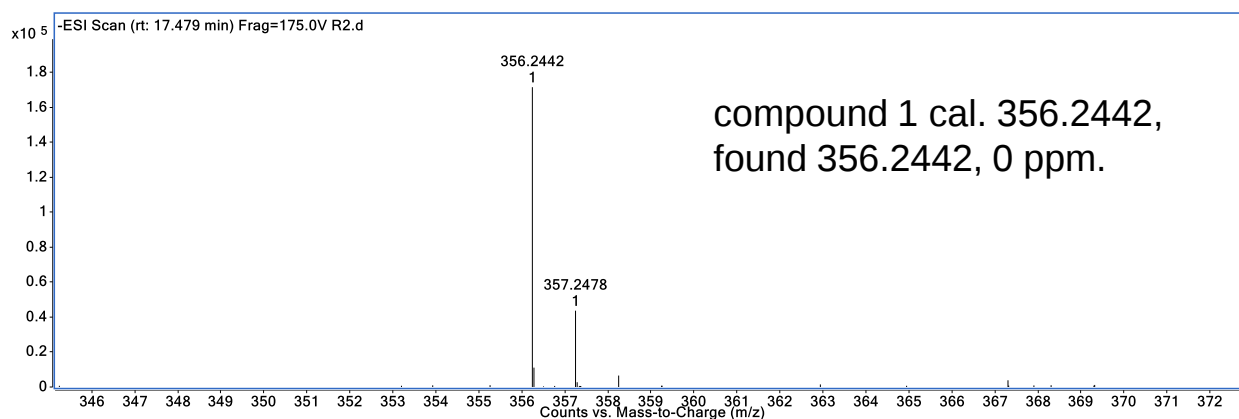


a



b

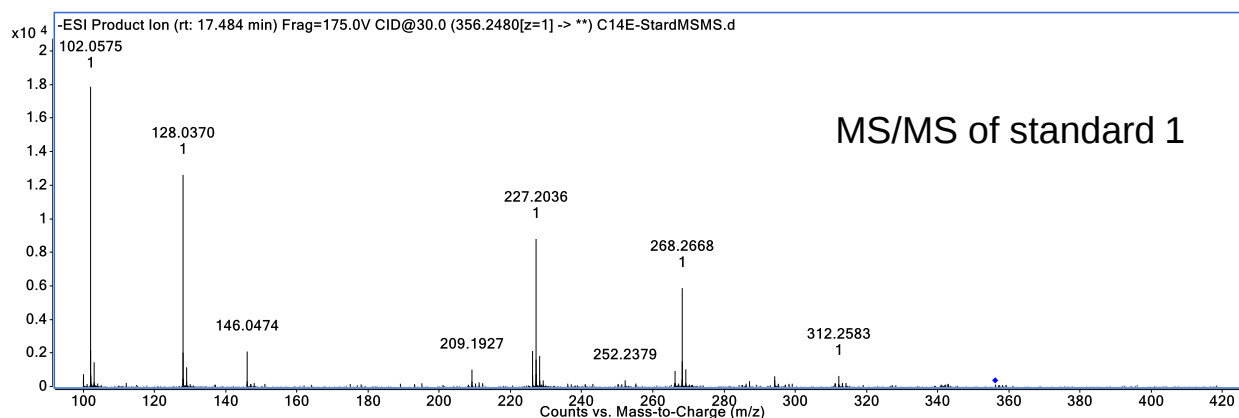
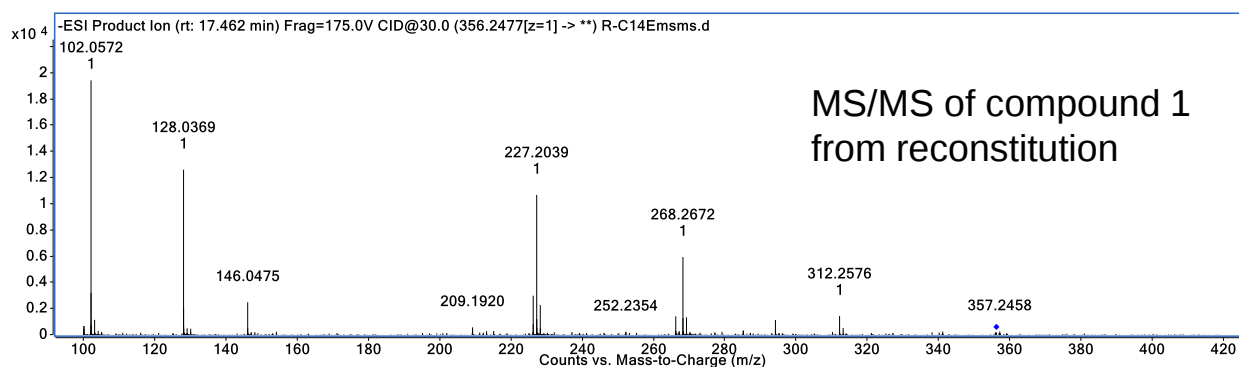


Figure S1. The mass spectrometry analysis of Compound 1. (a) High-resolution mass spectrum of Compound 1 in the reaction product. (b) Comparative MS/MS spectra: tandem mass spectrometry (MS/MS) data for compound 1 from PvdL reconstitution and standard show a maximum deviation below 20 ppm, confirming their structural equivalence.

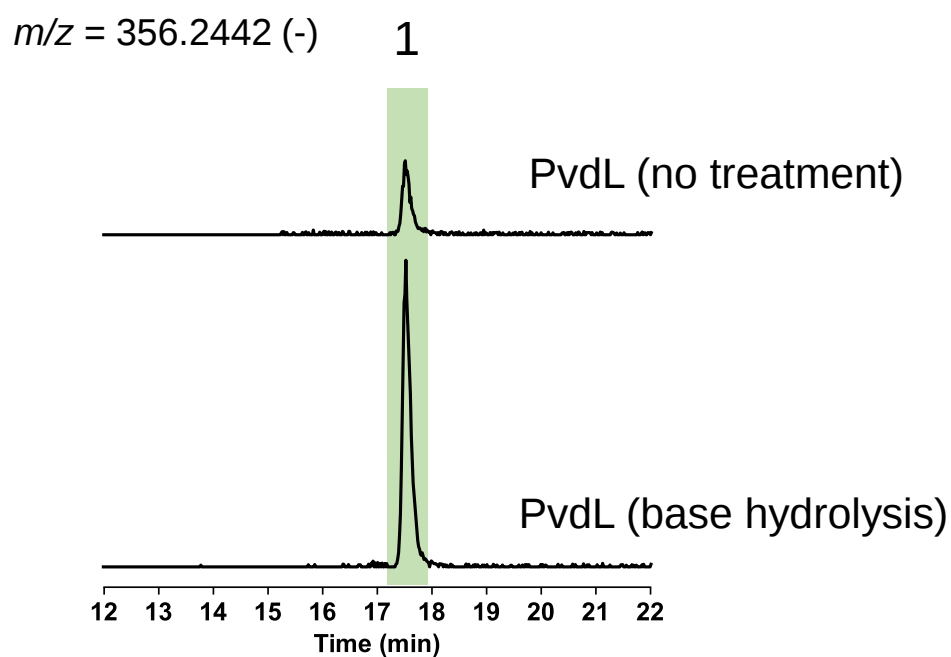


Figure S2. LC/HRMS analysis of compound 1 covalently linked to in-situ purified PvdL. The extracted ion chromatograms (EICs) obtained in negative ion mode at $m/z = 356.2442$ for compound 1 ($[M-H]^-$).

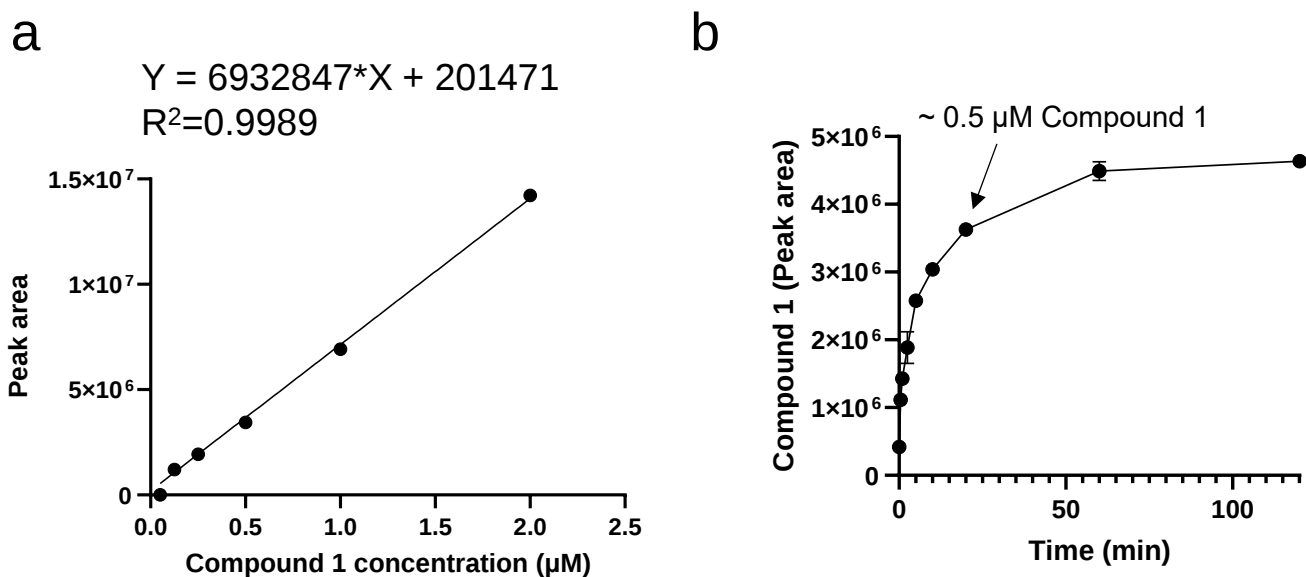


Figure S3. Quantitative analysis of biochemically reconstituted product Compound 1. (a) Standard curve and linear regression analysis for the concentration of compound 1. (b) Time course curve of Compound 1 synthesis by PvdL in vitro. The error bars represent the standard deviation. The concentration of Compound 1 was determined by substituting the peak area into the linear regression equation.

Tabel S1. The primers used in this article.

Primer name	Sequence
pK18-Linearization-SP	CTAGAGTCGACCTGCAGGCAT
pK18-Linearization-AP	CGGGTACCGAGCTCGAATT
PvdL-500TGA500-SP	CGAGCTCGGTACCCGACTGGCAGGATCCAAGTGGGC
PvdL-500TGA500-AP	GCAGGTCGACTCTAGGACGTGGCGTTCTTCCGCGTC
LcHF-SP	CCTTGTAATCATGGTGGTGATGATGGTGCAGCCCCCTCCAGT TCCGCCA
LcHF-AP	ACCATGATTACAAGGATGACGACGATAAGTGATGGGCAGGG GCGCTTG
PvdI-500TGA500-SP	TCGAGCTCGGTACCCGCAGTGCGTCATAGAACGACA
PvdI-500TGA500-SP	TGCAGGTCGACTCTAGCATCCACCTGCTGGATAACA
IcHF-SP	CCTTGTAATCATGGTGGTGATGATGGTGAGCCTCAGTCAATT CATTCA
IcHF-AP	GATTACAAGGATGACGACGATAAGTGAAATGGACAAGACAA CCG
PvdJ-500TGA500-SP	TCGAGCTCGGTACCCGCACCGGCAGGGCCGGCAGAT
PvdJ-500TGA500-AP	TGCAGGTCGACTCTAGGCTGGCCCATGAAGGTGTG
JcHF-SP	CCTTGTAATCATGGTGGTGATGATGGTGCTGGGTCAACTCG TCGATCTC
JcHF-AP	CACCATGATTACAAGGATGACGACGATAAGTAGCGCTCTGC GGGCCCCGG
PvdD-500TGA500-SP	CGAGCTCGGTACCCGATGCAGCGGGTCGCACTCG
PvdD-500TGA500-AP	GCAGGTCGACTCTAGATGAACTGGAGCTGGCCATCG
DcHF-SP	CCTTGTAATCATGGTGGTGATGATGGTGTGAAAGTTCAATGA AGCAGG
DcHF-AP	GATTACAAGGATGACGACGATAAGTGAGCTTTCGGGGCCGC CA
PP3808-500TGA500-AP	AGGTCGACTCTAGAGCACACGGTCTTCCTGCTCAT
PP3808-500TGA500-SP	AGCTCGGTACCCGGGTTCTTTACGCCAACCTTGC
PP3808cHF-SP	TTGTAATCATGGTGGTGATGATGGTGCTGGGCGGCAGCTTC GGCC
PP3808cHF-AP	ATGATTACAAGGATGACGACGATAAGTGACCGTGCTGAACC TGCTGTG
3808cHF2HS-AP	TGCGGGTGGCTCCACGAATCATGGTGGTGATGATGGTG
3808cHF2HS-SP	GCCACCCGCAGTTCGAAAAGTGACCGTGCTGAACCT

Tabel S2. The plasmids used in this article.

Plasmid Name	Description	Resistance
pK18-PvdLcHF	A derivative plasmid of pK18-mobsacB carrying a 1000 bp homologous gene fragment from the C-terminus of the PvdL protein fused with a C-terminal Flag tag + 6*His tag	Kan ^r
pK18-PvdIcHF	A derivative plasmid of pK18-mobsacB carrying a 1000 bp homologous gene fragment from the C-terminus of the PvdI protein fused with a C-terminal Flag tag + 6*His tag	Kan ^r
pK18-PvdJcHF	A derivative plasmid of pK18-mobsacB carrying a 1000 bp homologous gene fragment from the C-terminus of the PvdJ protein fused with a C-terminal Flag tag + 6*His tag	Kan ^r
pK18-PvdDcHF	A derivative plasmid of pK18-mobsacB carrying a 1000 bp homologous gene fragment from the C-terminus of the PvdD protein fused with a C-terminal Flag tag + 6*His tag	Kan ^r
pK18-PP3808cHF	A derivative plasmid of pK18-mobsacB carrying a 1000 bp homologous gene fragment near the PP3808 protein fused with a C-terminal Flag tag + 6*His tag	Kan ^r
pK18-PP3808cHS	A derivative plasmid of pK18-mobsacB carrying a 1000 bp homologous gene fragment near the PP3808 protein fused with a C-terminal Strep tag + 6*His tag	Kan ^r

Tabel S3. The mutant strain constructed in this article.

Mutant strain	Description
KT2440-PvdLcHF	The DNA coding sequence for a 6 × His+Flag tandem tag was inserted before the stop codon "TGA" at the C-terminus of the pvdL gene in <i>Pseudomonas putida</i> KT2440.
KT2440-PvdIcHF	The DNA coding sequence for a 6 × His+Flag tandem tag was inserted before the stop codon "TGA" at the C-terminus of the pvdI gene in <i>Pseudomonas putida</i> KT2440.
KT2440-PvdJcHF	The DNA coding sequence for a 6 × His+Flag tandem tag was inserted before the stop codon "TGA" at the C-terminus of the pvdJ gene in <i>Pseudomonas putida</i> KT2440.
KT2440-PvdDcHF	The DNA coding sequence for a 6 × His+Flag tandem tag was inserted before the stop codon "TGA" at the C-terminus of the pvdD gene in <i>Pseudomonas putida</i> KT2440.
KT2440-PP3808cHF	The DNA coding sequence for a 6 × His+Flag tandem tag was inserted before the stop codon "TGA" at the C-terminus of the pp_3808 gene in <i>Pseudomonas putida</i> KT2440.
KT2440-PP3808cHS	The DNA coding sequence for a 6 × His+Strep tandem tag was inserted before the stop codon "TGA" at the C-terminus of the pp_3808 gene in <i>Pseudomonas putida</i> KT2440.