Supporting Information

In vitro Reconstitution and Analysis of the 6-Deoxyerythronolide B Synthase

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Reagents and chemicals

1. Chemical Reagents

Isopropyl-β-D-1-thiogalactopyranoside (IPTG) was purchased from Gold Biotechnology, Inc. Sodium propionate, silica gel (200-425 mesh), *N*,*N*-dimethylformamide (anhydrous), and *N*-Boc glutamic acid benzyl ester were purchased from Sigma-Aldrich and used as received. Pentane, ethyl acetate, methanol (spectrophotometric grade), d_1-chloroform, and sodium sulfate were purchased from Fisher Scientific and used as received.

2. Plasmids

Phusion High Fidelity polymerase and Phusion Hot Start polymerase were purchased from Thermo Scientific. T5 Exonuclease was purchased from Epicentre, an Illumina Company. Taq DNA ligase was from New England Biolabs. All primers for cloning of gene constructs were synthesized by Elim Biopharmaceuticals.

3. Bacterial Culture and Protein Purification

All chemicals for preparation of buffers were from Sigma-Aldrich. Luria-Bertani (LB) Miller Broth was from Fisher Scientific. Complete protease inhibitor cocktail tablets were from Roche. Ni-NTA affinity resin was from MC Lab. HiTrap Q anion exchange chromatography columns for protein purification were purchased from GE Healthcare. SDS-PAGE Mini Protean TGX Precast Gels were purchased from BioRad, and Amicon Ultra centrifugal filters were purchased from Millipore. Mini-PROTEAN TGX precast polyacrylamide gels were purchased from Bio-Rad.

4. Protein Assays

Coenzyme A (CoASH), reduced β-nicotinamide adenine dinucleotide 2'-phosphate (NADPH), methylmalonic acid, and magnesium chloride hexahydrate were purchased from Sigma-Aldrich. Adenosine-5′-triphosphate (ATP) was purchased from Teknova. Bond-breaker tris(2-carboxyethyl)phosphine (TCEP) was purchased from Thermo Scientific. UVette cuvettes (2 mm x 10 mm path) were purchased from Eppendorf.

Methods

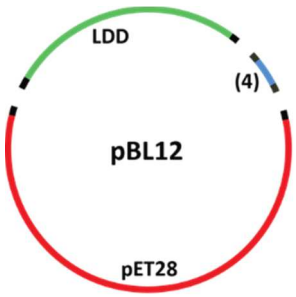
1. Plasmids

DNA constructs used to express DEBS proteins and their derivatives were constructed using either standard restriction digestion – ligation protocols or the Gibson assembly method[1]. For the latter, overlapping fragments of the final gene product were produced by PCR and purified. These fragments were designed to have 35-40 base pairs of overlapping sequence (denoted as black regions in plasmid diagrams shown below). For the assembly reaction,

approximately 100 ng of each DNA fragment was added to a reaction mixture containing buffer and enzymes (T5 exonuclease, Phusion high fidelity polymerase, and Taq ligase), and incubated for 45 minutes at 50 °C. The reaction mixture was transformed into chemically competent DH5α cells, and transformants were screened for correct plasmid assembly using standard colony PCR methods. See Table S1 for a list of plasmids and their associated primers and PCR templates. Also, see Table S2 for a list of amino acid sequences (with annotations) for engineered DEBS modules constructed in this work.

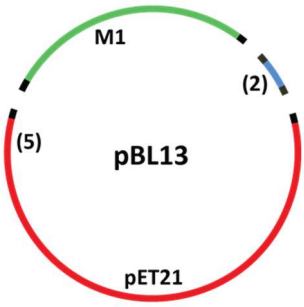
a. pBL12 – LDD(4)

Plasmid pBL12, encoding the gene for the recombinant LDD(4) protein (the loading didomain with the C-terminal docking domain from module 4 fused to its C-terminus), was assembled from three DNA fragments: LDD (green), the module 4 docking domain (4) (blue), and a pET28 vector backbone (red). LDD was PCR amplified from pBP144,[2] the module 4 docking domain was PCR amplified from pBP130,[2] and the vector was amplified from pET28. Fragments were added to the assembly mixture to synthesize the final circular plasmid.



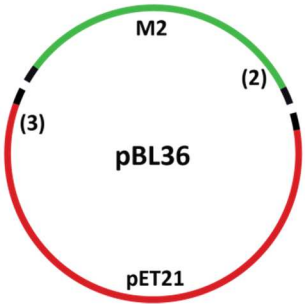
b. pBL13 – (5)M1(2)

Plasmid pBL13, encoding the gene for the recombinant (5)M1(2) protein (module 1 with the N-terminal docking domain from module 5 and the C-terminal docking domain from module 2), was assembled from three fragments: the module 5 N-terminal docking domain with a pET21 vector backbone (red), module 1 (green), and the module 2 C-terminal docking domain (blue). The module 5 docking domain with a pET21 vector was amplified from pFW100 (see below), module 1 was amplified from pBP144, and the module 2 docking domain pBP19.[3] Fragments were added to the assembly mixture to synthesize the final circular plasmid.



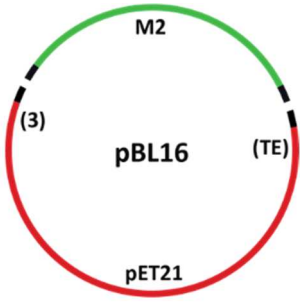
c. pBL36 – (3)M2(2)

Plasmid pBL36, encoding the gene for the recombinant (3)M2(2) protein (module 2 with the N-terminal docking domain from module 3 and the C-terminal docking domain from module 2), was assembled using two fragments: the module 3 N-terminal docking domain with a pET21 backbone (red) and the module 2 gene with its natural C-terminal docking domain (green). The module 3 docking domain with the pET21 vector was amplified from pRSG34,[4] and module 2 was amplified from pBP19. Fragments were added to the assembly mixture to synthesize the final circular plasmid.



d. pBL16 – (3)M2+TE

Plasmid pBL16, encoding the gene for the recombinant (3)M2+TE protein (module 2 with the N-terminal docking domain from module 3 and the C-terminal TE domain), was assembled from two fragments: the module 3 N-terminal docking domain with a pET21 backbone connected to the C-terminal TE domain (red) and module 2 (green). The module 3 docking domain with the pET21 vector and the TE was amplified from pRSG34 and module 2 was amplified from pBP19. Fragments were added to the assembly



mixture to synthesize the final circular plasmid.

e. pFW98 – DEBS2 and pFW100 – DEBS3

Plasmid, pFW98, encoding the gene for the recombinant DEBS2 protein was constructed by amplification of its gene from pBP130. The DEBS2 gene was inserted into pET21 as an NdeI/EcoRI fragment. Plasmid, pFW100, encoding the recombinant DEBS3 protein was constructed by amplification of its gene from pBP130. The DEBS3 gene was inserted into pET21 as an NdeI/EcoRI fragment. The gene encoding (3)M3+TE was reported previously.[4]

Plasmids containing the genes for the *S. coelicolor* MatB in pET28 and the *S. coelicolor* methylmalonyl-CoA epimerase in pET28 were gifts from Professor Michelle Chang's laboratory at the University of California Berkeley.

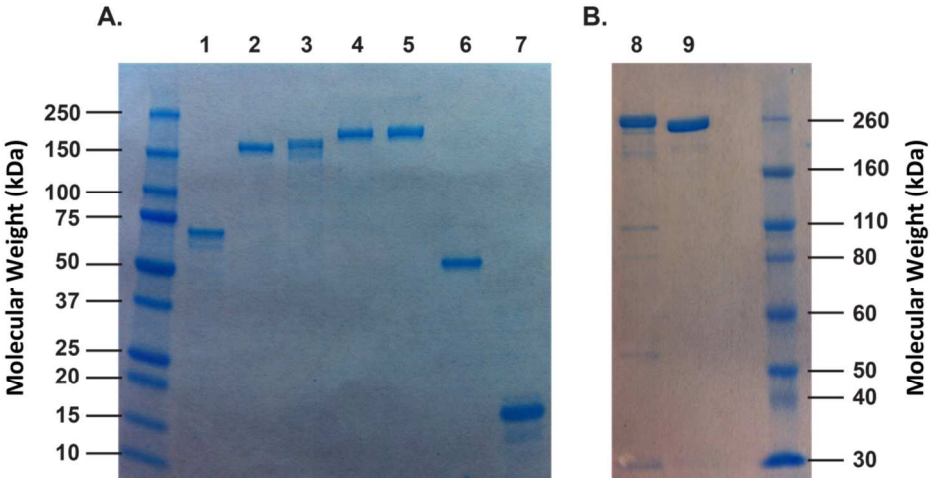
2. Bacterial Cell Culture and Protein Purification

All DEBS-derived proteins – LDD(4), (5)M1(2), (3)M2(2), DEBS2, DEBS3, (3)M3+TE and (3)M2+TE – were expressed and purified using similar protocols. Expression plasmids were introduced into *E. coli* BAP1 cells to allow phosphopantetheinyl modification of ACP domains.[2] Overnight seed cultures were used to inoculate a 1 L shake flask culture containing the appropriate antibiotic. Cells were grown to an approximate O.D. of 0.6 and then induced with 250 μL of 1M IPTG. After 12-15 h, cells were harvested by centrifugation at 4420 g and lysed by sonication in lysis buffer (50 mM sodium phosphate, 10 mM imidazole, 450 mM NaCl, 20% glycerol, pH = 7.6). The lysate was clarified by centrifugation at 25,000 g, and the supernatant was added to Ni-NTA agarose resin (2 mL of resin per L of culture) and allowed to incubate at 4 °C for 1 h. The incubating resin was applied to a Kimble-Kontes Flex column and washed with 20 column volumes of lysis buffer, 10 column volumes of wash buffer (50 mM phosphate, 25 mM imidazole, 300 mM NaCl, 10% glycerol, pH = 7.6), and eluted with 8 column volumes of elution buffer (75 mM phosphate, 500 mM imidazole, 20 mM NaCl, 10% glycerol, pH = 7.6). The eluate was further purified using anion exchange chromatography (HiTrap Q column, Buffer A: 50 mM phosphate, pH = 7.6, 10% glycerol and Buffer B: 50 mM phosphate, 500 mM NaCl, pH = 7.6, 10% glycerol) on an AKTA FPLC system. FPLC fractions were analyzed by SDS-PAGE, and fractions containing the desired protein were pooled and concentrated using an Amicon Ultra filter. Protein concentrations were measured using the Pierce™ BCA Protein Assay Kit (Thermo Scientific). Protein samples were aliquoted and stored at -80 °C. For DEBS2 and DEBS3, a Roche complete protease inhibitor tablet was added to the lysis buffer and Buffer B contained 1 M NaCl rather than 500 mM NaCl. For approximate protein yields, see table the adjacent table.

DEBS Protein	Approximate Yield (mg/L of culture)
LDD(4)	2
(5)M1(2)	6
(3)M2(2)	0.5
DEBS2	0.25
DEBS3	4
(3)M2+TE	1
M3+TE	10
MatB	40
Methylmalonyl-CoA Epimerase	40

The *S. coelicolor* MatB and methylmalonyl-CoA epimerase proteins were also purified using the above protocol but using the following buffer system – lysis buffer: 100 mM HEPES, 500 mM NaCl, 20% Glycerol, pH = 7.5, wash buffer: 100 mM HEPES, 300 mM NaCl, 15 mM imidazole 20% Glycerol, pH = 7.5, elution buffer: 100 mM HEPES, 300 mM NaCl, 300 mM Imidazole 20% Glycerol, pH = 7.5, Buffer A: 50 mM HEPES, 10% Glycerol, pH = 7.5, Buffer B:

50mM HEPES, 500 mM NaCl, 10% Glycerol, pH = 7.5. See the following graphic for SDS-PAGE gels (stained with SimplyBlue™ SafeStain from Invitrogen) of all purified proteins used in this study.



Purified DEBS Proteins. (A) SDS-PAGE Gel (4-20% polyacrylamide gradient) for **1**, LDD(4) – 67.6 kDa; **2**, (5)M1(2) – 168.1 kDa; **3**, (3)M2(2) – 163.7 kDa; **4**, (3)M2+TE – 184.4 kDa; **5**, (3)M3+TE – 185.8 kDa; **6**, MatB – 53.9 kDa; **7**, Methylmalonyl-CoA Epimerase – 18.1 kDa. (B) SDS-PAGE Gel (7.5% polyacrylamide) for **8**, DEBS2 – 376.6 kDa; **2**, DEBS3 – 334.1 kDa

3. Enzyme Assays

UV spectrophotometric assay: Kinetic parameters ($(V/[E]_0)_{max}$, K_{50}) were determined by fitting the data using MATLAB® to the equation: $V/[E]_0 = (V/[E]_0)_{max}$ [*Subunit*] / (K_{50} + [*Subunit*]), where $V/[E]_0$ is the initial rate normalized to the concentration of proteins in the assay that were held constant and [*Subunit*] is the concentration of the DEBS component that is being varied. $V/[E]_0$ vs. [*Subunit*] plots (see Figure 2 and Figure S8) were constructed such that only one protein was varied at a time while holding the others constant. Parameters are reported as the fitting value ± s.e. (n = 3) with standard error derived from the nonlinear curve fitting (Table 1). Error bars on graphs represent the mean ± s.d. (n = 3).

Assays to measure the initial rates of polyketide synthesis were performed as follows. Reactions were performed on a 70 µL scale containing 400 mM sodium phosphate (pH = 7.2), 5 mM TCEP, 10 mM $MgCl_2$, 500 µM Coenzyme A, and 4 mM ATP. Enzymes MatB (2 µM) and methylmalonyl-CoA epimerase (4 µM) were added to convert methylmalonate into racemic methylmalonyl-CoA.[5] The concentration of both these enzymes was selected to ensure that acyl-CoA supply was not rate limiting. The DEBS proteins were then added. For reactions performed in identifying the polyketide products, 2 µM of all DEBS proteins was added for each assembly line derivative (Figure S2 – Figure S4). For reactions performed to collect kinetic data on the full DEBS and trimodular DEBS systems, 2 µM was added for each DEBS protein held constant (Figure 2 and Figure S8). For reactions performed to collect kinetic data on the bimodular DEBS system, 1 µM was added for each DEBS protein held constant (Figure S8). Reactions were initiated upon simultaneous addition of propionyl-CoA (50 µM), methylmalonic acid (1 mM), and NADPH (500 µM), and the rate was monitored in an Eppendorf brand UVette cuvette at 340-nm (depletion of NADPH) using a Lambda-25 UV-Vis Spectrophotometer (Perkin-Elmer).

For polyketide product analysis, reactions were quenched with ethyl acetate, extracted twice with 450 μL, and dried *in-vacuo.*

***Substrate specificity assays*:** Assays to observe the incorporation of non-natural extender units into 6-dEB or its truncated derivatives were performed in an analogous fashion to kinetics assay described above. Reactions were performed on a 110 μL scale containing 400 mM sodium phosphate (pH = 7.2), 5 mM TCEP, 10 mM $MgCl_2$, 500 μM Coenzyme A, 4 mM ATP, and 500 μM NADPH. Enzymes MatB (2 μM) and methylmalonyl-CoA epimerase (4 μM) were added to convert the methylmalonate and ethylmalonate substrates into racemic methylmalonyl- and ethylmalonyl-CoA, respectively.[5] For each modular assembly line tested, the individual proteins were present at 2 μM except DEBS2, which was increased to 3 μM. Reactions were initiated upon addition of methylmalonate (500 μM), ethylmalonate (500 μM), and propionyl-CoA (50 μM) and incubated for 3 h, at which time they were quenched with ethyl acetate, extracted twice with 450 μL, then dried *in vacuo*. The reconstituted full DEBS under these assay conditions yields approximately 1 μg of 6-dEB.

Preparation of authentic 6-dEB: Authentic 6-dEB was prepared following previously described procedures.[2] Briefly, engineered *E. coli* strain BAP1 transformed with pBP130/pBP140 was fermented in the presence of 2.5 g/L sodium propionate and IPTG in LB media for 72 h. The cells were pelleted by centrifugation at 2170 g, and the supernatant was extracted 5 x 200 mL ethyl acetate per liter of supernatant. The ethyl acetate extracts were pooled and dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting oil was partially purified by silica gel chromatography (using a gradient elution of 15% ethyl acetate to 50% ethyl acetate in pentane), furnishing 6-dEB as a semi-solid yellow residue. 400 MHz ^{1}H NMR analysis (in $CDCl_3$) with *N*,*N*-dimethylformamide as internal standard revealed the isolated material to be 33% pure by mass.

LC-MS analysis of polyketides: Samples were reconstituted in methanol containing *N*-Boc glutamic acid benzyl ester (11.6μM), which served as an internal standard because its molecular weight and elution properties were similar to those of 6-dEB). The samples were separated on a Gemini-NX C18 column (Phenomenex, 5 um, 2 x 100 mm) connected to an Agilent 1260 HPLC over a 28 min linear gradient of acetonitrile from 3-95%, and then injected into a 6520 Accurate-Mass QTOF mass spectrometer. Fragmentation analysis of 6-deoxyerythronolide B and its ethyl-substituted analogs required a fragmenter voltage of 150.0V, a collision induced dissociation energy of 15.0V. Because of its relative abundance, fragmentation analysis was performed on the $[M+H-H_2O]$ ion.

Figures

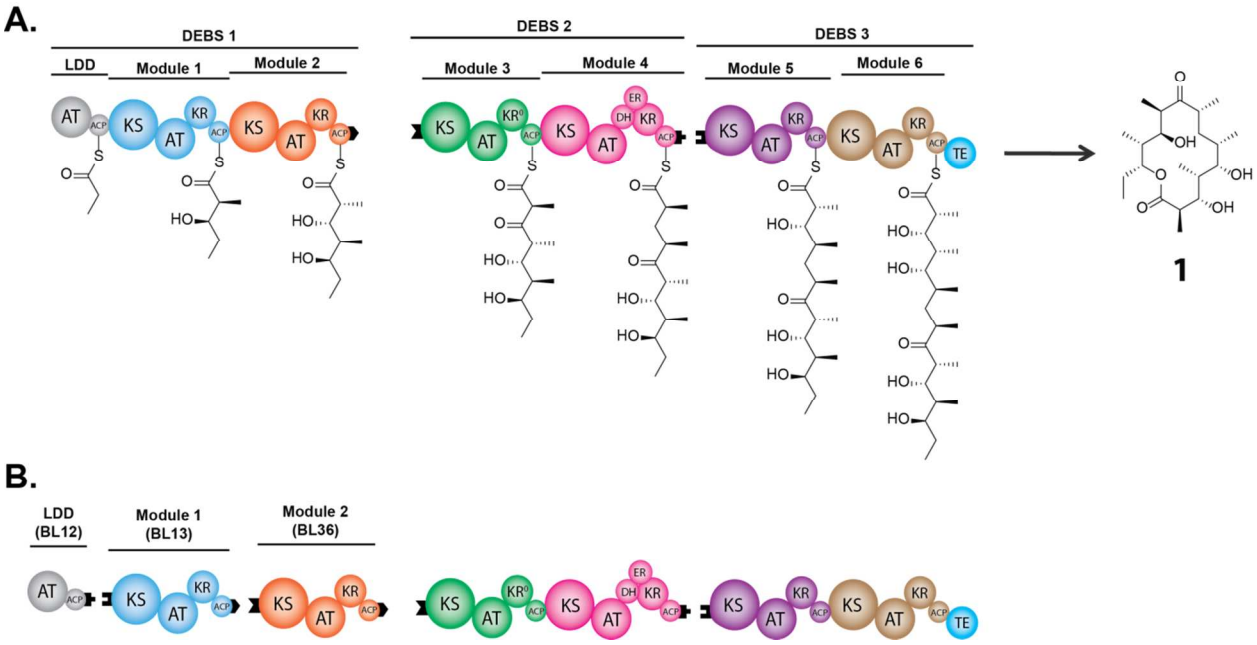


Figure S1. (A) The naturally occurring 6-deoxyerythronolide B synthase (DEBS) and (B) its engineered derivative used in this study, where DEBS1 is expressed as three distinct polypeptides. Docking domain pairs are shown as shape-complementing black tabs.

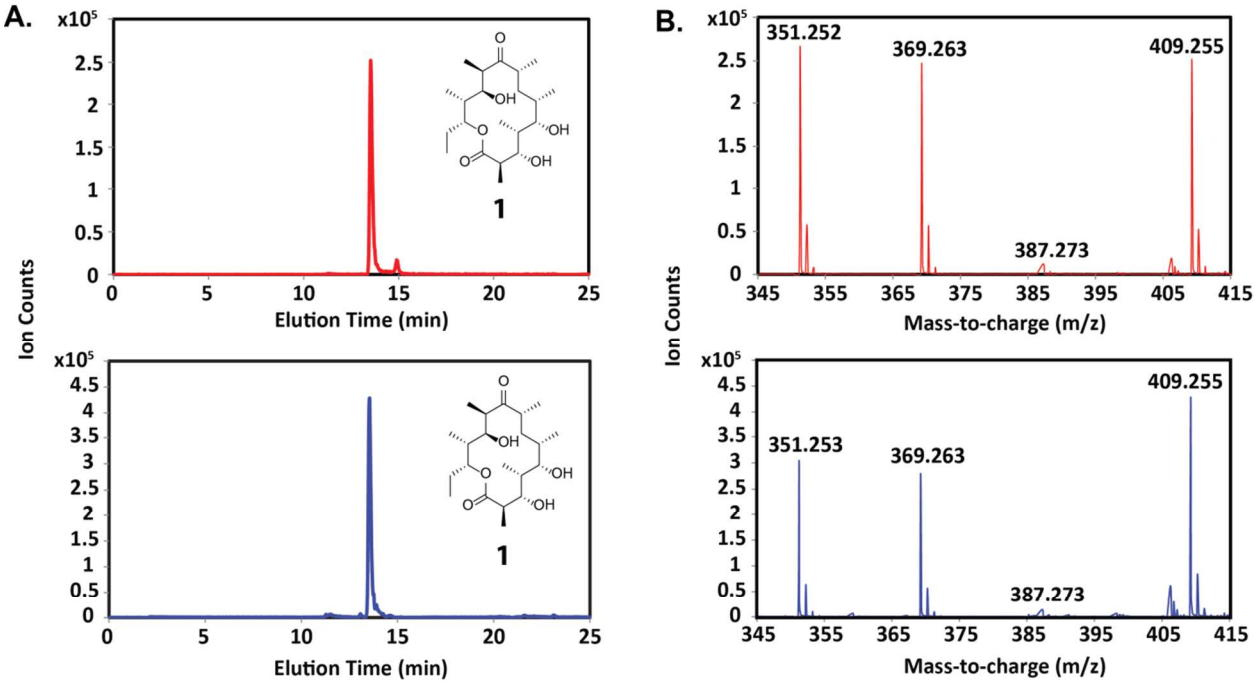


Figure S2. LC-MS analysis of 6-deoxyerythronolide B. (6-dEB; $C_{21}H_{38}O_6$; calculated MW 386.267) (A) Extracted ion chromatograms (obtained by extraction of the $[M+Na]^+$ species) for authentic standard (top) and the product of an *in vitro* reaction (bottom). (B) Full mass spectra of the 6-dEB peaks eluting at 13.5 min. The four major peaks, shown from left to right, are $[M+H-2H_2O]^+$, $[M+H-H_2O]^+$, $[M+H]^+$, and $[M+Na]^+$.

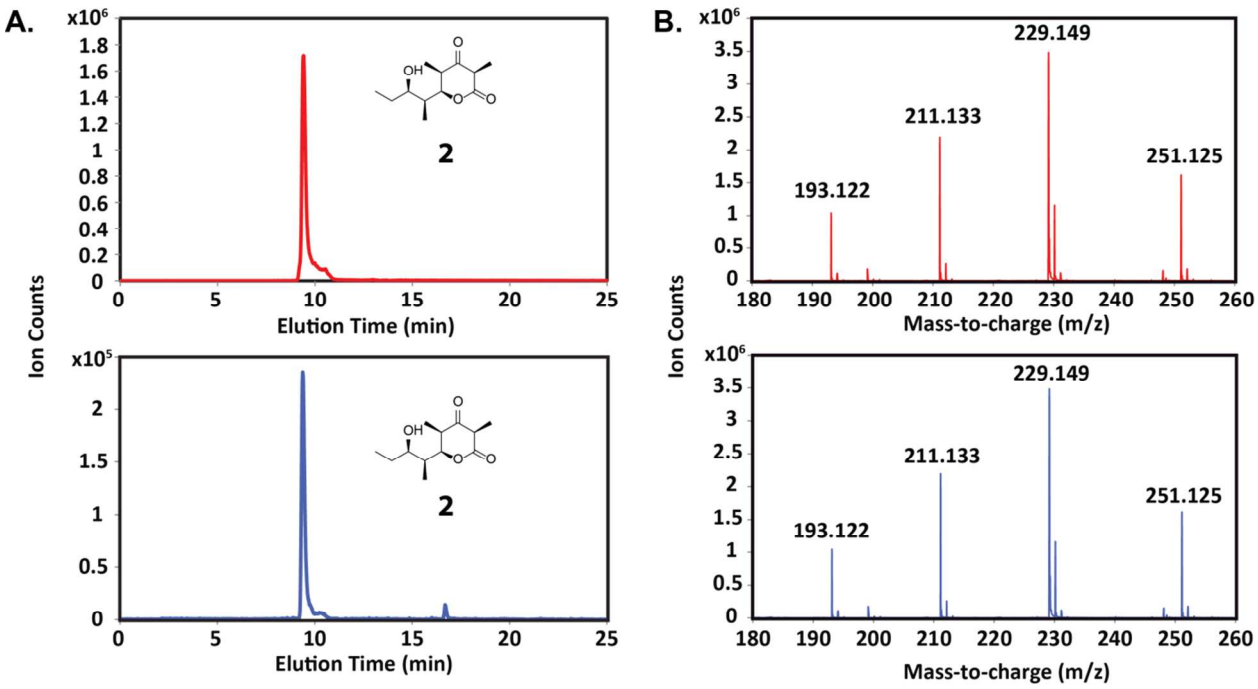


Figure S3. LC-MS analysis of the tetraketide lactone **2**. ($C_{12}H_{20}O_4$; calculated MW 228.136) (A) Extracted ion chromatograms (obtained by extraction of the $[M+Na]^+$ species) for authentic standard (top) and the product of an *in vitro* reaction (bottom). (B) Full mass spectra of the tetraketide lactone peaks eluting at 9.4 min. The four major peaks, shown from left to right, are $[M+H-2H_2O]^+$, $[M+H-H_2O]^+$, $[M+H]^+$, and $[M+Na]^+$.

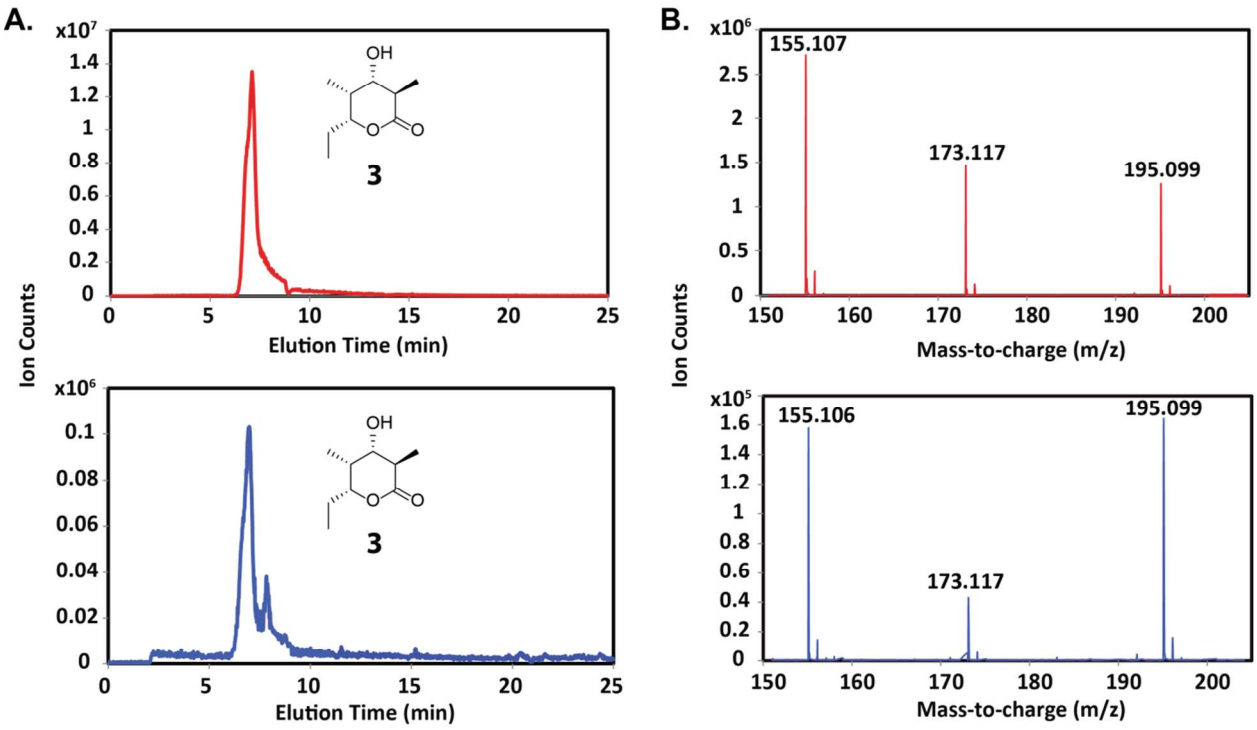


Figure S4. LC-MS analysis of the triketide lactone **3**. ($C_9H_{16}O_3$; calculated MW 172.110) (A) Extracted ion chromatograms (obtained by extraction of the $[M+Na]^+$ species) for authentic standard (top) and the product of an *in vitro* reaction (bottom). (B) Full mass spectra of the triketide lactone peaks eluting at 7.1 min. The three major peaks, shown from left to right, are $[M+H-H_2O]^+$, $[M+H]^+$, and $[M+Na]^+$.

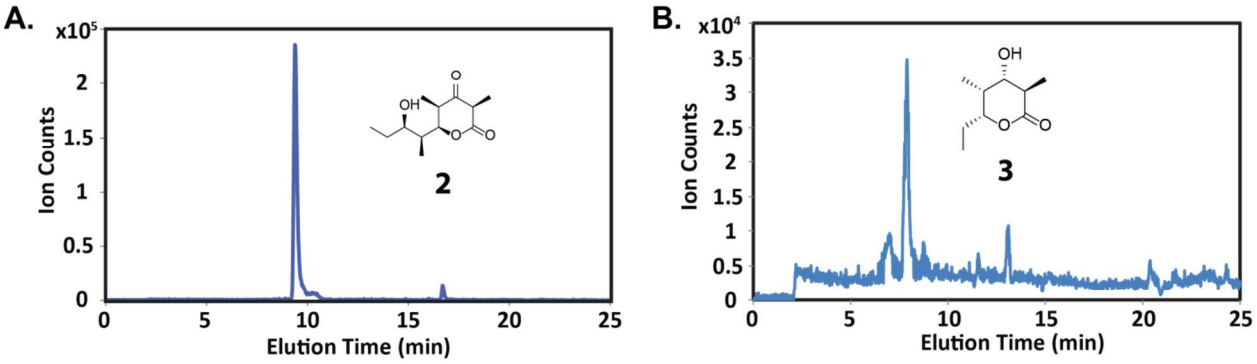


Figure S5. LC-MS comparison of tetraketide lactone **2** and triketide lactone **3** produced by the trimodular DEBS derivative. Extracted ion chromatograms for (A) tetraketide lactone and (B) triketide lactone produced within the same reaction. Ion counts by integration of the extracted ion peaks for **2** and **3** indicate that the minor product **3** is approximately 10% of the major product **2**.

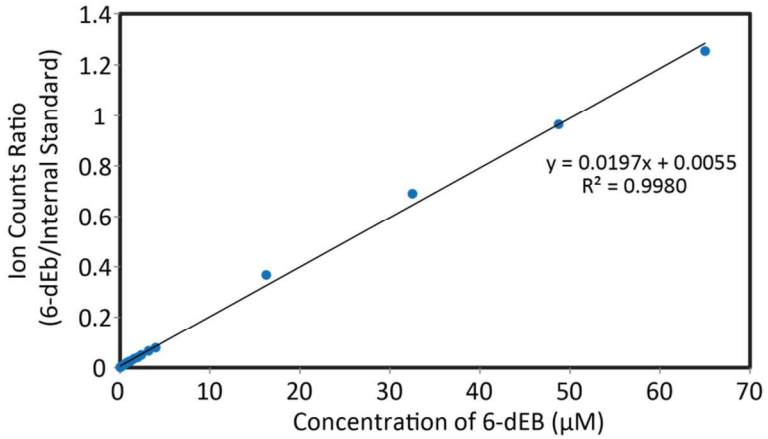


Figure S6. LC-MS calibration curve of an authentic standard of 6-deoxyerythronolide B.

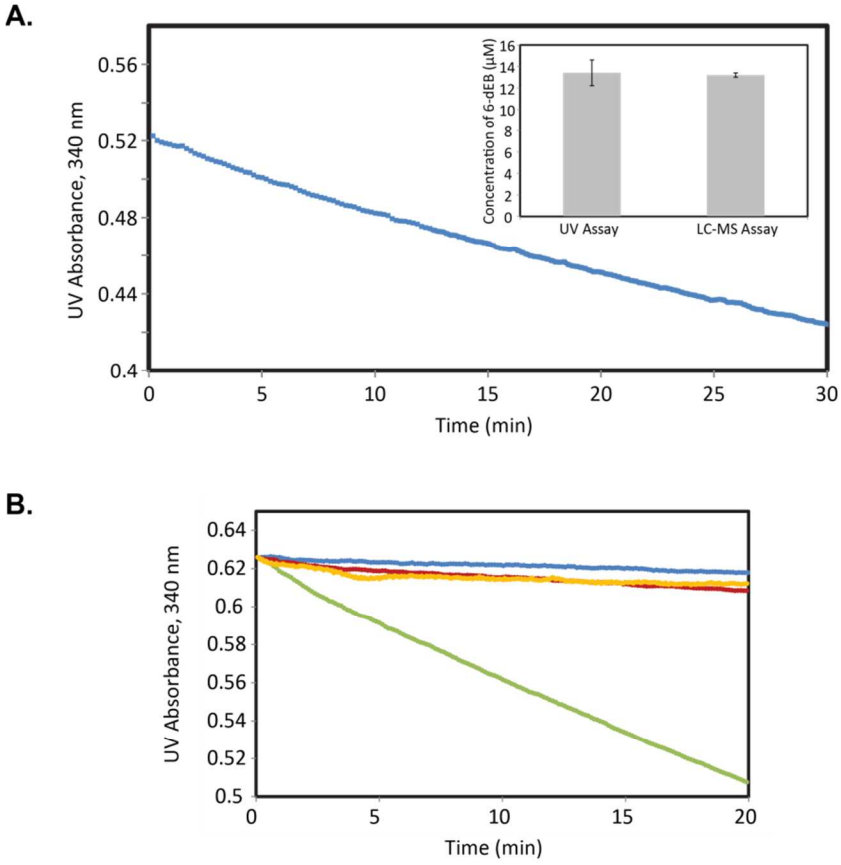


Figure S7. (A) Stoichiometric equivalence between 6-deoxyerythronolide B formation and NADPH consumption. Equivalence was established for all DEBS proteins at a constant concentration of 2 µM. A 30 minute spectrophotometric time course for NADPH consumption by DEBS is shown. At the end of the time course, production of compound **1** was quantified by LC-MS and compared to production of **1** predicted by NADPH consumption within the continuous UV spectrophotometric assay (inset). (B) NADPH consumption time-courses for

LDD-M1-M2-M3+TE. Full system: (green) [LDD], [M1], [M2], [M3+TE] = 2 μM, [NADPH] = 500 μM, all substrates. Control reactions: (yellow) "background consumption" - [LDD], [M1], [M2], [M3+TE] = 2 μM, no methylmalonic acid or propionyl-CoA; (blue) [LDD], [M1], [M3+TE] = 2 μM, [M2] = 0 μM, all substrates; (red) [LDD], [M1], [M2] = 2 μM, [M3+TE] = 0 μM, all substrates.

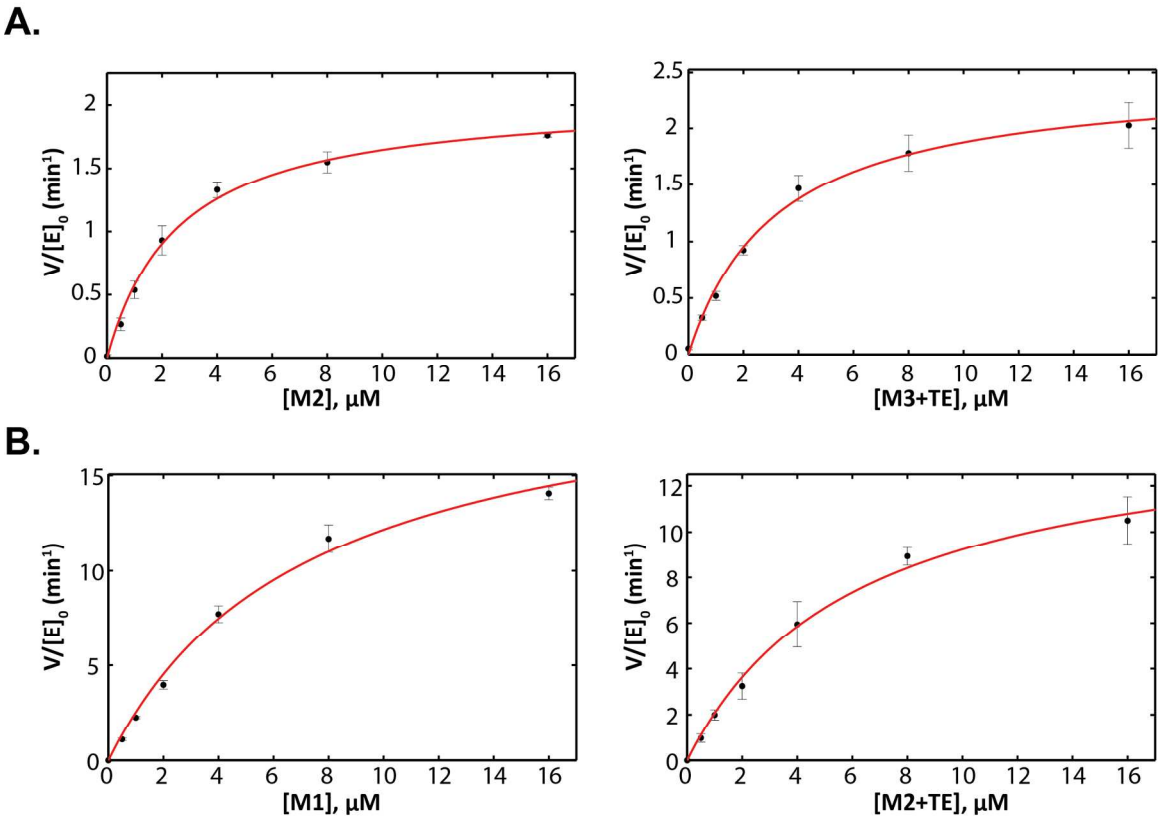


Figure S8. Effect of titrating individual subunits on the turnover rates of a (A) trimodular and (B) bimodular derivative of DEBS. Except for the titrant protein, all other proteins were held at a constant concentration of (A) 2 μM or (B) 1 μM. Kinetic parameters: (A) M2, $(V/[E]_0)_{max} = 2.1 \pm 0.1$ min^{-1} and $K_{50} = 2.6 \pm 0.2$ μM; M3+TE, $(V/[E]_0)_{max} = 2.5 \pm 0.1$ min^{-1} and $K_{50} = 3.2 \pm 0.4$ μM. (B) M1, $(V/[E]_0)_{max} = 21.0 \pm 0.9$ min^{-1} and $K_{50} = 7.3 \pm 0.7$ μM; M2+TE, $(V/[E]_0)_{max} = 15.0 \pm 0.7$ min^{-1} and $K_{50} = 6.3 \pm 0.6$ μM.

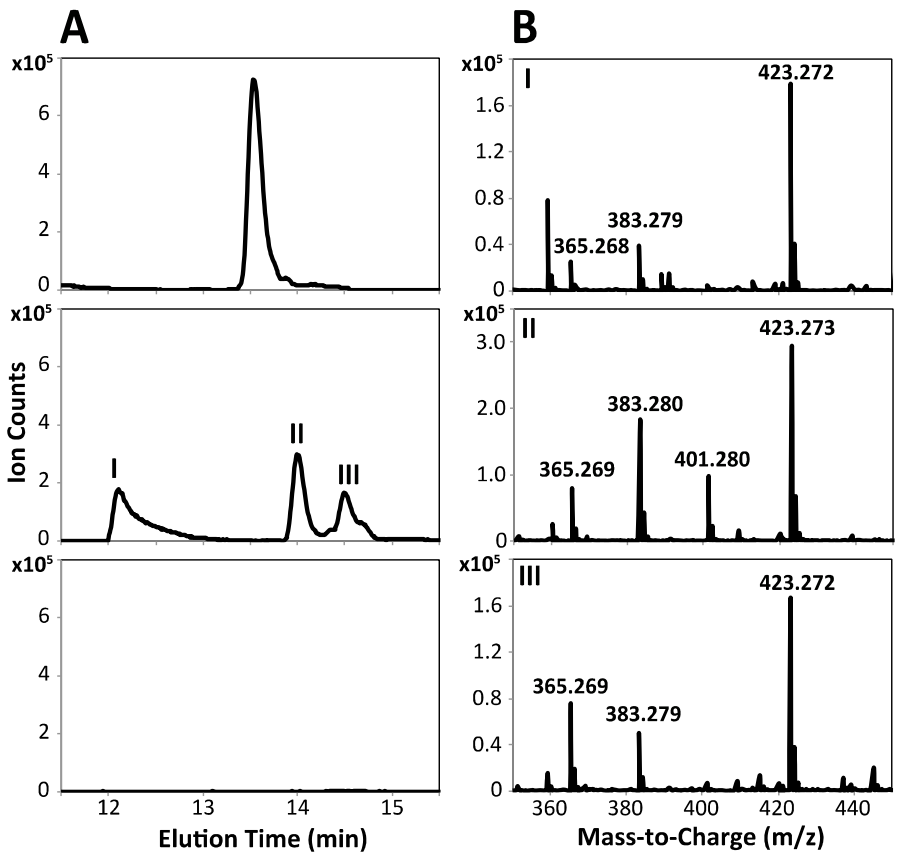


Figure S9: Product profile of DEBS in the presence of comparable concentrations of methylmalonyl and ethylmalonyl extender units. (A) Extracted Ion Chromatogram (obtained by extraction of the $[M+Na]^+$ species) for 6-deoxyerythronolide B (top), analogs with the molecular formula $C_{22}H_{40}O_6$, corresponding to products derived from incorporation of a single ethylmalonyl extender (middle), and analogs with the molecular formula $C_{22}H_{40}O_6$ extracted from a control in which no ethylmalonyl was present (bottom). (B) Full mass spectrum of the most abundant analogs corresponding Peak I (12.1 min; top), Peak II (14.0 min; middle), and Peak III (14.5 min; bottom). In each case, ions corresponding to at least three of the following species were observed: $[M+H-2H_2O]^+$, $[M+H-H_2O]^+$, $[M+H]^+$, and $[M+Na]^+$.

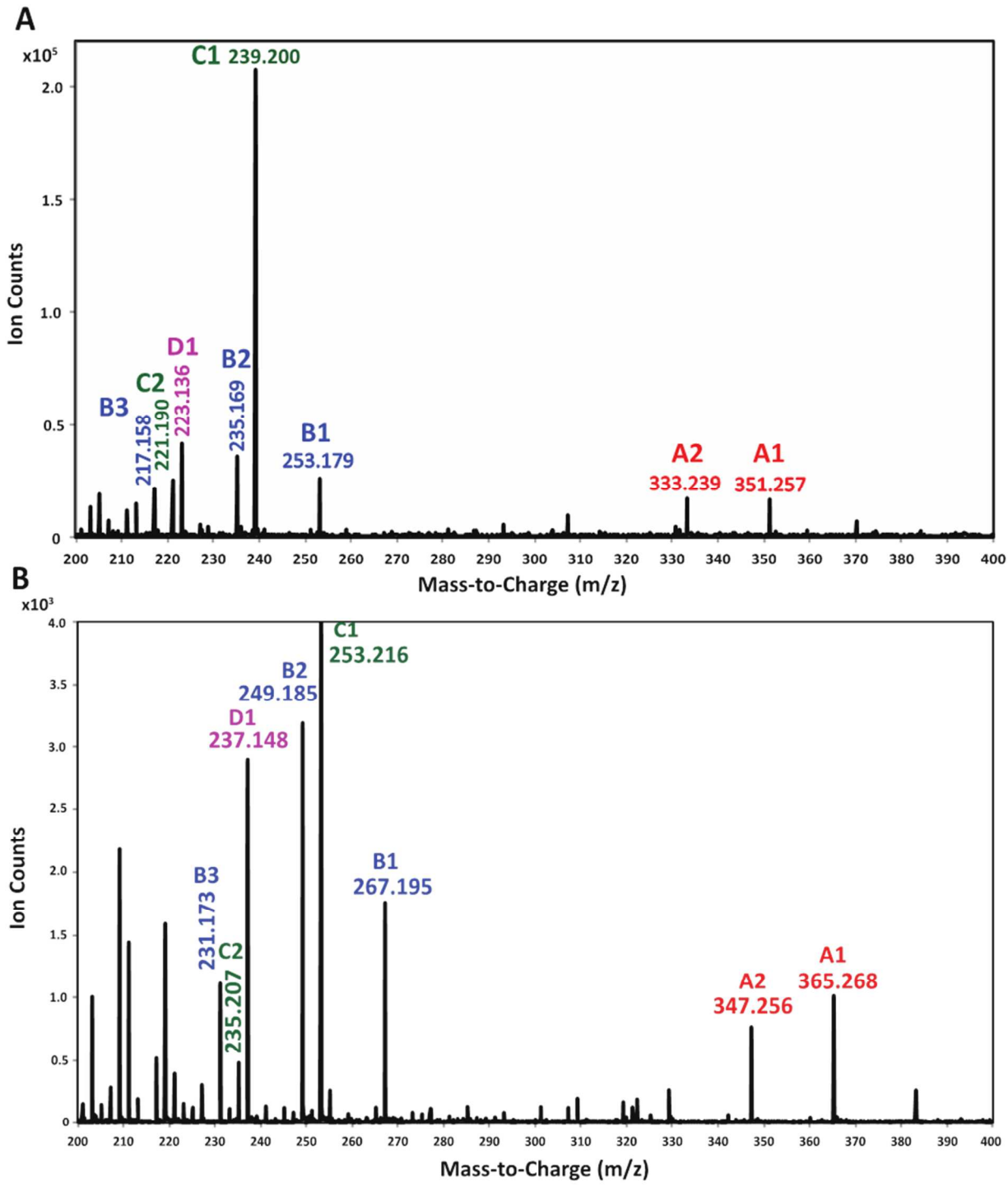
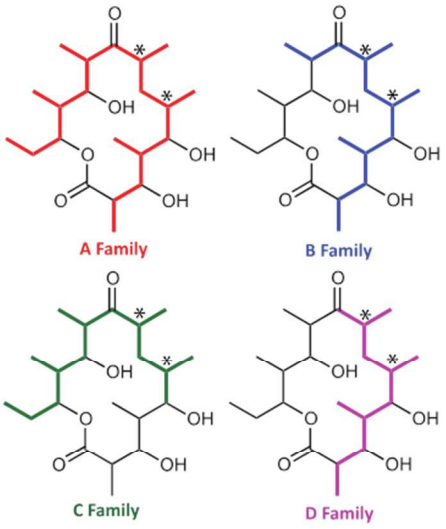


Figure S10: ESI-MS fragmentation patterns of (A) the 6-deoxyerythronolide B standard (16 μM), and (B) the analog corresponding to the 14.0 min peak in Figure 3.



Family	6-dEB	6-dEB+CH_2	Mass Change
A1	351.257	365.268	14.011
A2	333.239	347.256	14.017
B1	253.179	267.195	14.016
B2	235.169	249.185	14.016
B3	217.158	231.173	14.015
C1	239.200	253.216	14.016
C2	221.190	235.207	14.017
D1	223.136	237.148	14.012

Figure S11: Interpretation of fragmentation patterns shown in Figure S10. The asterisked carbons in the 6-deoxyerythronolide B structures correspond to those positions where incorporation of an ethylmalonyl extender unit is predicted to result in a mass increase of one methylene unit for all four fragment ion families. For a detailed description of these fragment ion families, see Ashley *et al.* 2004.[6]

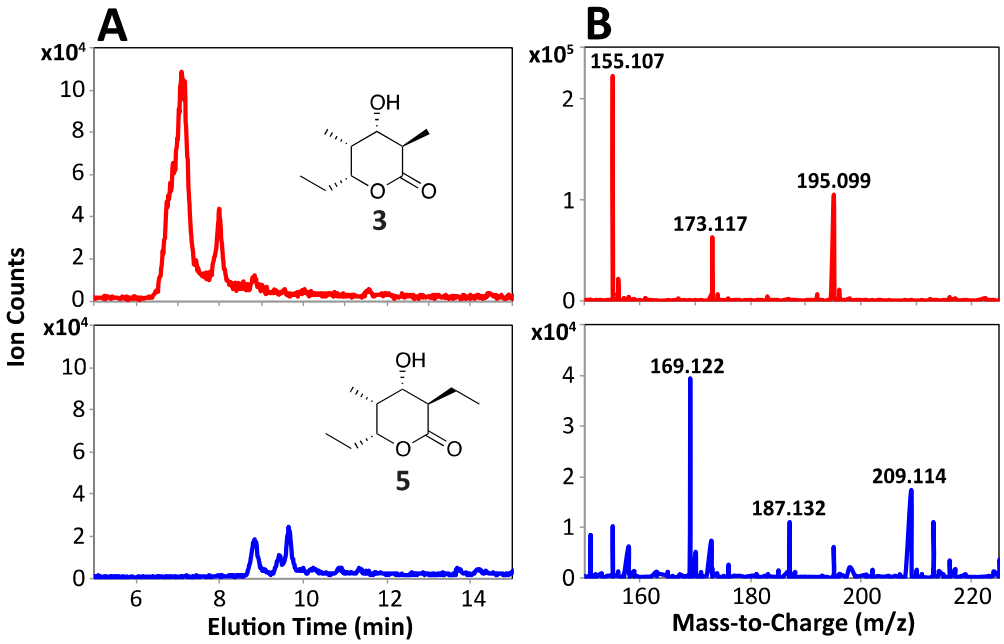


Figure S12: Product profiles of the bimodular derivative of DEBS in the presence of comparable concentrations of methylmalonyl and ethylmalonyl extender units. (A) Extracted Ion Chromatograms (obtained by extraction of the $[M+Na]^+$ species) for triketide **3** (top) and analogs (one proposed structure shown as **5**) with the molecular formula $C_{10}H_{18}O_3$, corresponding to products derived from incorporation of a single ethylmalonyl extender (bottom). (B) Full mass spectrum of the triketide **3** (7.1 min, top) and the most abundant analog corresponding to (8.8

min; bottom). In each case, ions corresponding to the following species were observed: $[M+H-H_2O]^+$, $[M+H]^+$, and $[M+Na]^+$.

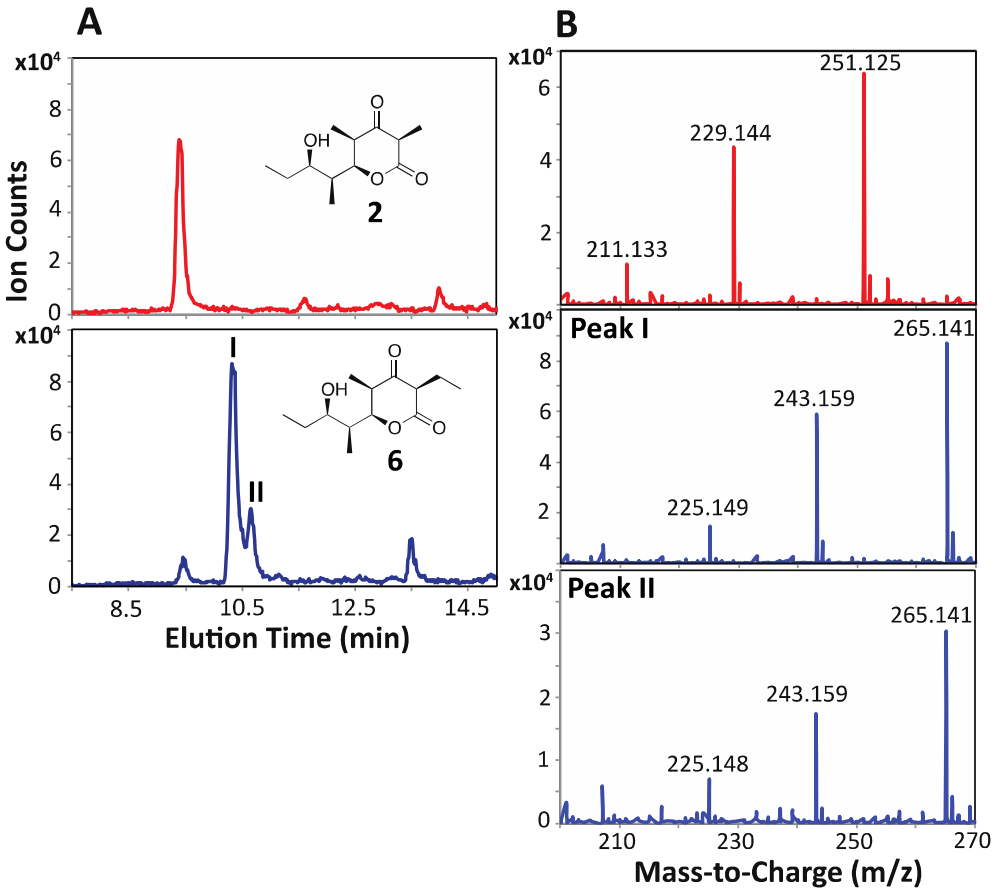


Figure S13: Product profiles of the trimodular derivative of DEBS in the presence of comparable concentrations of methylmalonyl and ethylmalonyl extender units. (A) Extracted Ion Chromatograms (obtained by extraction of the $[M+Na]^+$ species) for tetraketide **2** (top) and analogs (one proposed structure shown as **6**) with the molecular formula $C_{13}H_{22}O_4$, corresponding to products derived from incorporation of a single ethylmalonyl extender (bottom). (B) Full mass spectrum of the tetraketide **2** (top) and the most abundant analogs corresponding to Peak I (9.4 min; middle) and Peak II (10.3 min; bottom). In each case, ions corresponding to the following species were observed: $[M+H-H_2O]^+$, $[M+H]^+$, and $[M+Na]^+$.

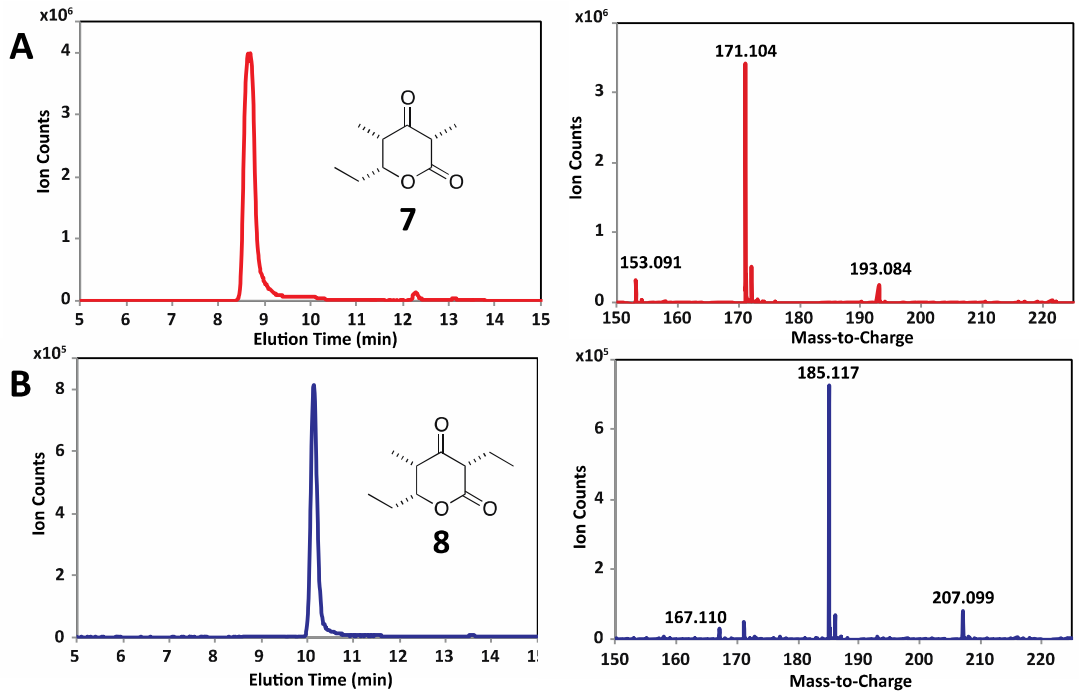


Figure S14: DEBS M3+TE incubated with the N-acetylcysteamine thioester of (2*S*,3*R*)-2-methyl-3-hydroxypentanoic acid (a known substrate of this module) and comparable concentrations of methylmalonyl and ethylmalonyl extender units. Extracted Ion Chromatograms (obtained by extraction of the $[M+H]^+$ species) (left) and full mass spectra (right) for the expected methylmalonyl-derived triketide **7** (top) and the ethylmalonyl-derived triketide **8** (bottom) are shown. In each case, ions corresponding to the following species were observed: $[M+H-H_2O]^+$, $[M+H]^+$, and $[M+Na]^+$.

Table S1. Plasmid Construct Design and Primers

Plasmid Encoded Protein	Cloning Method	PCR Fragment	Primer Name	Primer Sequence	PCR Template
pBL12 LDD(4)	3-fragment Gibson Assembly	1. LDD	pBL12_LDD_Nterm	GCCGCGCGGCAGCCATATGGCG GACCTGTCAAAGCTC	pBP144[2]
			pBL12_LDD_Cterm	CCGCCGGTGAGGCCGCGAATTC CCGTTGTGCGACCTCGG	
		2. M4 DD	pBL12_M4DD_Nterm	CCGAGGTCGCACAACGGGAATT CGCGGCCTCACCGGCGG	pBP130[2]
			pBL12_M4DD_Cterm	GTCGACGGAGCTCGAATTCTCA CAGGTCCTCTCCCCCGCC	
		3. pET28 Vector	pBL12_pET28_Nterm	GGCGGGGGAGAGGACCTGTGA GAATTCGAGCTCCGTCGAC	pET28
			pBL12_pET28_Cterm	GAGCTTTGACAGGTCCGCCATA TGGCTGCCGCGCGGC	
pBL13 (5)M1(2)	3-fragment Gibson Assembly	1. M5 DD and pET21 vector	pBL13_Vector_Nterm	CGACGGCGATCCGAATTCGAGC TCCGTCGACAAGC	pFW100
			pBL13_Vector_Cterm	GCGACGACCGCGACCGGTTCAC CGGCCCGGTGCTCGA	
		2. M1	pBL13_M1_Nterm	TCGAGCACCGGGCCGGTGAACC GGTCGCGGTCGTCGC	pBP144[2]
			pBL13_M1_Cterm	GCCTCCCCCCGGACCTCGGTGC CGAGTTCGGCGGCCAGG	
		3. M2 DD	pBL13_M2DD_Nterm	CCTGGCCGCCGAACTCGGCACC GAGGTCCGGGGGGAGGC	pBP19[3]
			pBL13_M2DD_Cterm	GCTTGTCGACGGAGCTCGAATT CGGATCGCCGTCG	
pBL36 (3)M2(2)	2-fragment Gibson Assembly	1. M3 DD and pET21	pBL36_Vector_Nterm	CTCGACGGCGATCCGAATTCGA GCTCCGTCGACAAGC	pRSG34[4]
			pBL36_Vector_Cterm	CGACGATCGCGATCGGCTC GGATTCCAGCTCGCGGATGCG	
		2. M2	pBL36_M2_Nterm	CGCATCCGCGAGCTGGAATCC GAGCCGATCGCGATCGTCG	pBP19
			pBL36_M2_Cterm	GCTTGTCGACGGAGCTCGAATT CGGATCGCCGTCGAG	
pBL16 (3)M2+TE	2-fragment Gibson Assembly	1. M3 DD and pET21 and TE	pBL16_Vector_Nterm	CCTCACTAGTGAGCTCGGC AGCGGGACTCCCGCCCGGG	pRSG34
			pBL16_Vector_Cterm	CCGACGATCGCGATCGGCTC GGATTCCAGCTCGCGGATGC	
		2. M2	pBL16_M2_Nterm	GCATCCGCGAGCTGGAATCC GAGCCGATCGCGATCGTCGG	pBP19
			pBL16_M2_Nterm	CCCGGGCGGGAGTCCCGCTGCC GAGCTCACTAGTGAGGAAACCG	
pFW98	Restriction Digestion / Ligation	DEBS2 NdeI / EcoRI Insert	(3)mod3_fwd	AAAAACATATGGCTAGCACTGA CAGCGAG	pBP130[2]
			mod4C	TTTTTGAATTCGCCAGGTCCTCT CCCCCGCC	
pFW100	Restriction Digestion / Ligation	DEBS3 NdeI / EcoRI Insert	mod5N	AAAAACATATGAGCGGTGACA ACGGCATG	pBP130[2]
			mod6C	TTTTTAAGCTTTGAATTCCCTCC GCCCAG	

Table S2. Protein Sequences and Diagrams for Engineered DEBS Modules

Table Color Coding:
Module Internal Sequence
Module 2 C-terminal Docking Domain Sequence
Module 3 N-terminal Docking Domain Sequence
Module 4 C-terminal Docking Domain Sequence
Module 5 N-terminal Docking Domain Sequence
Docking domain fusion sites denoted using double bars - ||

DEBS Protein	Amino Acid Sequence
LDD(4)	GSSHHHHHHSSGLVPRGSHMADLSKLSDSRTAQPGRIVRPWPLSGCNESALRARARQLRA HLDRFPDAGVEGVGAALAHDEQADAGPHRAVVVASSTSELLDGLAAVADGRPHASVVRGV ARPSAPVVFVFPGQGAQWAGMAGELLGESRVFAAAMDACARAFEPVTDWTLAQVLDSPE QSRRVEVVQPALFAVQTSLAALWRSFGVTPDAVVGHSIGELAAAHVCGAAGAADAARAAA LWSREMIPLVGNGDMAAVALSADEIEPRIARWDDDVVLAGVNGPRSVLLTGSPEPVARRV QELSAEGVRAQVINVSMAAHSAQVDDIAEGMRSALAWFAPGGSEVPFYASLTGGAVDTRE LVADYWRRSFRLPVRFDEAIRSALEVGPGTFVEASPHPVLAAALQQTLDAEGSSAAVVPTL QRGQGGMRRFLLAAAQAFTGGVAVDWTAAYDDVGAEPGSLPEFAPAEEEDEPAESGVDW NAPPHVLRERLLAVVNGETAALAGREADAEATFRELGLDSVLAAQLRAKVSAAIGREVNIA LLYDHPTPRALAEALAAGTEVAQRE||FAASPAVDIGDRLDELEKALEALSAEDGHDDVGQR LESLLRRWNSRRADAPSTSAISEDASDDELFSMLDQRFGGGEDL
(5)M1(2)	MSGDNGMTEEKLRRYLKRTVTELDSVTARLREVEHRAG||EPVAVVAMACRLPGGVSTPEE FWELLSEGRDAVAGLPTDRGWDLDSLFHPDPTRSGTAHQRGGGFLTEATAFDPAFFGMSP REALAVDPQQRLMLELSWEVLERAGIPPTSLQASPTGVFVGLIPQEYGPRLAEGGEGVEGY LMTGTTTSVASGRIAYTLGLEGPAISVDTACSSSLVAVHLACQSLRRGESSLAMAGGVTVM PTPGMLVDFSRMNSLAPDGRCKAFSAGANGFGMAEGAGMLLLERLSDARRNGHPVLAVL RGTAVNSDGASNGLSAPNGRAQVRVIQQALAESGLGPADIDAVEAHGTGTRLGDPIEARAL FEAYGRDREQPLHLGSVKSNLGHTQAAAGVAGVIKMVLAMRAGTLPRTLHASERSKEID WSSGAISLLDEPEPWPAGARPRRAGVSSFGISGTNAHAIIEEAPQVVEGERVEAGDVVAPW VLSASSAEGLRAQAARLAAHLREHPGQDPRDIAYSLATGRAALPHRAAFAPVDESAALRVL DGLATGNADGAAVGTSRAQQRAVFVFPGQGWQWAGMAVDLLDTSPVFAAALRECADAL EPHLDFEVIPFLRAEAARREQDAALSTERVDVVQPVMFAVMVSLASMWRAHGVEPAAVIG HSQGEIAAACVAGALSLDDAARVVALRSRVIATMPGNKGMASIAAPAGEVRARIGDRVEIA AVNGPRSVVVAGDSDELDRLVASCTTECIRAKRLAVDYASHSSHVETIRDALHAELGEDFH PLPGFVPFFSTVTGRWTQPDELDAGYWYRNLRRTVRFADAVRALAEQGYRTFLEVSAHPI LTAAIEEIGDGSGADLSAIHSLRRGDGSLADFGEALSRAFAAGVAVDWESVHLGTGARRVP LPTYPFQRERVWLEPKPVARRSTEVDEVSALRYRIEWRPTGAGEPARLDGTWLVAKYAGT ADETSTAAREALESAGARVRELVVDARCGRDELAERLRSVGEVAGVLSLLAVDEAEPEEAP LALASLADTLSLVQAMVSAELGCPLWTVTESAVATGPFERVRNAAHGALWGVGRVIALEN PAVWGGLVDVPAGSVAELARHLAAVVSGGAGEDQLALRADGVYGRRWVRAAAPATDDE WKPTGTVLVTGGTGGVGGQIARWLARRGAPHLLLVSRSGPDADGAGELVAELEALGART TVAACDVTDRESVRELLGGIGDDVPLSAVFHAAATLDDGTVDTLTGERIERASRAKVLGAR NLHELTRELDLTAFVLFSSFASAFGAPGLGGYAPGNAYLDGLAQQRRSDGLPATAVAWGT WAGSGMAEGPVADRFRRHGVIEMPPETACRALQNALDRAEVCPIVIDVRWDRFLLAYTAQ RPTRLFDEIDDARRAAPQAAAEPRVGALASLPAPEREKALFELVRSHAAAVLGHASAERVP ADQAFAELGVDSLSALELRNRLGAATGVRLPTTTVFDHPDVRTLAAHLAAELG||TEVRGEA PSALAGLDALEAALPEVPATEREELVQRLERMLAALRPVAQAADASGTGANPSGDDLGEA GVDELLEALGRELDGDANSSSVDKLAAALEHHHHHH
(3)M2(2)	MASTDSEKVAEYLRRATLDLRAARQRIRELES||EPIAIVGMACRLPGEVDSPERLWELITSG RDSAAEVPDDRGWVPDELMASDAAGTRRAHGNFMAGAGDFDAAFFGISPREALAMDPQQ RQALETTWEALESAGIPPETLRGSDTGVFVGMSHQGYATGRPRPEDGVDGYLLTGNTASV ASGRIAYVLGLEGPALTVDTACSSSLVALHTACGSLRDGDCGLAVAGGVSVMAGPEVFTEF SRQGALSPDGRCKPFSDEADGFGLGEGSAFVVLQRLSDARREGRRVLGVVAGSAVNQDGA SNGLSAPSGVAQQRVIRRAWARAGITGADVAVVEAHGTGTRLGDPVEASALLATYGKSRG SSGPVLLGSVKSNIGHAQAAAGVAGVIKVLLGLERGVVPPMLCRGERSGLIDWSSGEIELA DGVREWSPAADGVRRAGVSAFGVSGTNAHVIIAEPPEPEPVPQPRRMLPATGVVPVVLSAR TGAALRAQAGRLADHLAAHPGIAPADVSWTMARARQHFEERAAVLAADTAEAVHRLRAV ADGAVVPGVVTGSASDGGSVFVFPGQGAQWEGMARELLPVPVFAESIAECDAVLSEVAGF

	SVSEVLEPRPDAPSLERVDVVQPVLFAVMVSLARLWRACGAVPSAVIGHSQGEIAAAVVAG ALSLEDGMRVVARRSRAVRAVAGRGSMLSVRGGRSDVEKLLADDSWTGRLEVAAVNGPD AVVVAGDAQAAREFLEYCEGVGIRARAIPVDYASHTAHVEPVRDELVQALAGITPRRAEVP FFSTLTGDFLDGTELDAGYWYRNLRHPVEFHSAVQALTDQGYATFIEVSPHPVLASSVQET LDDAESDAAVLGTLERDAGDADRFLTALADAHTRGVAVDWEAVLGRAGLVDLPGYPFQG KRFWLLPDRTTPRDELDGWFYRVDWTEVPRSEPAALRGRWLVVVPEGHEEDGWTVEVRS ALAEAGAEPEVTRGVGGLVGDCAGVVSLLALEGDGAVQTLVLVRELDAEGIDAPLWTVTF GAVDAGSPVARPDQAKLWGLGQVASLERGPRWTGLVDLPHMPDPELRGRLTAVLAGSED QVAVRADAVRARRLSPAHVTATSEYAVPGGTILVTGGTAGLGAEVARWLAGRGAEHLAL VSRRGPDTEGVGDLTAELTRLGARVSVHACDVSSREPVRELVHGLIEQGDVVRGVVHAAG LPQQVAINDMDEAAFDEVVAAKAGGAVHLDELCSDAELFLLFSSGAGVWGSARQGAYAA GNAFLDAFARHRRGRGLPATSVAWGLWAAGGMTGDEEAVSFLRERGVRAMPVPRALAA LDRVLASGETAVVVTDVDWPAFAESYTAARPRPLLDRIVTTAPSERAGEPETESLRDRLAG LPRAERTAELVRLVRTSTATVLGHDDPKAVRATTPFKELGFDSLAAVRLRNLLNAATGLRL PSTLVFDHPNASAVAGFLTSELG||TEVRGEAPSALAGLDALEAALPEVPATEREELVQRLER MLAALRPVAQAADASGTGANPSGDDLGEAGVDELLEALGRELDGDPNSSSVDKLAAALEH HHHHH
DEBS2	MASTDSEKVAEYLRRATLDLRAARQRIRELESDPIAIVSMACRLPGGVNTPQRLWELLREG GETLSGFPTDRGWDLARLHHPDPDNPGTSYVDKGGFLDDAAGFDAEFFGVSPREAAAMDP QQRLLLETSWELVENAGIDPHSLRGTATGVFLGVAKFGYGEDTAAAEDVEGYSVTGVAPA VASGRISYTMGLEGPSISVDTACSSSLVALHLAVESLRKGESSMAVVGGAAVMATPGVFVD FSRQRALAADGRSKAFGAGADGFGFSEGVTLVLLERLSEARRNGHEVLAVVRGSALNQDG ASNGLSAPSGPAQRRVIRQALESCGLEPGDVDAVEAHGTGTALGDPIEANALLDTYGRDRD ADRPLWLGSVKSNIGHTQAAAGVTGLLKVVLALRNGELPATLHVEEPTPHVDWSSGGVAL LAGNQPWRRGERTRRAAVSAFGISGTNAHVIVEEAPEREHRETTAHDGRPVPLVVSARSTA ALRAQAAQIAELLERPDADLAGVGLGLATTRARHEHRAAVVASTREEAVRGLREIAAGAA TADAVVEGVTEVDGRNVVFLFPGQGSQWAGMGAELLSSSPVFAGKIRACDESMAPMQDW KVSDVLRQAPGAPGLDRVDVVQPVLFAVMVSLAELWRSYGVEPAAVVGHSQGEIAAAHV AGALTLEDAAKLVVGRSRLMRSLSGEGGMAAVALGEAAVRERLRPWQDRLSVAAVNGPR SVVVSGEPGALRAFSEDCAAEGIRVRDIDVDYASHSPQIERVREELLETTGDIAPRPARVTFH STVESRSMDGTELDARYWYRNLRETVRFADAVTRLAESGYDAFIEVSPHPVVVQAVEEAVE EADGAEDAVVVGSLHRDGGDLSAFLRSMATAHVSGVDIRWDVALPGAAPFALPTYPFQRK RYWLQPAAPAAASDELAYRVSWTPIEKPESGNLDGDWLVVTPLISPEWTEMLCEAINANG GRALRCEVDTSASRTEMAQAVAQAGTGFRGVLSLLSSDESACRPGVPAGAVGLLTLVQAL GDAGVDAPVWCLTQGAVRTPADDDLARPAQTTAHGFAQVAGLELPGRWGGVVDLPESVD DAALRLLVAVLRGGGRAEDHLAVRDGRLHGRRVVRASLPQSGSRSWTPHGTVLVTGAAS PVGDQLVRWLADRGAERLVLAGACPGDDLLAAVEEAGASAVVCAQDAAALREALGDEPV TALVHAGTLTNFGSISEVAPEEFAETIAAKTALLAVLDEVLGDRAVEREVYCSSVAGIWGG AGMAAYAAGSAYLDALAEHHRARGRSCTSVAWTPWALPGGAVDDGYLRERGLRSLSADR AMRTWERVLAAGPVSVAVADVDWPVLSEGFAATRPTALFAELAGRGGQAEAEPDSGPTG EPAQRLAGLSPDEQQENLLELVANAVAEVLGHESAAEINVRRAFSELGLDSLNAMALRKRL SASTGLRLPASLVFDHPTVTALAQHLRARLVGDADQAAVRVVGAADESEPIAIVGIGCRFPG GIGSPEQLWRVLAEGANLTTGFPADRGWDIGRLYHPDPDNPGTSYVDKGGFLTDAADFDP GFFGITPREALAMDPQQRLMLETAWEAVERAGIDPDALRGTDTGVFVGMNGQSYMQLLA GEAERVDGYQGLGNSASVLSGRIAYTFGWEGPALTVDTACSSSLVGIHLAMQALRRGECSL ALAGGVTVMSDPYTFVDFSTQRGLASDGRCKAFSARADGFALSEGVAALVLEPLSRARAN GHQVLAVLRGSAVNQDGASNGLAAPNGPSQERVIRQALAASGVPAADVDVVEAHGTGTEL GDPIEAGALIATYGQDRDRPLRLGSVKTNIGHTQAAAGAAGVIKVVLAMRHGMLPRSLHA DELSPHIDWESGAVEVLREEVPWPAGERPRRAGVSSFGVSGTNAHVIVEEAPAEQEAARTE RGPLPFVLSGRSEAVVAAQARALAEHLRDTPELGLTDAAWTLATGRARFDVRAAVLGDDR AGVCAELDALAEGRPSADAVAPVTSAPRKPVLVFPGQGAQWVGMARDLLESSEVFAESMS RCAEALSPHTDWKLLDVVRGDGGPDPHERVDVLQPVLFSIMVSLAELWRAHGVTPAAVV GHSQGEIAAAHVAGALSLEAAAKVVALRSQVLRELDDQGGMVSVGASRDELETVLARWD GRVAVAAVNGPGTSVVAGPTAELDEFFAEAEAREMKPRRIAVRYASHSPEVARIEDRLAAE LGTITAVRGSVPLHSTVTGEVIDTSAMDASYWYRNLRRPVLFEQAVRGLVEQGFDTFVEVS PHPVLLMAVEETAEHAGAEVTCVPTLRREQSGPHEFLRNLLRAHVHGVGADLRPAVAGG RPAELPTYPFEHQRFWPRPHRPADVSALGVRGAEHPLLLAAVDVPGHGGAVFTGRLSTDE QPWLAEHVVGGRTLVPGSVLVDLALAAGEDVGLPVLEELVLQRPLVLAGAGALLRMSVG APDESGRRTIDVHAAEDVADLADAQWSQHATGTLAQGVAAGPRDTEQWPPEDAVRIPLDD HYDGLAEQGYEYGPSFQALRAAWRKDDSVYAEVSIAADEEGYAFHPVLLDAVAQTLSLGA LGEPGGGKLPFAWNTVTLHASGATSVRVVATPAGADAMALRVTDPAGHLVATVDSLVVR STGEKWEQPEPRGGEGELHALDWVRLAEPGSTGRVVAADASDLDAVLRSGEPEPDAVLVR

	YEPEGDDPRAAARHGVLWAAALVRRWLEQEELPGATLVIATSGAVTVSDDDSVPEPGAAA MWGVIRCAQAESPDRFVLLDTDAEPGMLPAVPDNPQLALRGDDVFVPRLSPLAPSALTLPA GTQRLVPGDGAIDSVAFEPAPDVEQPLRAGEVRVDVRATGVNFRDVLLALGMYPQKADM GTEAAGVVTAVGPDVDAFAPGDRVLGLFQGAFAPIAVTDHRLLARVPDGWSDADAAAVPI AYTTAHYALHDLAGLRAGQSVLIHAAAGGVGMAAVALARRAGAEVLATAGPAKHGTLRA LGLDDEHIASSRETGFARKFRERTGGRGVDVVLNSLTGELLDESADLLAEDGVFVEMGKT DLRDAGDFRGRYAPFDLGEAGDDRLGEILREVVGLLGAGELDRLPVSAWELGSAPAALQH MSRGRHVGKLVLTQPAPVDPDGTVLITGGTGTLGRLLARHLVTEHGVRHLLLVSRRGAD APGSDELRAEIEDLGASAEIAACDTADRDALSALLDGLPRPLTGVVHAAGVLADGLVTSIDE PAVEQVLRAKVDAAWNLHELTANTGLSFFVLFSSAASVLAGPGQGVYAAANESLNALAAL RRTRGLPAKALGWGLWAQASEMTSGLGDRIARTGVAALPTERALALFDSALRRGGEVVF PLSINRSALRRAEFVPEVLRGMVRAKLRAAGQAEAAGPNVVDRLAGRSESDQVAGLAELV RSHAAAVSGYGSADQLPERKAFKDLGFDSLAAVELRNRLGTATGVRLPSTLVFDHPTPLAV AEHLRDRLFAASPAVDIGDRLDELEKALEALSAEDGHDDVGQRLESLLRRWNSRRADAPST SAISEDASDDELFSMLDQRFGGGEDLGNSSSVDKLAAALEHHHHHH
DEBS3	MSGDNGMTEEKLRRYLKRTVTELDSVTARLREVEHRAGEPIAIVGMACRFPGDVDSPESF WEFVSGGGDAIAEAPADRGWEPDPDARLGGMLAAAGDFDAGFFGISPREALAMDPQQRI MLEISWEALERAGHDPVSLRGSATGVFTGVGTVDYGPRPDEAPDEVLGYVGTGTASSVAS GRVAYCLGLEGPAMTVDTACSSGLTALHLAMESLRRDECGLALAGGVTVMSSPGAFTEFR SQGGLAADGRCKPFSKAADGFGLAEGAGVLVLQRLSAARREGRPVLAVLRGSAVNQDGAS NGLTAPSGPAQQRVIRRALENAGVRAGDVDYVEAHGTGTRLGDPIEVHALLSTYGAERDP DDPLWIGSVKSNIGHTQAAAGVAGVMKAVLALRHGEMPRTLHFDEPSPQIEWDLGAVSVV SQARSWPAGERPRRAGVSSFGISGTNAHVIVEEAPEADEPEPAPDSGPVPLVLSGRDEQAMR AQAGRLADHLAREPRNSLRDTGFTLATRRSAWEHRAVVVGDRDDALAGLRAVADGRIAD RTATGQARTRRGVAMVFPGQGAQWQGMARDLLRESQVFADSIRDCERALAPHVDWSLTD LLSGARPLDRVDVVQPALFAVMVSLAALWRSHGVEPAAVVGHSQGEIAAAHVAGALTLED AAKLVAVRSRVLRRLGGQGGMASFGLGTEQAAERIGRFAGALSIASVNGPRSVVVAGESG PLDELIAECEAEGITARRIPVDYASHSPQVESLREELLTELAGISPVSADVALYSTTTGQPIDT ATMDTAYWYANLREQVRFQDATRQLAEAGFDAFVEVSPHPVLTVGIEATLDSALPADAGA CVVGTLRRDRGGLADFHTALGEAYAQGVEVDWSPAFADARPVELPVYPFQRQRYWLPIPT GGRARDEDDDWRYQVVWREAEWESASLAGRVLLVTGPGVPSELSDAIRSGLEQSGATVLT CDVESRSTIGTALEAADTDALSTVVSLLSRDGEAVDPSLDALALVQALGAAGVEAPLWVLT RNAVQVADGELVDPAQAMVGGLGRVVGIEQPGRWGGLVDLVDADAASIRSLAAVLADPR GEEQVAIRADGIKVARLVPAPARAARTRWSPRGTVLVTGGTGGIGAHVARWLARSGAEHL VLLGRRGADAPGASELREELTALGTGVTIAACDVADRARLEAVLAAERAEGRTVSAVMHA AGVSTSTPLDDLTEAEFTEIADVKVRGTVNLDELCPDLDAFVLFSSNAGVWGSPGLASYAA ANAFLDGFARRRRSEGAPVTSIAWGLWAGQNMAGDEGGEYLRSQGLRAMDPDRAVEELH ITLDHGQTSVSVVDMDRRRFVELFTAARHRPLFDEIAGARAEARQSEEGPALAQRLAALST AERREHLAHLIRAEVAAVLGHGDDAAIDRDRAFRDLGFDSMTAVDLRNRLAAVTGVREAA TVVFDHPTITRLADHYLERLVGAAEAEQAPALVREVPKDADDPIAIVGMACRFPGGVHNPG ELWEFIVGGGDAVTEMPTDRGWDLDALFDPDPQRHGTSYSRHGAFLDGAADFDAAFFGIS PREALAMDPQQRQVLETTWELFENAGIDPHSLRGSDTGVFLGAAYQGYGQDAVVPEDSEG YLLTGNSSAVVSGRVAYVLGLEGPAVTVDTACSSSLVALHSACGSLRDGDCGLAVAGGVSV MAGPEVFTEFSRQGGLAVDGRCKAFSAEADGFGFAEGVAVVLLQRLSDARRAGRQVLGV VAGSAINQDGASNGLAAPSGVAQQRVIRKAWARAGITGADVAVVEAHGTGTRLGDPVEAS ALLATYGKSRGSSGPVLLGSVKSNIGHAQAAAGVAGVIKVVLGLNRGLVPPMLCRGERSPL IEWSSGGVELAEAVSPWPPAADGVRRAGVSAFGVSGTNAHVIIAEPPEPEPLPEPGPVGVLA AANSVPVLLSARTETALAAQARLLESAVDDSVPLTALASALATGRAHLPRRAALLAGDHEQ LRGQLRAVAEGVAAPGATTGTASAGGVVFVFPGQGAQWEGMARGLLSVPVFAESIAECD AVLSEVAGFSASEVLEQRPDAPSLERVDVVQPVLFSVMVSLARLWGACGVSPSAVIGHSQG EIAAAVVAGVLSLEDGVRVVALRAKALRALAGKGGMVSLAAPGERARALIAPWEDRISVA AVNSPSSVVVSGDPEALAELVARCEDEGVRAKTLPVDYASHSRHVEEIRETILADLDGISAR RAAIPLYSTLHGERRDGADMGPRYWYDNLRSQVRFDEAVSAAVADGHATFVEMSPHPVLT AAVQEIAADAVAIGSLHRDTAEEHLIAELARAHVHGVAVDWRNVFPAAPPVALPNYPFEPQ RYWLAPEVSDQLADSRYRVDWRPLATTPVDLEGGFLVHGSAPESLTSAVEKAGGRVVPVA SADREALAAALREVPGEVAGVLSVHTGAATHLALHQSLGEAGVRAPLWLVTSRAVALGES EPVDPEQAMVWGLGRVMGLETPERWGGLVDLPAEPAPGDGEAFVACLGADGHEDQVAI RDHARYGRRLVRAPLGTRESSWEPAGTALVTGGTGALGGHVARHLARCGVEDLVLVSRR GVDAPGAAELEAELVALGAKTTITACDVADREQLSKLLEELRGQGRPVRTVVHTAGVPES RPLHEIGELESVCAAKVTGARLLDELCPDAETFVLFSSGAGVWGSANLGAYSAANAYLDAL AHRRRAEGRAATSVAWGAWAGEGMATGDLEGLTRRGLRPMAPERAIRALHQALDNGDT CVSIADVDWERFAVGFTAARPRPLLDELVTPAVGAVPAVQAAPAREMTSQELLEFTHSHVA

	AILGHSSPDAVGQDQPFTELGFDSLTAVGLRNQLQQATGLALPATLVFEHPTVRRLADHIG QQLDSGTPAREASSALRDGYRQAGVSGRVRSYLDLLAGLSDFREHFDGSDGFSLDLVDMA DGPGEVTVICCAGTAAISGPHEFTRLAGALRGIAPVRAVPQPGYEEGEPLPSSMAAVAAVQ ADAVIRTQGDKPFVVAGHSAGALMAYALATELLDRGHPPRGVVLIDVYPPGHQDAMNAW LEELTATLFDRETVRMDDTRLTALGAYDRLTGQWRPRETGLPTLLVSAGEPMGPWPDDS WKPTWPFEHDTVAVPGDHFTMVQEHADAIARHIDAWLGG**GNSSSVDKLAAALEHHHHHH**
(3)M2+TE	**MASTDSEKVAEYLRRATLDLRAARQRIRELES**||EPIAIVGMACRLPGEVDSPERLWELITSG RDSAAEVPDDRGWVPDELMASDAAGTRRAHGNFMAGAGDFDAAFFGISPREALAMDPQQ RQALETTWEALESAGIPPETLRGSDTGVFVGMSHQGYATGRPRPEDGVDGYLLTGNTASV ASGRIAYVLGLEGPALTVDTACSSSLVALHTACGSLRDGDCGLAVAGGVSVMAGPEVFTEF SRQGALSPDGRCKPFSDEADGFGLGEGSAFVVLQRLSDARREGRRVLGVVAGSAVNQDGA SNGLSAPSGVAQQRVIRRAWARAGITGADVAVVEAHGTGTRLGDPVEASALLATYGKSRG SSGPVLLGSVKSNIGHAQAAAGVAGVIKVLLGLERGVVPPMLCRGERSGLIDWSSGEIELA DGVREWSPAADGVRRAGVSAFGVSGTNAHVIIAEPPEPEPVPQPRRMLPATGVVPVVLSAR TGAALRAQAGRLADHLAAHPGIAPADVSWTMARARQHFEERAAVLAADTAEAVHRLRAV ADGAVVPGVVTGSASDGGSVFVFPGQGAQWEGMARELLPVPVFAESIAECDAVLSEVAGF SVSEVLEPRPDAPSLERVDVVQPVLFAVMVSLARLWRACGAVPSAVIGHSQGEIAAAVVAG ALSLEDGMRVVARRSRAVRAVAGRGSMLSVRGGRSDVEKLLADDSWTGRLEVAAVNGPD AVVVAGDAQAAREFLEYCEGVGIRARAIPVDYASHTAHVEPVRDELVQALAGITPRRAEVP FFSTLTGDFLDGTELDAGYWYRNLRHPVEFHSAVQALTDQGYATFIEVSPHPVLASSVQET LDDAESDAAVLGTLERDAGDADRFLTALADAHTRGVAVDWEAVLGRAGLVDLPGYPFQG KRFWLLPDRTTPRDELDGWFYRVDWTEVPRSEPAALRGRWLVVVPEGHEEDGWTVEVRS ALAEAGAEPEVTRGVGGLVGDCAGVVSLLALEGDGAVQTLVLVRELDAEGIDAPLWTVTF GAVDAGSPVARPDQAKLWGLGQVASLERGPRWTGLVDLPHMPDPELRGRLTAVLAGSED QVAVRADAVRARRLSPAHVTATSEYAVPGGTILVTGGTAGLGAEVARWLAGRGAEHLAL VSRRGPDTEGVGDLTAELTRLGARVSVHACDVSSREPVRELVHGLIEQGDVVRGVVHAAG LPQQVAINDMDEAAFDEVVAAKAGGAVHLDELCSDAELFLLFSSGAGVWGSARQGAYAA GNAFLDAFARHRRGRGLPATSVAWGLWAAGGMTGDEEAVSFLRERGVRAMPVPRALAA LDRVLASGETAVVVTDVDWPAFAESYTAARPRPLLDRIVTTAPSERAGEPETESLRDRLAG LPRAERTAELVRLVRTSTATVLGHDDPKAVRATTPFKELGFDSLAAVRLRNLLNAATGLRL PSTLVFDHPNASAVAGFLTSELGSGTPAREASSALRDGYRQAGVSGRVRSYLDLLAGLSDFR EHFDGSDGFSLDLVDMADGPGEVTVICCAGTAAISGPHEFTRLAGALRGIAPVRAVPQPGY EEGEPLPSSMAAVAAVQADAVIRTQGDKPFVVAGHSAGALMAYALATELLDRGHPPRGVV LIDVYPPGHQDAMNAWLEELTATLFDRETVRMDDTRLTALGAYDRLTGQWRPRETGLPT LLVSAGEPMGPWPDDSWKPTWPFEHDTVAVPGDHFTMVQEHADAIARHIDAWLGG**GNSS SVDKLAAALEHHHHHH**

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