CHEMBIOCHEM

Supporting Information

A Retro-biosynthesis-Based Route to Generate Pinene-Derived Polyesters

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Sequences of CHMO$_{Acineto}$_QM

The gene and protein sequences of CHMO$_{Acineto}$_QM are given below:

5'-
atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcagccatatggctagcatgactg
gtggacagcaaatgggtcgcggatccatgtcacaaaaaatggattttgatgctatcgtgattggtggtggttttggcgga
ctttatgcagtcaaaaaattaagagacgagctcgaacttaaggttcaggcttttgataaagccacggatgtcgcaggta
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gtcaccactgaatatggtgataagtacacggcgcgtttcctcatcactgctttaggcttattgtctgcgcctaacttgccaa
acatcaaaggcattaatcagtttaaaggtgagctgcatcataccagccgctggccagatgacgtaagttttgaaggta
aacgtgtcggcgtgattggtacgggttccaccggtgttcaggttattacggctgtggcacctctggctaaacacctcactg
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gaagaacaatggactcaaacttgcgccaatattgcggaaatgaccttattccctaaagcgcaatcctggatttttggtgc
gaatatcccgggcaagaaaaacacggtttacttctatctcggtggtttaaaagaatatcgcagtgcgctagccaactgc
aaaaaccatgcctatgaaggttttgatattcaattacaacgttcagatatcaagcaacctgccaatgcctaa-3'

Translated sequence of CHMO$_{Acineto}$_QM:

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMSQKMDFDAIVIGGGFGGL
YAVKKLRDELELKVQAFDKATDVAGTWYWNRYPGALTDTETHLYCYSWDKELLQS
LEIKKKYVQGPDVRKYLQQVAEKHDLKKSYQFNTAVQSAHYNEADALWEVTTEYG
DKYTARFLITALGLLSAPNLPNIKGINQFKGELHHTSRWPDDVSFEGKRVGVIGTGST
GVQVITAVAPLAKHLTVFQRSAQYSVPIGNDPLSEEDVKKIKDNYDKIWDGVWNSAL
AFGLNESTVPAMSVSAEERKAVFEKAWQTGGGFRFMFETFGDIATNMEANIEAQNF
IKGKIAEIVKDPAIAQKLMPQDLYAKRPLCDSGYYNTFNRDNVRLEDVKANPIVEITEN
GVKLENGDFVELDMLILATGFDAVDGNYVRMDIQGKNGLAIKDYWKEGPSSYMGV
CVNNYPNMFMVLGPNGPFTNLPPSIESQVEWISDTIQYTVENNVESIECTKEAEEQ
WTQTCANIAEMTLFPKAQSWIFGANIPGKKNTVYFYLGGLKEYRSALANCKNHAYE
GFDIQLQRSDIKQPANA

For reference, the original protein sequence of wild type CHMO$_{Acineto}$ (GenBank: BAA86293.1[1]), is given below:

MSQKMDFDAIVIGGGFGGLYAVKKLRDELELKVQAFDKATDVAGTWYWNRYPGAL
TDTETHLYCYSWDKELLQSLEIKKKYVQGPDVRKYLQQVAEKHDLKKSYQFNTAVQ
SAHYNEADALWEVTTEYGDKYTARFLITALGLLSAPNLPNIKGINQFKGELHHTSRW
PDDVSFEGKRVGVIGTGSTGVQVITAVAPLAKHLTVFQRSAQYSVPIGNDPLSEEDV

KKIKDNYDKIWDGVWNSALAFGLNESTVPAMSVSAEERKAVFEKAWQTGGGFRFM
FETFGDIATNMEANIEAQNFIKGKIAEIVKDPAIAQKLMPQDLYAKRPLCDSGYYNTF
NRDNVRLEDVKANPIVEITENGVKLENGDFVELDMLICATGFDAVDGNYVRMDIQGK
NGLAMKDYWKEGPSSYMGVTVNNYPNMFMVLGPNGPFTNLPPSIESQVEWISDTI
QYTVENNVESIEATKEAEEQWTQTCANIAEMTLFPKAQSWIFGANIPGKKNTVYFYL
GGLKEYRSALANCKNHAYEGFDIQLQRSDIKQPANA

Construction of the F246A variant

Primers in the 5’ to 3’ direction are specified below:

F246A_f

ggtgtatggaattcagcccttgccGCGggcctgaatg

F246A_r

ggcactgtgctttcattcaggccCGCggcaagg

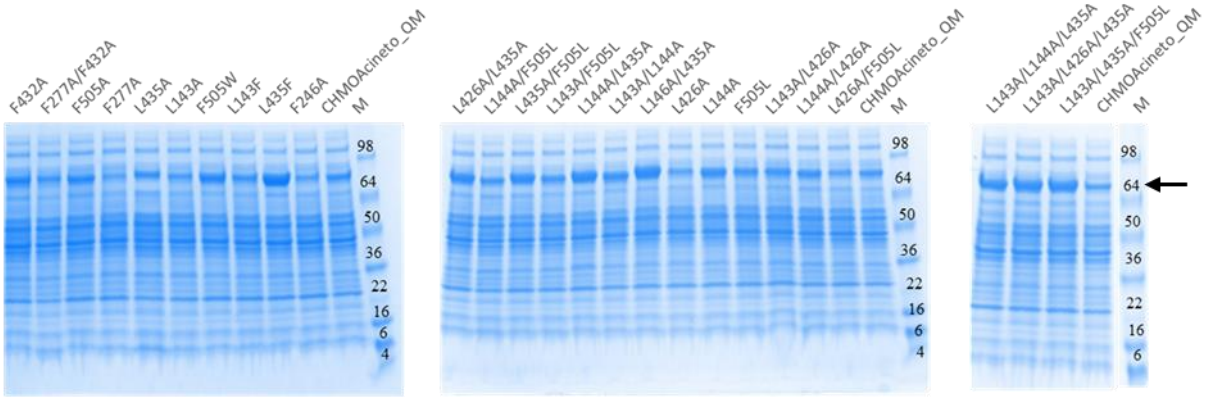


Figure S1. SDS-PAGE analysis (4-15%) of CHMO$_{Acineto}$_QM and its variants expressed in *E. coli* BL21(DE3). Samples were taken after 20 h of induction (with 0.05 mM IPTG) at 25°C. *Lane M*, pre-stained protein molecular ladder SeeBlue Plus 2. Relevant bands of CHMO$_{Acineto}$_QM and variants are indicated by the arrow.

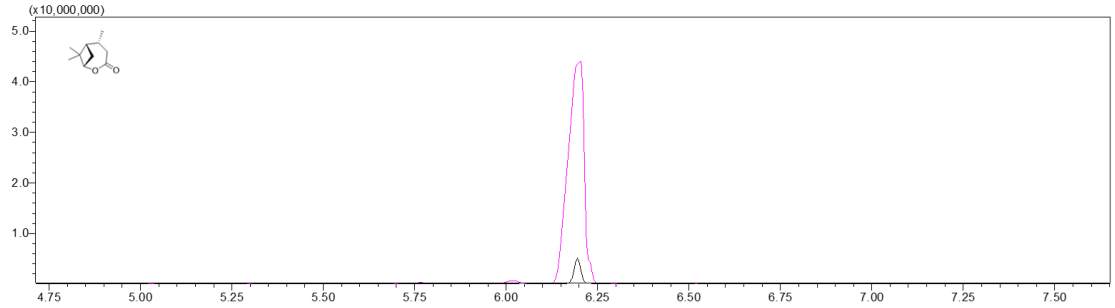


Figure S2. GC-spectra of the "normal lactone" **10** chemically (*purple*) and enzymatically (*black*) produced.

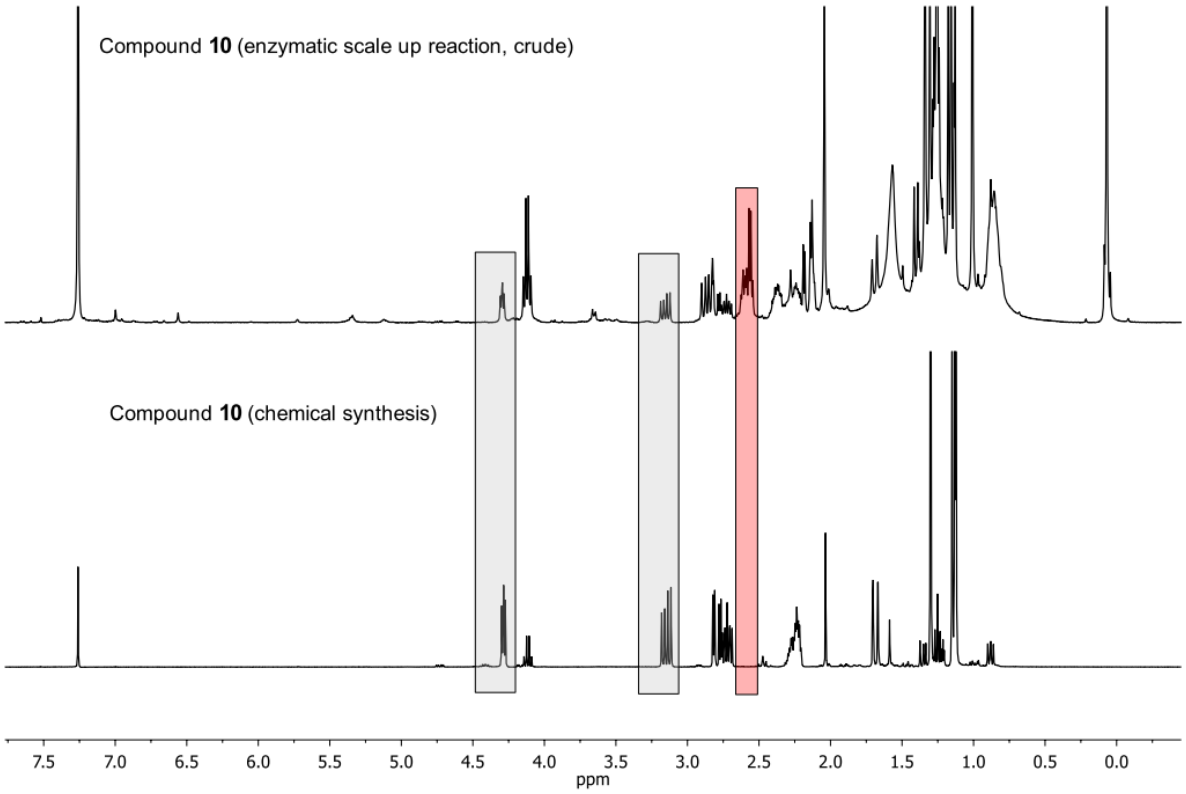


Figure S3. ^{1}H-NMR spectra of extracted reaction mixture from enzymatic upscaling (top) compared to ^{1}H-NMR of compound **10** (bottom). Rectangles used to highlight the characteristic peaks of compound **10** (grey) and **9** (red).

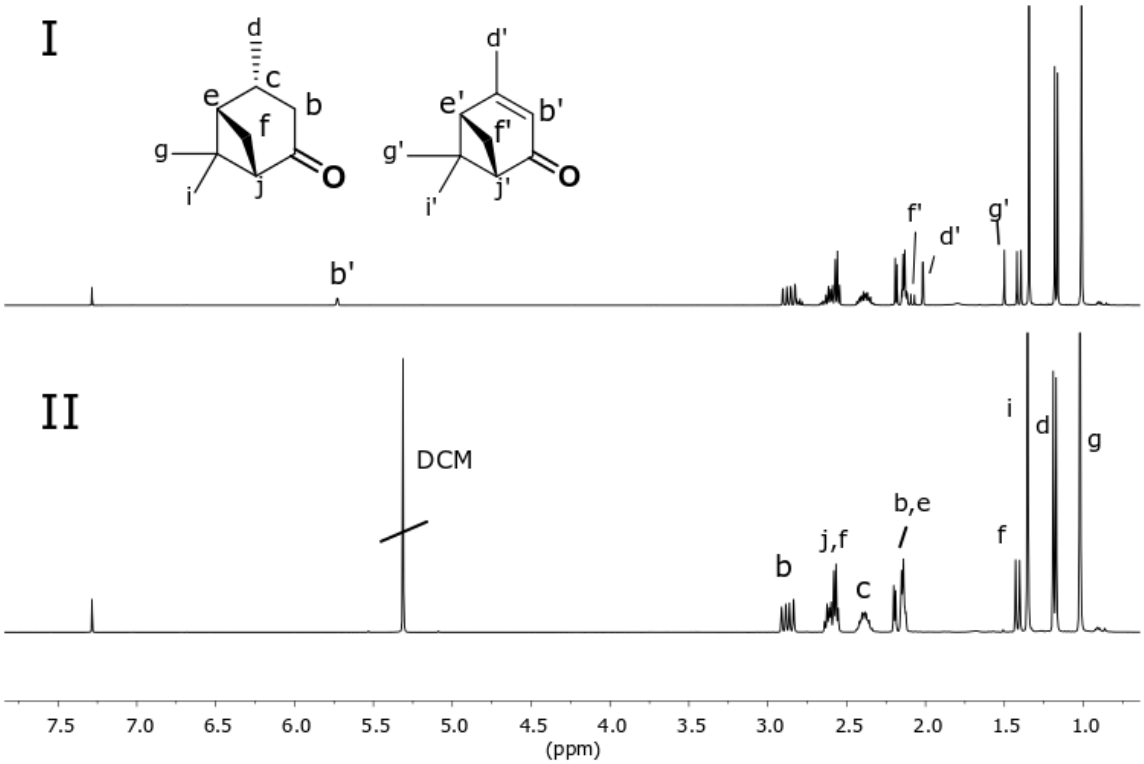


Figure S4. [1]H-NMR spectra of (I.) a mixture containing both **8** and **9** and (II.) the chemically reduced mixture of (I.) in order to contain only **9.**

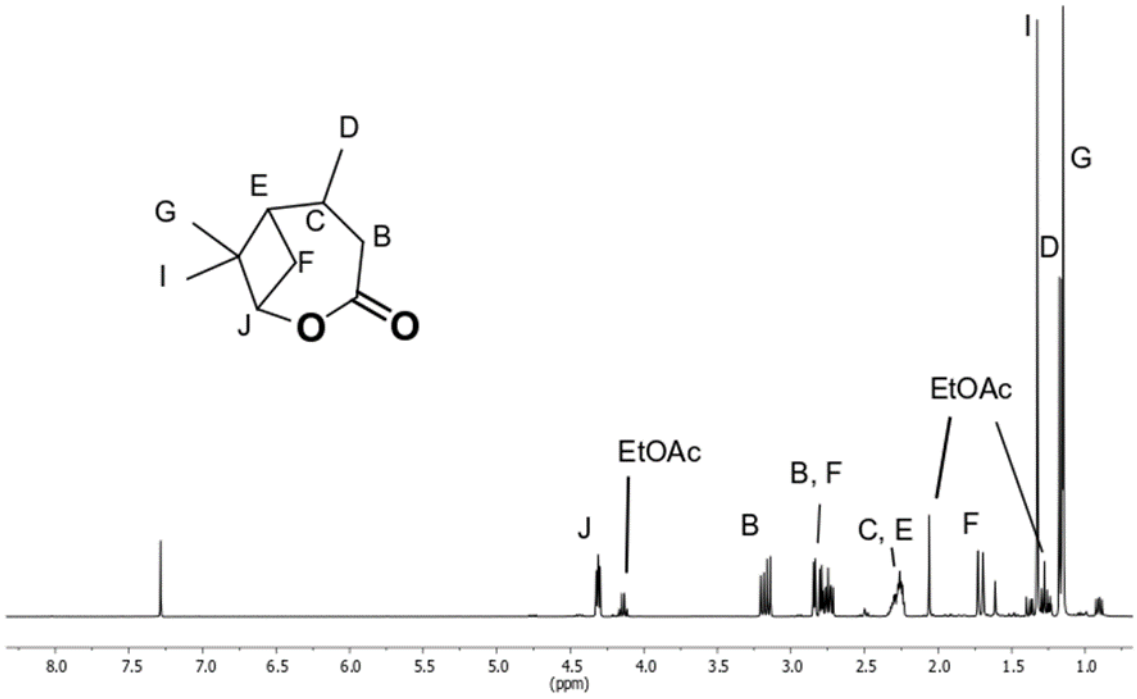
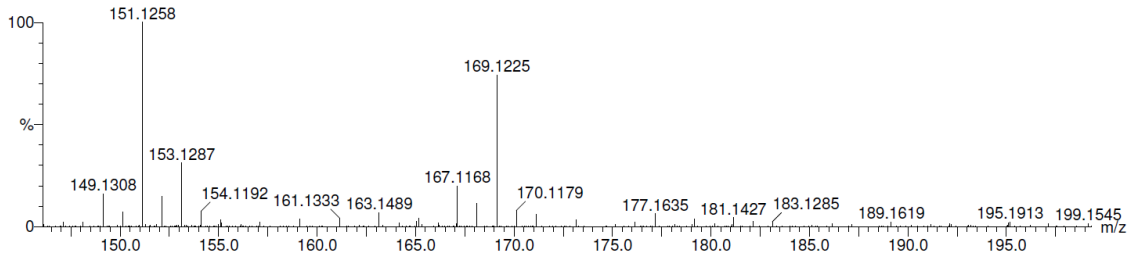


Figure S5 [1]H-NMR spectra of **10.**



Minimum:				-1.5		
Maximum:		5.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
169.1225	169.1229	-0.4	-2.1	2.5	1	C10 H17 O2

Figure S6. High-resolution GC/MS-ToF mass spectrum of **10.** Spectra was acquired with chemical ionization and methane as a reagent gas.

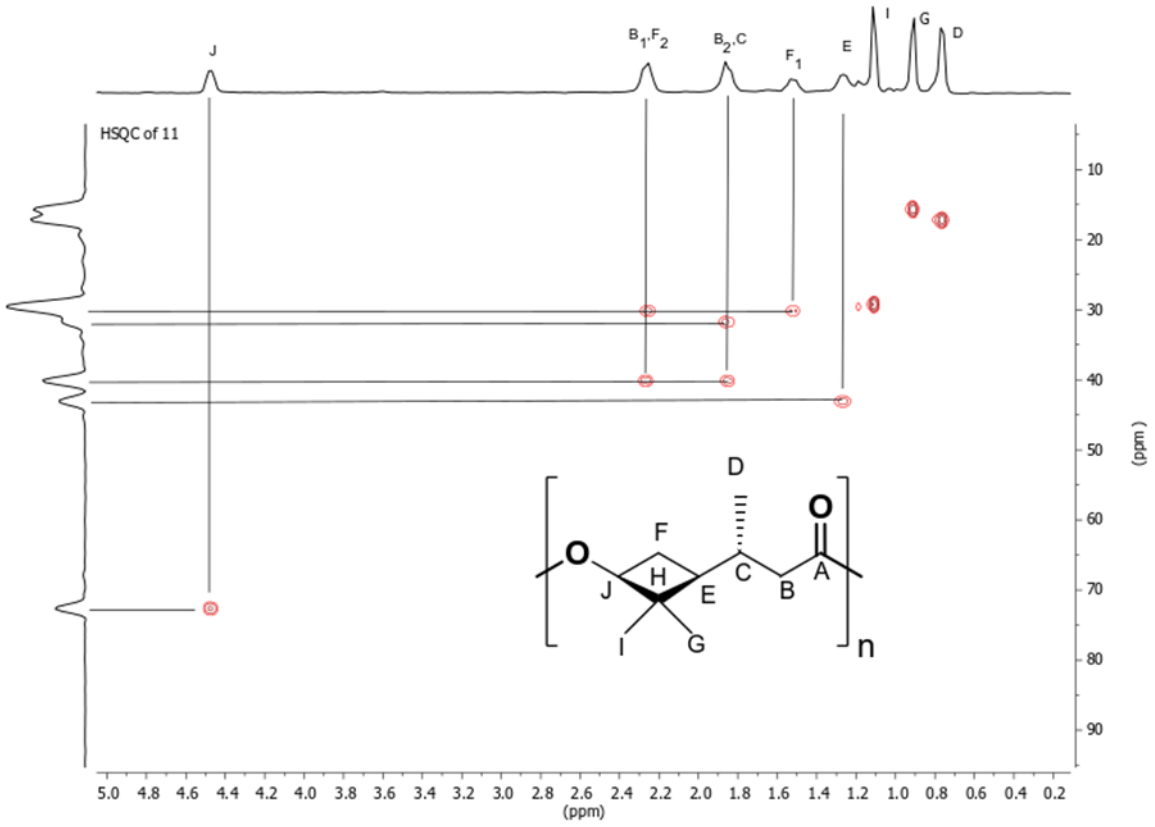


Figure S7. HSQC correlation plot of **11** polymerized using MSA/benzyl alcohol for 24 h at 70 °C.

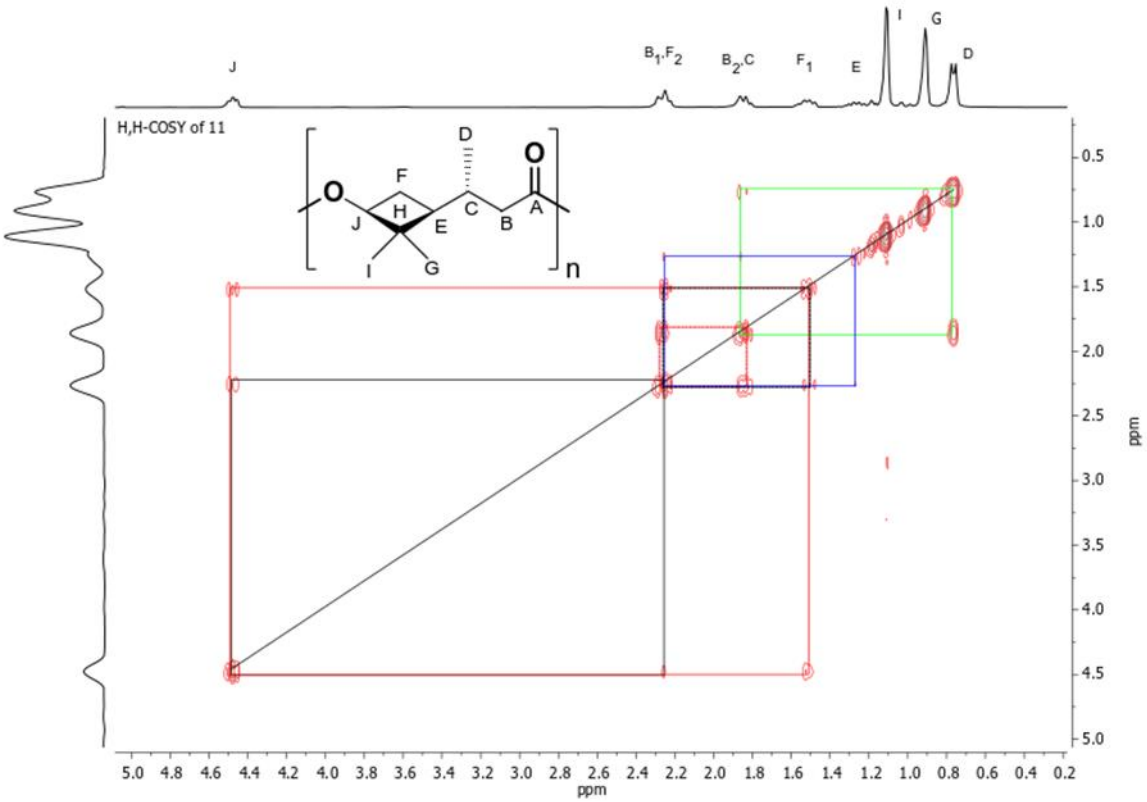


Figure S8. H,H-COSY of **11** polymerized with MSA/benzyl alcohol for 24 h at 70 °C. Rectangles indicate the coupling between the specific protons. Large black: J-F_2 coupling, large red: J-F_1 coupling, small black: F_1-F_2 coupling, small red: B_1-B_2 coupling, blue: E-F_2 coupling and green: C-D coupling.

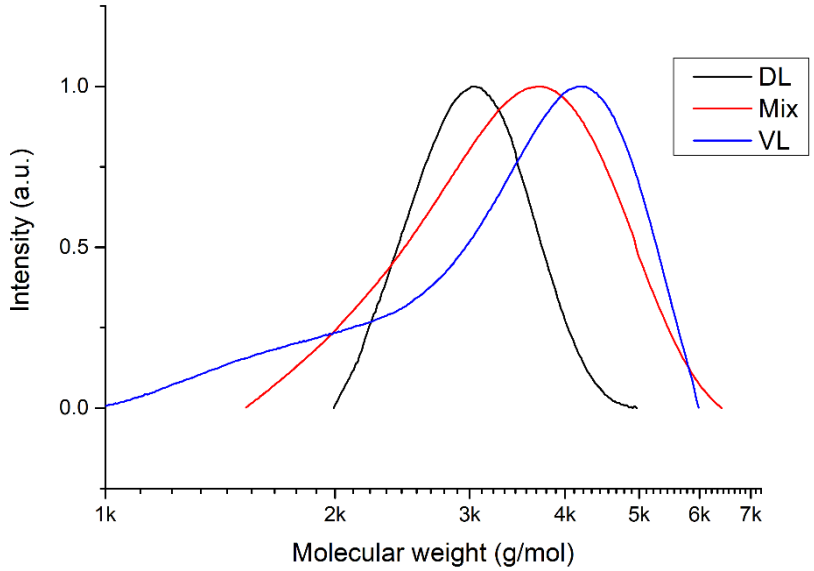


Figure S9. Molecular weight distributions for the synthesized polyesters. Black: poly-εDL; Red: poly(εDL-εVaL); Blue: poly-εVaL.

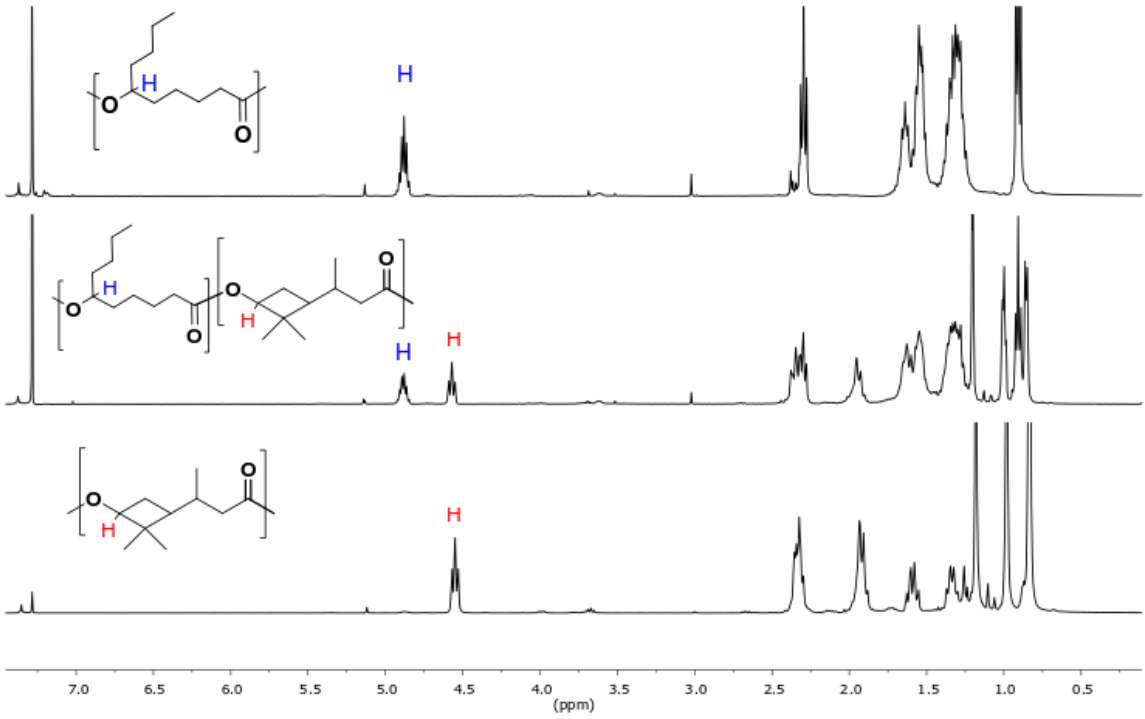


Figure S10. ^{1}H-NMR spectra of precipitated polymers (top: poly-εDL; middle: poly(εDL-εVaL); bottom: poly-εVaL) polymerized using MSA/benzyl alcohol for 24 h at 70 °C.

Supplementary References

[1] Y. C. Chen, O. P. Peoples, C. T. Walsh, *J. Bacteriol.* **1988**, *170*, 781-789.