}H and ^{13}C-NMR ($CDCl_3$) spectra of compound **13T** (*threo* isomer).

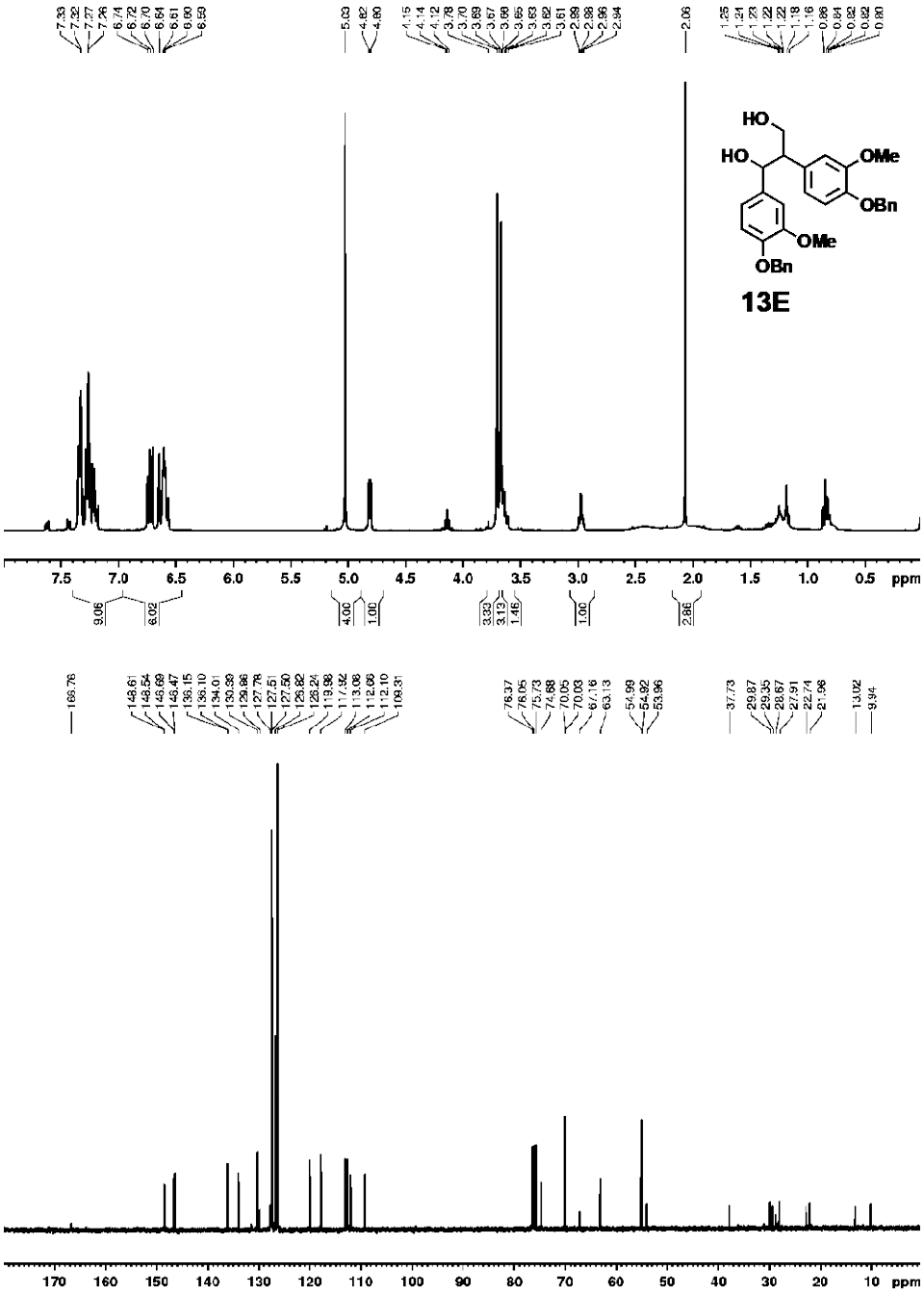

Figure S15. ^{1}H and ^{13}C-NMR ($CDCl_3$) spectra of compound **13E** (*erythro* isomer).

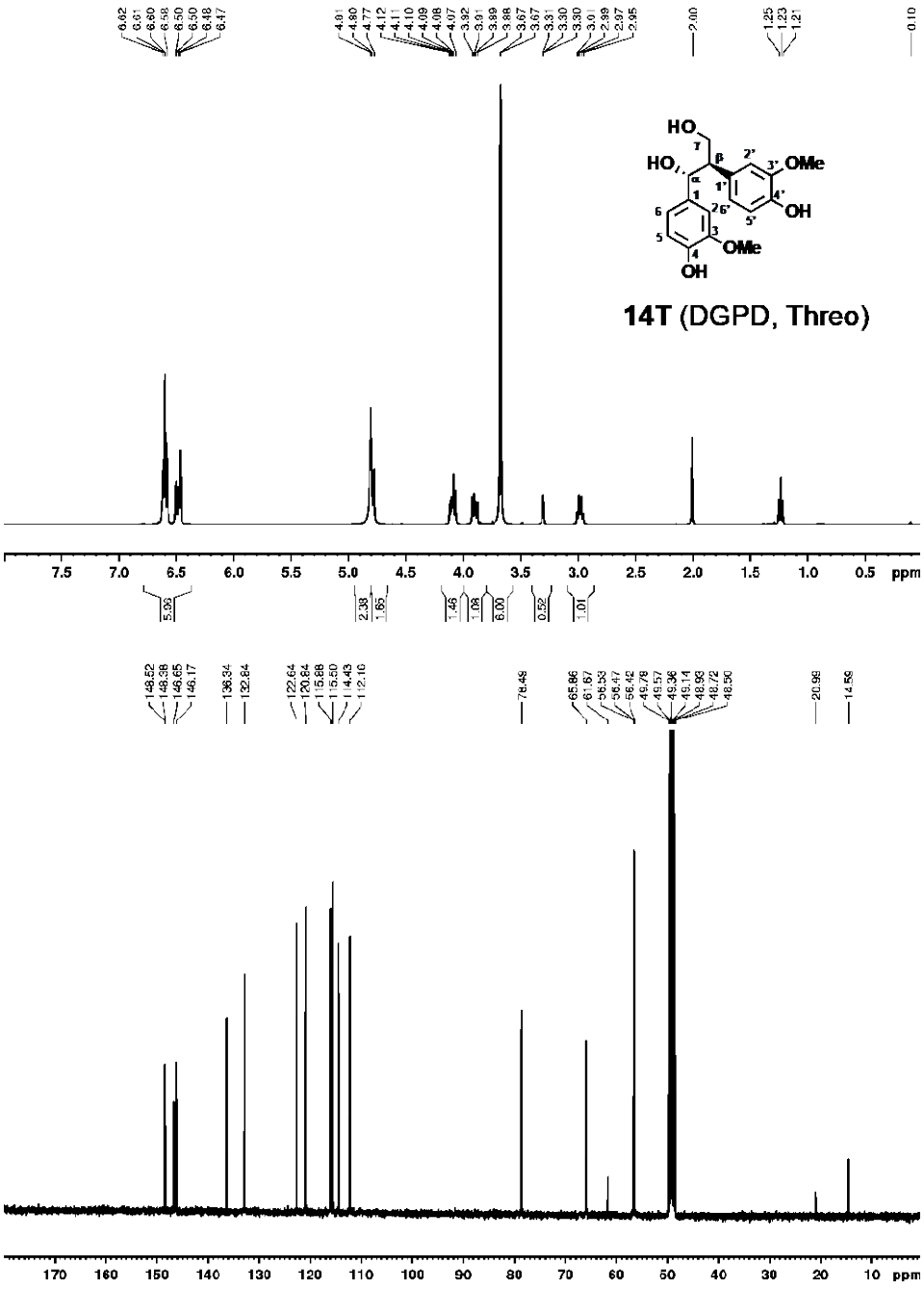

Figure S16. ^{1}H and ^{13}C-NMR (CD_3OD) spectra of compound **14T** (*threo* isomer).

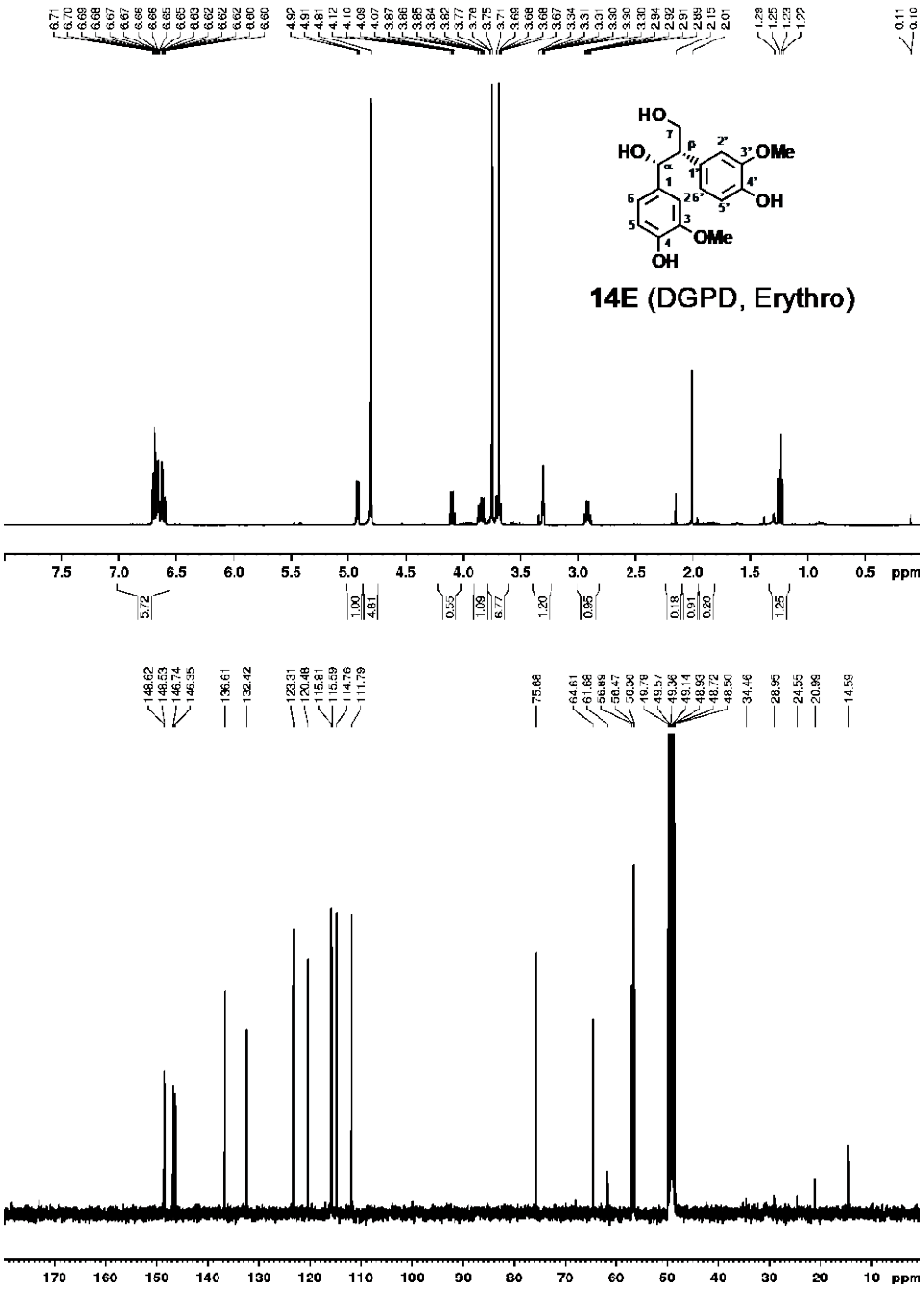

Figure S17. ^{1}H and ^{13}C-NMR (CD_3OD) spectra of compound **14E** (*erythro* isomer).

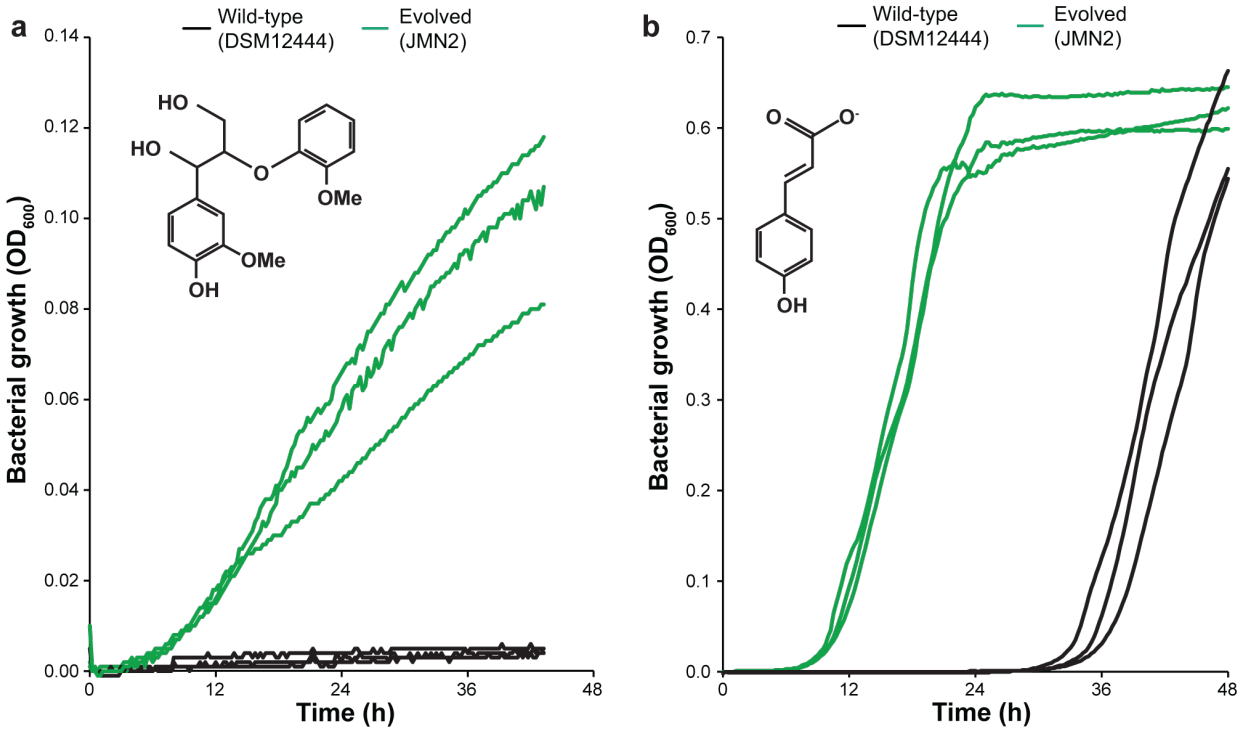

Figure S18. Evolved *N. aromaticivorans* strain JMN2 shows improved growth with a variety of lignin-derived aromatic compounds. Wildtype DSM12444 and evolved JMN2 were grown in minimal medium with 1 g/L of either (a) GGE or (b) *p*-coumaric acid as the sole carbon source. Three biological replicates are shown for each strain and medium.

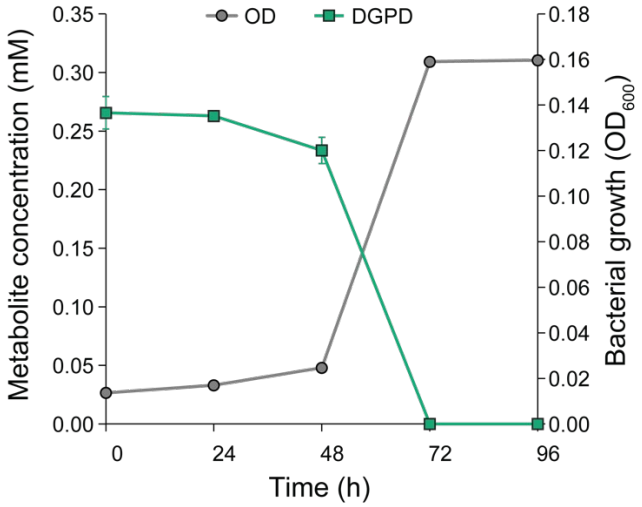

Figure S19. In minimal medium, DGPD consumption by JMN2 is growth-associated. Strain JMN2 was grown in minimal medium with DGPD as the sole carbon source. Cultures were assayed every 24 hours for cell growth and residual DGPD concentration. Error bars show one standard deviation, calculated from three biological replicates.

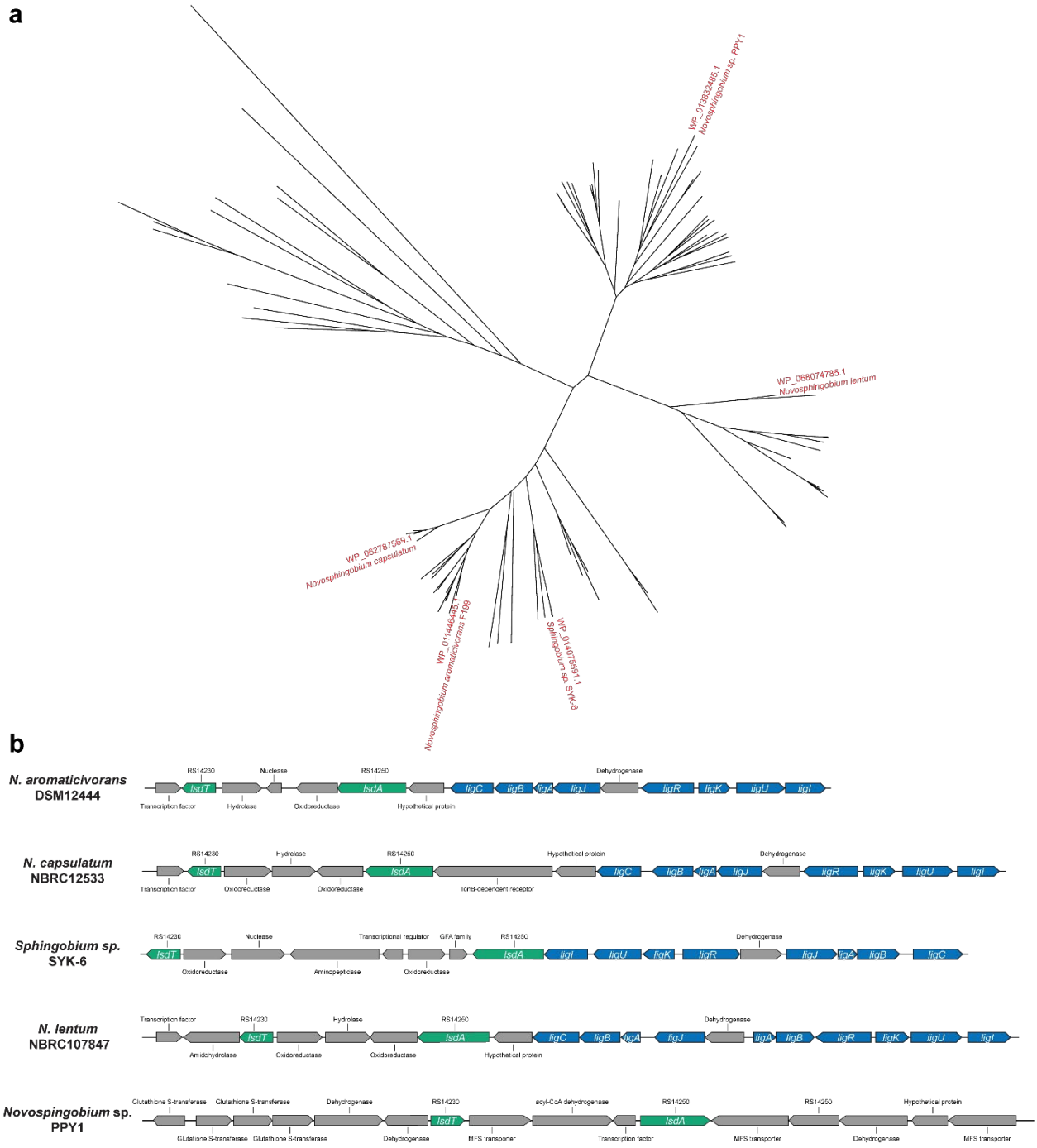

Figure S20. (**a**) Maximum-likelihood phylogenetic tree of LsdE sequences. Representative strains from the major branches are highlighted in red and were examined in more detail. (**b**) Genomic arrangement of *lsdE* and *lsdA* in selected genome sequences. Genes for the PCA 4,5-cleavage pathway are shown in blue. *lsdE* was not found in close proximity to *lsdA* or the PCA degradation pathway genes in the fourth cluster of (**a**).

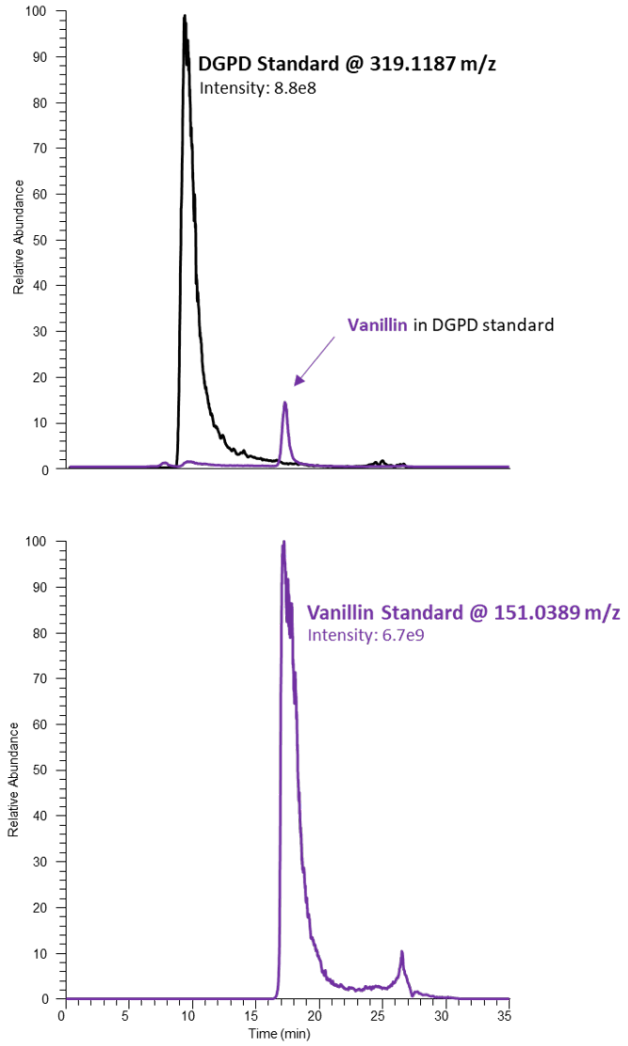

Figure S21. LC-MS analysis of DGPD stock identifies trace contamination with vanillin. The DGPD stock used for experiments with *E. coli* lysates expressing heterologous LsdA and LsdE contained small amounts of vanillin before addition of lysate.

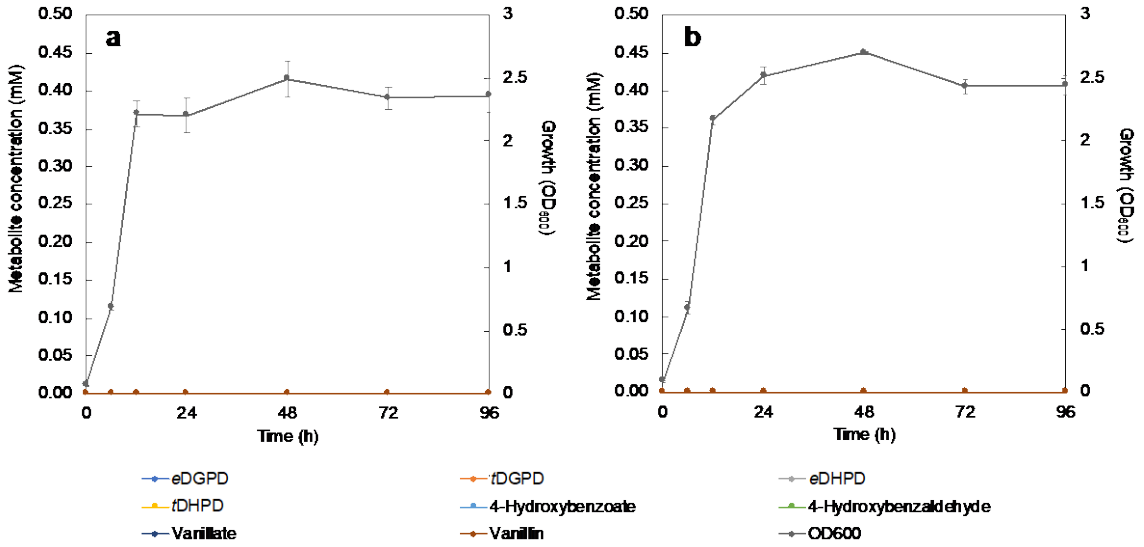

Figure S22. Growth of (**a**) *P. putida* wild-type and (**b**) AW006 (*P. putida fpvA*::P_{tac}:*lsdT:lsdA*) in M9 minimal media supplemented with 20 mM glucose and 2% (v/v) methanol. Metabolite concentrations of all analytes and growth (optical density, 600nm) are plotted. Error bars represent the standard derivation across biological triplicates.

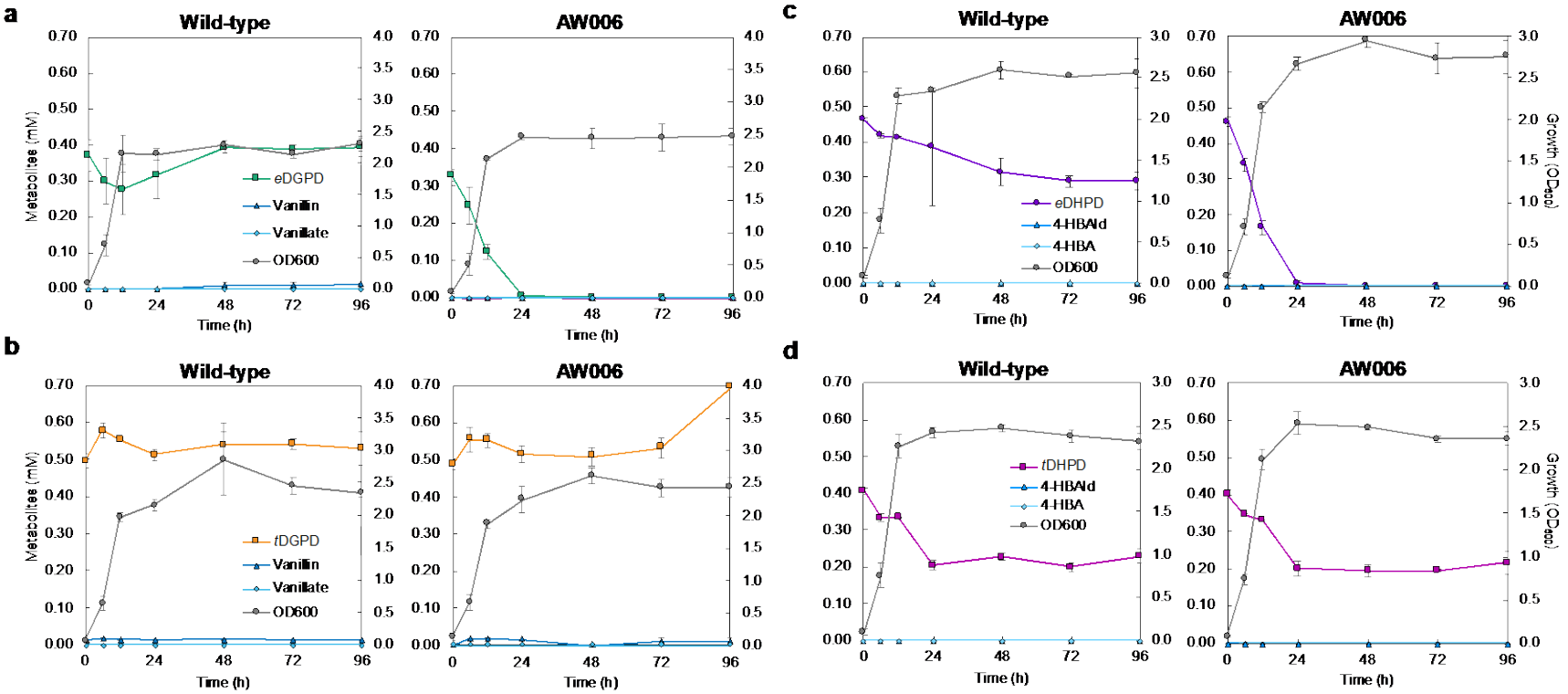

Figure S23. Metabolite concentration and growth (OD_{600}) across the 96 h experiment. Wild-type *P. putida* or AW006 (*P. putida fpvA*::P_{tac}:*RS14230:RS14250*) cultivations were performed in M9 minimal media supplemented with 20 mM glucose and (**a**) *erythro*-DGPD (*e*DGPD), (**b**) *threo*-DGPD (*t*DGPD), (**c**) *erythro*-DHPD (*e*DHPD), or (**d**) *threo*-DHPD (*t*DHPD). Metabolites were quantified from the cultivation supernatant. Error bars represent the standard deviation across biological triplicates. Abbreviations: 4-HBAld, 4-hydrobenzaldehyde; 4-HBA, 4-hydroxybenzoate; OD600, optical density (600 nm).

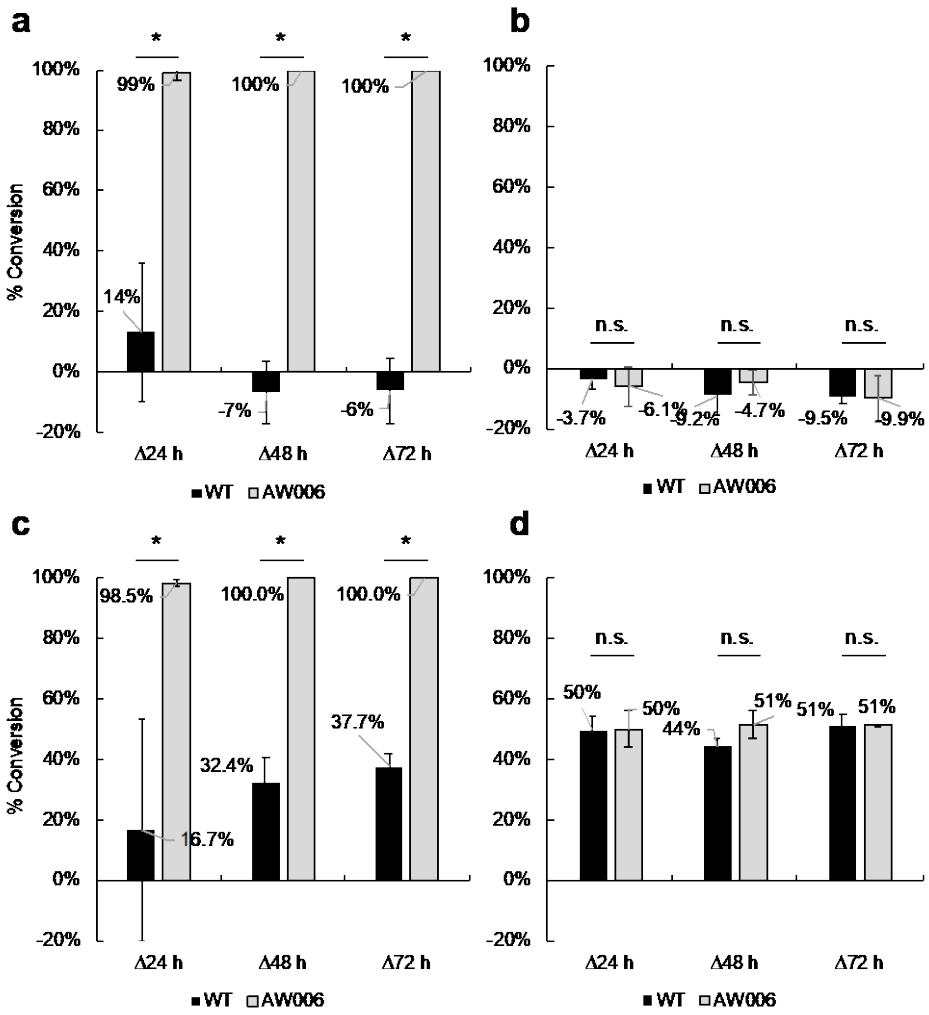

Figure S24. Dimer conversion (%) after 24 h (Δ24 h), 48 h (Δ48 h), or 72 h (72 h) for wild-type *P. putida* or AW006 (*P. putida fpvA*::P_{tac}:*lsdT:lsdA*) cultivated in M9 minimal medium supplemented with 20 mM glucose and (**a**) *erythro*-DGPD, (**b**) *threo*-DGPD, (**c**) *erythro*-DHPD, or (**d**) *threo*-DHPD. Error bars represent the standard deviation across biological triplicates. * indicates $p \leq 0.05$ and n.s. indicates $p > 0.05$ from a paired one-tailed Student's *t*-test comparing the percent conversion observed by WT versus AW006 cultivations.

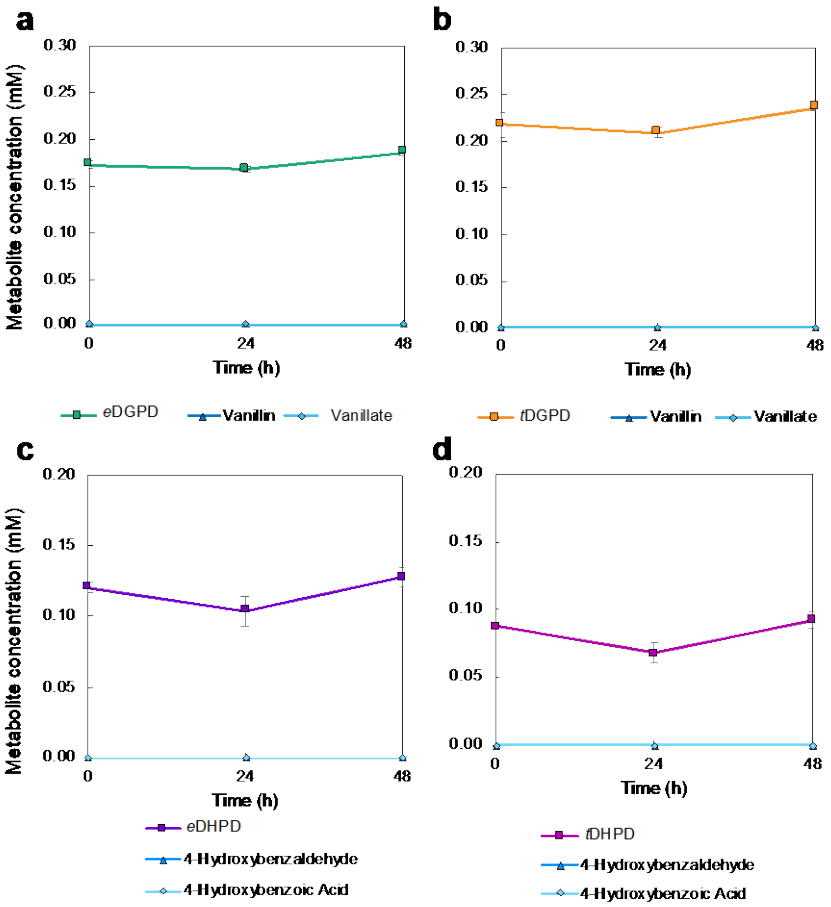

Figure S25. Metabolite concentration over time in non-inoculated controls. Cultivations without the addition of cells were performed in M9 minimal media supplemented with 20 mM glucose and (**a**) *erythro*-DGPD, (**b**) *threo*-DGPD, (**c**) *erythro*-DHPD, or (**d**) *threo*-DHPD. Error bars represent the standard deviation across biological triplicates.

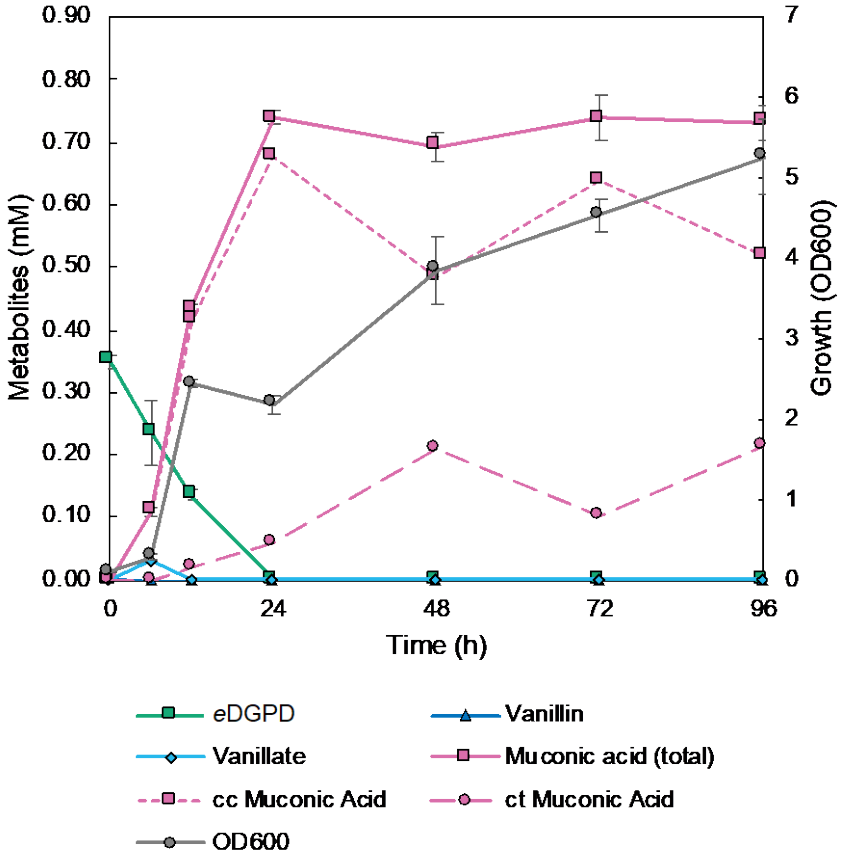

Figure S26. Growth and metabolite concentration across 96 h of AW049 (*P. putida* KT2440 Δ*catRBC*::P_{tac}:*catA* Δ*pcaHG*::P_{tac}:*aroY*:*ecdBD* Δ*crc*::P_{tac}:*lsdT*:*lsdA fpvA*::P_{tac}:*vanAB*) cultivations in M9 minimal media supplemented with 20 mM glucose and 0.35 mM *erythro*-DGPD (*e*DGPD). Glucose was fed to 20 mM every 24 h. Metabolites were quantified from the cultivation supernatant. Muconate was quantified as both *cis,cis*- (cc Muconic acid) and *cis,trans*- (ct Muconic acid) muconic acid. Error bars represent the standard deviation across biological triplicates.

Supplemental Text

SI Text 1. Dimer synthesis and NMR acquisition details. See Scheme 1-2 (**Figure S1-S2**) for compound number references.

General information for synthesis of model compounds
All chemicals and solvents were used without additional purification. Flash chromatography was performed using Teledyne CombiFlash equipped with Teledyne 80 and 120 g prepared column for further purification. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE 400 MHz spectrometer equipped with a 5 mm BBO probe (^{1}H 400 MHz, ^{13}C 100 MHz). Chemical shifts for ^{1}H NMR spectra are reported in parts per million (ppm) from tetramethylsilane as a reference of 0 ppm. ^{1}H-NMR spectra were recorded with a relaxation delay 1.0 s and an acquisition time of 4.09 s with 64 scans. Chemical shifts for ^{13}C NMR spectra are reported in ppm from the solvent as a reference ($CDCl_3$: δ 77.23 ppm, $(CD_3)_2CO$: 29.92 ppm, CD_3OD: 49.15 ppm). The acquisition parameters for ^{13}C-NMR included a 90° pulse width, a relaxation delay 1.0 s and an acquisition time of 1.36 s with 1024 scan. 2D-NMR spectra [gradient correlation spectroscopy (gCOSY), heteronuclear single quantum coherence (HSQC)] were recorded using standard Bruker implementation.

Synthesis method for 1,2-di(p-hydroxyphenyl)propane-1,3-diol (compound 7, DHPD)
*Benzylation (compound **2**)*
To the solution of 4-hydroxybenzaldehyde (10.0 g, 82.3 mmol) in DMF (5 mL), benzyl bromide (12.0 mL, 98.7 mmol), potassium carbonate (17.1 g, 123.4 mmol) and potassium iodate (1.36 g, 8.23 mmol) were added at room temperature. After stirring for 19.5 hours, the reaction mixture was filtered to remove excess K_2CO_3. The reaction mixture was diluted with deionized H_2O and extracted with ethyl acetate three times. The organic layer was washed with brine two times and then dried over Na_2SO_4. A crude oil obtained was recrystallized from ethanol to yield a white powder (compound **2**, 13.1 g) with 76.6 mol% yield.

*Benzylation (compound **4**, **Figure S3**)*
Compound **3** was benzylated in the same method of compound **2**. To the solution of methyl 4-hydroxyphenylacetate (compound **3**, 3.0 g, 18.1 mmol) in DMF (5 mL), benzyl bromide (2.64 mL, 21.7 mmol), potassium carbonate (3.75 g, 27.2 mmol) and potassium iodate (0.3 g, 1.81 mmol) were added at room temperature. After stirring for 22 hours, the reaction mixture was treated in the same manner as compound **1**. The reaction mixture was recrystallized from mixture of ethyl acetate and hexane to obtain a white powder (compound **4**, 2.48 g, 56.1 mol%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.43 (5H, m), 7.19 (2H, d, *J*=8.6Hz, Arom-H), 6.93 (2H, d, *J*=8.6Hz, Arom-H), 5.04 (2H, s, CH_2), 3.68 (3H, s, $COOCH_3$), 3.56 (2H, s, Hα). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5 (Cβ), 158.2, 137.2, 130.5, 128.8, 128.1, 127.6, 126.6, 115.2, 70.2 (CH_2), 52.2 ($COOCH_3$), 40.5 (Cα) ppm.

*Coupling (compound **5**, **Figure S4-5**)*
To the solution of lithium diisopropylamide (2 M in THF, 3.73 mL, 7.46 mmol) in anhydrous THF (10 mL), benzylated methyl 4-O-benzylphenylacetate (compound **4,** 0.91 g, 3.73 mmol) in anhydrous THF (10 mL) was added dropwisely for over 15 min at -78°C. After stirring for 30 min, benzylated vanillin (compound **2,** 0.79 g, 3.73 mmol) was added drop-wise for over 1 hour. After stirring for 2 hours, the reaction solution was heated from -78°C to ambient temperature, and quenched by addition of H_2O (50 mL). The reaction mixture was acidified with 1 N HCl, extracted with ethyl acetate three times, washed with brine, and dried over Na_2SO_4. A crude oil was purified by a silica gel column to obtain of γ-methyl ester (compound **5**) with the *threo* isomer (0.682 g, 39.1 mol%) and the *erythro* isomer (0.283 mg, 16.2 mol%). *Threo* isomer (**5T**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.39 (10H, m), 7.01 (2H, d, *J*=8.7Hz, Arom-H), 6.98 (2H, d, *J*=8.7Hz, Arom-H), 6.78 (2H, d, *J*=8.8Hz, Arom-H), 6.77 (2H, d, *J*=8.8Hz, Arom-H), 5.08 (H, dd, *J*=9.4, 3.8Hz, Ha), 4.98 (2H, s, CH_2), 4.96 (2H, s, CH_2), 3.79 (H, d, *J*=9.4Hz, Hb), 3.71 (3H, s, $COOCH_3$), 2.92 (H, d, *J*=3.8Hz, OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4 (Cγ), 158.5, 158.3, 137.1, 137.0, 133.5, 129.8, 128.8, 128.7, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 115.0, 114.7, 76.4 (Cα), 70.1 (CH_2), 59.3 (Cβ), 52.5 ($COOCH_3$) ppm. *Erythro* isomer (**5E**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17-7.36 (10H, m), 7.18 (4H, dd, *J*=19.2, 8.7Hz), 6.85 (4H, dd, *J*=14.0, 8.7Hz), 5.11 (H, dd, *J*=7.8, 2.1Hz, Hα), 4.98 (2H, s, CH_2), 4.97 (2H, s, CH_2), 3.71 (H, d, *J*=7.8Hz, Hβ), 3.44 (3H, s, $COOCH_3$), 2.36 (H, d, *J*=2.3Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2 (Cγ), 158.8, 158.7, 137.2, 137.1, 133.6, 130.4, 128.8, 128.7, 128.22, 128.16, 128.1, 127.7, 127.3, 115.2, 114.8, 74.9 (Cα), 70.2 (CH_2), 70.1 (CH_2), 59.1 (Cβ), 52.2 ($COOCH_3$) ppm.

*Reduction (compound **6**, **Figure S6-7**)*
To a solution of the γ-methyl ester (compound **5T**, *threo* isomer, 0.62 mg, 1.32 mmol) in anhydrous dichloromethane (15 mL), DIBAL in DCM (5.3 mL, 5.3 mmol) was added drop-wise at 0°C. After stirring for 4 hours at the same temperature, deionized H_2O (20 mL) was added to quench the reaction. The reaction mixture was gelated by acidification with saturated citric acid. After dilution with deionized H_2O (30 mL), the solution was extracted with ethyl acetate (50 mL) three times. The combined organic layer was washed with brine and dried over Na_2SO_4. A crude oil was recrystallized from mixture of ethyl acetate and hexane to obtain a white powder (compound **6T**, *threo* isomer, 0.365 g, 62.6 mol%). The *erythro* isomer of compound **5E** was reduced in the same way to obtain a white crystal with 92.1 mol% yield. *Threo* isomer (**6T**): ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 7.20-7.36 (10H, m), 6.91-7.03 (4H, m, Arom-H), 6.69-6.74 (4H, m, Arom-H), 4.93 (2H, s, CH_2), 4.92 (2H, s, CH_2), 4.89 (H, dd, *J*=8.8, 3.2Hz, Hα), 4.00 (H, m, Hγ), 3.79 (H, m, Hγ), 2.98 (H, m, Hβ). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 158.7, 158.3, 138.6, 138.5, 137.6, 133.9, 130.7, 129.3, 129.0, 128.6, 128.5, 128.4, 115.1, 114.8, 78.4 (Cα), 70.3 (CH_2), 66.6 (Cγ), 55.4 (Cβ) ppm. *Erythro* isomer (**6E**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-7.37 (10H, m), 7.06-7.11 (4H, m, Arom-H), 6.83-6.89 (4H, m, Arom-H), 4.98 (4H, s, 2xCH_2), 4.83 (H, d, *J*=7.2Hz, Hα), 3.63 (2H, m, Hγ), 3.01 (H, dt, *J*=7.2, 6.6Hz, Hβ). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 158.4, 137.2, 137.1, 134.7, 131.0, 130.3, 128.8, 128.2, 128.1, 127.7, 127.6, 115.3, 115.0, 75.7 (Cα), 70.3 (CH_2), 64.5 (Cγ), 55.2 (Cβ) ppm.

*Deprotection (compound 7, DHPD, **Figure S8-9**)*
To the solution of compound **6T**- *threo* (108 mg, 0.245 mmol) in 3 mL of methanol/THF (1:1, v/v), 10% Pd-C (72 mg) was added and stirred for 6 hours under H_2 atmosphere. The reaction mixture was filtered, and the filtrate was dried to obtain a white powder (compound **7T**, 0.06 g, 94.0 mol%). To the solution of compound **6E**- *erythro* (0.2 g, 0.455 mmol) in 5 mL of methanol/THF (1:1, v/v), 10% Pd-C (80 mg) was added and stirred for 3 hours under H_2 atmosphere to obtain white powder (compound **7E**, 0.0669 g, 56.6 mol%). *Threo* isomer (**7T**): ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 6.85 (2H, d, *J*=8.5Hz, Arom-H), 6.74 (2H, d, *J*=8.5Hz, Arom-H), 6.46-6.50 (4H, m, Arom-H), 4.76 (H, d, *J*=8.8Hz, Hα), 3.95 (H, dd, *J*=10.8, 7.5Hz, Hγ), 3.72 (H, dd, *J*=10.8, 5.2Hz, Hγ), 2.88 (H, m, Hβ). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 157.1 (C4), 156.6 (C4'), 135.8 (C1), 132.0 (C1'), 130.5 (C2',6'), 128.9 (C2,6), 115.7 (C3',5'), 115.3 (C3,5), 78.8 (Cα), 66.8 (Cγ), 55.3 (Cβ) ppm. *Erythro* isomer (**7E**): ^{1}H NMR (400

MHz, CD_3OD) δ 7.00 (2H, d, *J*=8.5Hz, Arom-H), 6.98 (2H, d, *J*=8.6Hz, Arom-H), 6.68 (2H, d, *J*=8.6Hz, Arom-H), 6.67 (2H, d, *J*=8.6Hz, Arom-H), 4.87 (H, d, *J*=6.3Hz, Hα), 3.75 (H, dd, *J*=10.8, 6.2Hz, Hγ), 3.61 (H, dd, *J*=10.8, 7.4Hz, Hγ), 2.93 (H, dt, *J*=7.4, 6.5Hz, Hβ). ^{13}C NMR (100 MHz, CD_3OD) δ 157.7 (C4), 157.1 (C4'), 135.9 (C1), 132.0 (C1'), 131.6 (C2',6'), 129.0 (C2,6), 115.9 (C3',5'), 115.8 (C3,5), 75.7 (Cα), 64.7 (Cγ), 56.5 (Cβ) ppm.

Synthesis method for 1,2-diguaiacylpropane-1,3-diol (compound 14, DGPD)

*Benzylation (compound **9**)*

To the solution of vanillin (6.5 g, 42.7 mmol) in DMF (7 mL), benzyl bromide (6.16 mL, 51.3 mmol), potassium carbonate (8.86 g, 64.1 mmol) and potassium iodate (0.71 g, 4.27 mmol) were added at room temperature. After stirring for 20 hours, the reaction mixture was treated with the same purification protocol for compound **1** to yield white crystals (compound **9**, 7.94 g) in a 76.9 mol% yield.

*Methyl ester (compound **10**, **Figure S10**)*

To the solution of 4-hydroxy-3-methoxyphenyl acetic acid (2.0 g, 10.8 mmol) in methanol (25 mL), acetyl chloride (0.92 mL, 12.9 mmol) and 96% H_2SO_4 (1.5 mL) were added at room temperature. After refluxing for 18 hours, the reaction mixture was diluted with deionized H_2O (60 mL) and extracted with ethyl acetate (60 mL) four times. The combined ethyl acetate layer was washed with brine and then dried over Na_2SO_4. A crude oil was recrystallized from ethanol/hexane (1:1, v/v) to obtain a white powder (compound **10**, 2.1 g, 97.5 mol%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72-6.84 (3H, m), 5.82 (1H, s, OH), 3.82 (3H, s, OMe), 3.66 (3H, s, $COOCH_3$), 3.53 (2H, s, Hα). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6 (Cβ), 146.7, 144.9, 125.8, 122.2, 114.6, 111.9, 55.9 (OMe), 52.1 ($COOCH_3$), 40.8 (Cα) ppm.

*Benzylation (compound **11**, **Figure S11**)*

Compound **10** was benzylated in the same method of compound **2**. To the solution of methyl homovanillate (compound **10**, 2.0 g, 10.3 mmol) in DMF (5 mL), benzyl bromide (1.51 mL, 12.4 mmol), potassium carbonate (2.14 g, 15.5 mmol) and potassium iodate (0.17 g, 1.03 mmol) were added at room temperature. After stirring for 21 hours, the reaction mixture was treated in the same manner as compound **1**. The reaction mixture was recrystallized from ethanol to obtain a white powder (compound **11**, 2.03 g, 68.5 mol%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17-7.36 (5H, m), 6.65-6.76 (3H, m, Arom-H), 5.06 (2H, s, CH_2), 3.81 (3H, s, OMe), 3.61 (3H, s, $COOCH_3$), 3.47 (2H, s, Hα). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4 (Cβ), 149.8, 147.5, 137.4, 128.7, 128.0, 127.4, 127.2, 121.5, 114.2, 113.1, 71.2 (CH_2), 56.2 (OMe), 52.2 ($COOCH_3$), 40.9 (Cα) ppm.

*Coupling (compound **12**, **Figure S12-13**)*

To the solution of lithium diisopropylamide (2 M in THF, 5.4 mL, 10.8 mmol) in anhydrous THF (10 mL), benzylated methyl homovanillate (compound **11,** 1.03 g, 3.6 mmol) in anhydrous THF (13 mL) was added drop-wise for over 1 hour at -78°C. After stirring for 1 hour, benzylated vanillin (compound **9,** 1.05 g, 4.32 mmol) in anhydrous THF (13 mL) was added drop-wise for over 1 hour. After 27 hours of stirring, the reaction solution was heated from -78°C to room temperature, and quenched by addition of H_2O (80 mL). The reaction mixture was acidified with 1 N HCl, extracted with ethyl acetate three times, washed with brine, and dried over Na_2SO_4. A crude oil was purified by flash chromatography to obtain of γ-methyl ester (compound **12**) with *threo* isomer (0.630 g, 33.1 mol%) and *erythro* isomer (0.274 g, 14.4 mol%). *Threo* isomer (**12T**): ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 7.14-7.31 (10H, m), 6.51-6.69 (6H, m, Arom-H), 4.99 (H, dd, *J*=10.2, 4.1Hz, Hα), 4.88 (4H, s, 2xCH_2), 3.66 (H, d, *J*=10.2Hz, Hβ), 3.56 (3H, s, OMe), 3.55 (3H, s, OMe), 3.54 (3H, s, $COOCH_3$). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 172.6 (Cγ), 149.1, 149.0, 147.2, 147.1, 137.3, 137.2, 135.3, 128.6, 127.8, 127.7, 127.12, 127.09, 127.04, 127.03, 120.5, 118.8, 113.3, 113.0, 112.8, 110.7, 75.6 (Cα), 69.98 (CH_2), 69.95 (CH_2), 59.3 (Cβ), 54.8 (OMe), 54.7 (OMe), 50.5 ($COOCH_3$) ppm. *Erythro* isomer (**12E**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18-7.37 (10H, m), 6.71-6.81 (6H, m, Arom-H), 5.09 (H, dd, *J*=7.6, 1.4Hz, Hα), 5.07 (2H, s, CH_2), 5.06 (2H, s, CH_2), 3.78 (3H, s, OMe), 3.73 (3H, s, OMe), 3.67 (H, d, *J*=7.6Hz, Hβ), 3.46 (3H, s, $COOCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2 (Cγ), 149.9, 149.8, 148.3, 148.0, 137.4, 137.3, 128.8, 128.7, 128.1, 128.0, 127.8, 127.5, 127.4, 121.7, 119.2, 114.1, 114.0, 112.9, 110.6, 75.1 (Cα), 71.3 (CH_2), 71.2 (CH_2), 59.4 (Cβ), 56.3 (OMe), 56.2 (OMe), 52.2 ($COOCH_3$) ppm.

*Reduction (compound **13**, **Figure S14-15**)*

To the solution of LAH (229 mg, 5.73 mmol) in THF (5 mL), a solution of the γ-methyl ester (compound **12T**, *threo* isomer, 381 mg, 0.722 mmol) in anhydrous THF (14 mL), was added drop-wise at 0°C for over 40 min. After stirring for 4 hours at the same temperature, the reaction mixture was allowed to warm to room temperature. Remaining LAH was quenched by adding deionized H_2O (3 mL). After acidification with 1 N HCl, the reaction mixture was extracted with ethyl acetate (30 mL) four times. The combined organic layer was washed with brine and dried over Na_2SO_4. A crude oil was purified by preparative TLC with 5% acetone/DCM to obtain a white powder (compound **13T**, *threo* isomer, 197 mg, 55.9 mol%). To a solution of the γ-methyl ester (compound **12E**, *erythro* isomer, 104 mg, 0.196 mmol) in anhydrous DCM (15 mL), DIBAL in DCM (1.88 mL, 1.88 mmol) was added drop-wise at 0°C for over 30 min. After stirring for 19.5 hours at the same temperature, deionized H_2O (30 mL) was added to quench the reaction. The reaction mixture was gelated. The gel status was changed to liquid solution by acidification with saturated citric acid. The solution was extracted with ethyl acetate (30 mL) four times. Combined organic layer was washed with brine and dried over Na_2SO_4. A crude oil was purified by preparative TLC with 5% acetone/DCM to obtain a white powder (compound **13E**, *erythro* isomer, 25.9 mg, 27.0 mol%). *Threo* isomer (**13T**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17-7.31 (10H, m), 6.37-6.63 (6H, m, Arom-H), 4.97 (2H, s, CH_2), 4.96 (2H, s, CH_2), 4.77 (H, d, *J*=8.7Hz, Hα), 4.03 (H, m, Hγ), 3.83 (H, dd, *J*=10.9, 4.4Hz, Hγ), 3.63 (3H, s, OMe), 3.62 (3H, s, OMe), 2.92 (H, dt, *J*=8.1, 4.7Hz, Hβ). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.4, 148.3, 146.4, 146.0, 136.2, 136.1, 135.1, 131.4, 127.5, 126.8, 126.2, 119.3, 117.8, 113.0, 112.5, 111.8, 109.3, 78.3 (Cα), 70.0 (CH_2), 69.9 (CH_2), 65.3 (Cγ), 55.0 (OMe), 54.9 (OMe), 53.5 (Cβ) ppm. *Erythro* isomer (**13E**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17-7.35 (10H, m), 6.57-6.74 (6H, m, Arom-H), 5.03 (4H, s, 2xCH_2), 4.81 (H, d, *J*=6.6Hz, Hα), 3.70 (3H, s, OMe), 3.67 (3H, s, OMe), 3.66-3.70 (H, m, Hγ), 3.63 (H, dd, *J*=10.9, 6.6Hz, Hγ), 2.97 (H, t, *J*=6.6Hz, Hβ). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.6, 148.5, 146.7, 146.5, 136.2, 136.1, 134.0, 130.4, 127.51, 127.5, 126.8, 126.2, 120.0, 117.9, 113.1, 112.7, 112.1, 109.3, 74.7 (Cα), 70.04 (CH_2), 70.03 (CH_2), 63.1 (Cγ), 55.0 (OMe), 54.9 (OMe), 54.0 (Cβ) ppm.

*Deprotection (compound **14**, DGPD, **Figure S16-17**)*

To the solution of compound **13T**-*threo* (196 mg, 0.392 mmol) in 5 ml of methanol, 10% Pd-C (80 mg) was added and stirred 4.5 hours under H_2 atmosphere. The reaction mixture was filtered, and the filtrate was dried to obtain a crude oil. The mixture was purified by preparative TLC developed with ethyl acetate/hexane (2:1, v/v) to obtain a white powder (compound **14T**, 118 mg, 94.4 mol%). To the solution of compound **13E**-*erythro* (112 mg, 0.224 mmol) in 12 mL of methanol/THF (1:1, v/v), 10% Pd-C (80 mg) was added and stirred for 6 hours under H_2 atmosphere to

obtain a crude oil. The crude oil was purified by preparative TLC developed with ethyl acetate to obtain a syrup (compound **14E**, 46.0 mg, 64.0 mol%). *Threo* isomer (**14T**): ^{1}H NMR (400 MHz, CD_3OD) δ 6.47-6.62 (6H, m, Arom-H), 4.79 (H, d, *J*=8.7Hz, Hα), 4.09 (H, dd, *J*=10.9, 6.7Hz, Hγ), 3.90 (H, dd, *J*=10.8, 6.6Hz, Hγ), 3.68 (3H, s, OMe), 3.67 (3H, s, OMe), 2.98 (H, dt, *J*=8.7, 6.7Hz, Hβ). ^{13}C NMR (100 MHz, CD_3OD) δ 148.5 (C3), 148.4 (C3'), 146.7 (C4), 146.2 (C4'), 136.3 (C1), 132.8 (C1'), 122.6 (C6'), 120.8 (C6), 115.9 (C5'), 115.5 (C5), 114.4 (C2'), 112.2 (C2), 78.5 (Cα), 65.9 (Cγ), 56.53 (Cβ), 56.47 (OMe), 56.4 (OMe) ppm. *Erythro* isomer (**14E**): ^{1}H NMR (400 MHz, CD_3OD) δ 6.60-6.71 (6H, m, Arom-H), 4.92 (H, d, *J*=5.7Hz, Hα), 3.84 (H, dd, *J*=10.8, 6.6Hz, Hγ), 3.75 (3H, s, OMe), 3.69 (3H, s, OMe), 3.69 (H, dd, *J*=10.8, 6.6Hz, Hγ), 2.91 (H, dt, *J*=6.7, 5.7Hz, Hβ). ^{13}C NMR (100 MHz, CD_3OD) δ 148.6 (C3), 148.5 (C3'), 146.7 (C4), 146.4 (C4'), 136.6 (C1), 132.4 (C1'), 123.3 (C6'), 120.5 (C6), 115.8 (C5'), 115.6 (C5), 114.8 (C2'), 111.8 (C2), 75.7 (Cα), 64.6 (Cγ), 56.9 (Cβ), 56.5 (OMe), 56.4 (OMe) ppm.

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