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Analysis of the Molecular Recognition Features of Individual Modules Derived from the Erythromycin Polyketide Synthase

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Abstract: 6-Deoxyerythronolide B synthase (DEBS), the multifunctional enzyme responsible for the biosynthesis of the macrolide aglycon of the antibiotic erythromycin, is an excellent model system for studying the properties of modular polyketide synthases. In these studies, we analyzed the substrate specificity of selected individual modules of DEBS. Unexpectedly, we observed (i) a high degree of similarity in the specificity of all modules tested, despite the diverse structural features of their natural substrates, and (ii) a distinct preference by all modules for syn diketides over anti diketides. The implications of these results are analyzed from an evolutionary and a protein engineering perspective.

Introduction

Polyketide synthases have generated significant attention in recent years because of their striking ability to catalyze multistep biosynthetic reactions and because of their immense potential for the combinatorial biosynthesis of complex molecules. The source of this combinatorial potential lies in the unique modular architecture of these biosynthetic enzymes. 6-Deoxyerythronolide B synthase (DEBS), the multifunctional enzyme that produces 6-deoxyerythronolide B (6-dEB, **1**), the aglycon precursor of the antibiotic erythromycin, is an excellent model system for examining the structure and function of modular polyketide synthases. This megasynthase consists of three polypeptide subunits (DEBS1, DEBS2, and DEBS3), each containing two distinct elongation modules per polypeptide (Figure 1). In addition to these six modules, a loading didomain at the N-terminus of DEBS1 is responsible for priming the synthase with a propionyl unit, and a thioesterase (TE) domain at the C-terminus of DEBS3 is reponsible for chain release and macrocyclization. Each elongation module can be further subdivided into structurally distinct domains. The "core" domains, ketosynthase (KS), acyltransferase (AT), and acyl carrier protein (ACP), are responsible for catalyzing the decarboxylative condensation of a methylmalonyl unit onto the growing chain. In addition, there are several variable sets of post-condensation modification domains, including ketoreductase (KR), dehydratase (DH), and enoylreductase (ER) domains, within certain modules.

The realization of the combinatorial potential of modular polyketide synthases is dependent on the verification of two main assumptions: (1) that individual domains and modules of these megasynthases can be interchanged without disrupting

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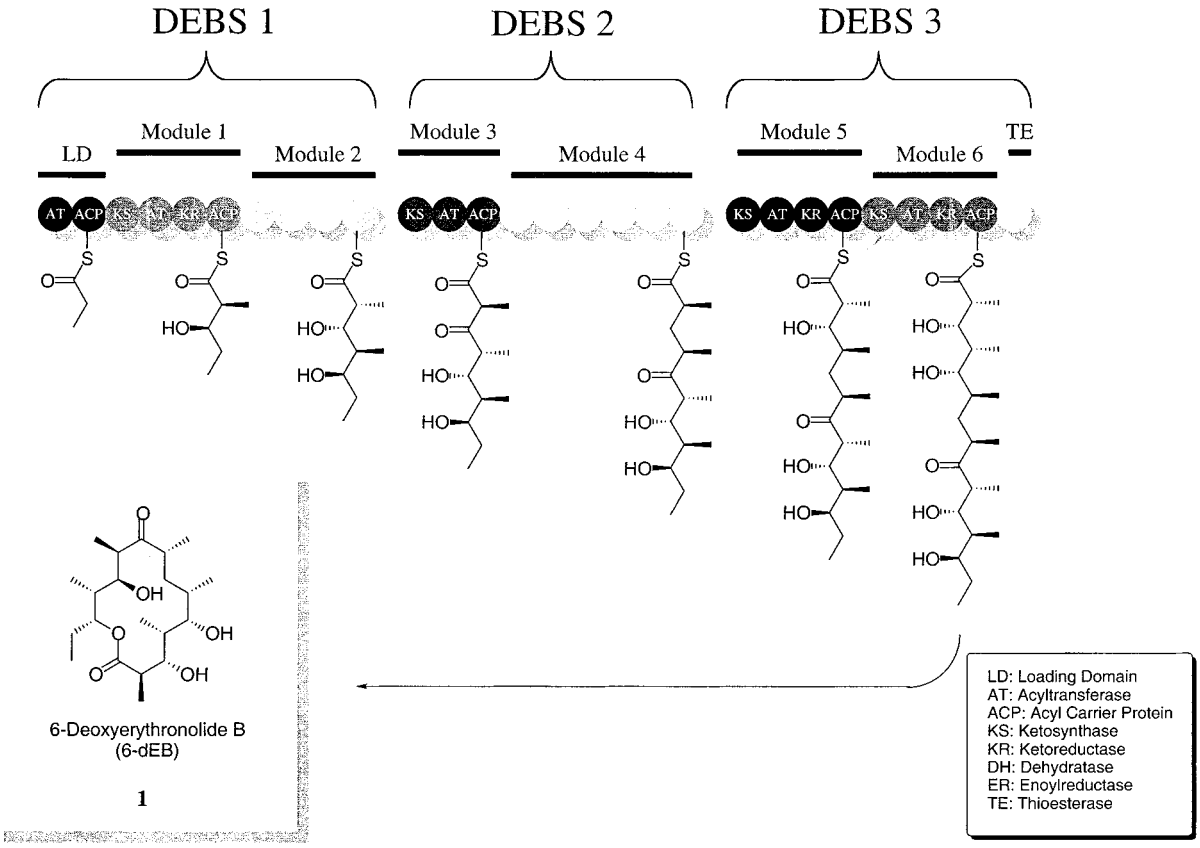

Figure 1. Schematic diagram of DEBS, its intermediates, and its final product.

their structural integrity and (2) that the individual domains and modules are able to accept and process unnatural substrates. There is a growing body of evidence indicating that genetic manipulation of domains and modules is a tractable problem.[1−14] In addition, there are a number of examples, both in vivo and in vitro, that demonstrate the tolerance of modular polyketide synthases for unnatural substrates.[15] For example, the tolerance of downstream modules for substrates with unnatural degrees of reduction is well documented both in vivo and in vitro.[1,6,7,13,16,17] There are also several documented cases in which domains (e.g., loading domain and acyltransferase) with alternative substrate specificity were substituted into existing polyketide synthase frameworks, confirming the relaxed specificity of both the recombinant module and the downstream modules.[4,8−10,13,14] And finally, precursor-directed experiments both in vivo and in vitro have delineated a reasonably broad range of substrate tolerance by downstream DEBS modules.[16−21]

Despite the previous work on the tolerance and specificity of modular PKSs, these experiments have not been able to probe these megasynthases at the level of individual modules. In this paper, we looked at the substrate specificity of individual modules of the DEBS system. Specifically, we wished to address the following questions: (1) Do individual modules have intrinsic substrate specificity? (2) If these modules are selective toward incoming acyl chains, is there a correlation between this specificity and the structure and stereochemistry of the natural substrates of these modules? The structural features of the substrates that we were interested in probing are the stereochemistry at the α- and *β*-positions as well as the chain length of the carbon backbone. To probe the effects of these different features, we chose compounds **2**−**6** (shown in Figures 2 and 4) as model substrates. Compounds **2**−**5** cover all possible diastereomers at the α- and *β*-positions, whereas compounds **2** and **6** examine the effect of increasing chain length. As enzymes, we chose modules 2, 3, 5, and 6 from the DEBS system. In each case, the TE domain from the C-terminus of DEBS3 was fused to the C-terminus of the individual modules to facilitate turnover. In their natural context each of these modules recognizes and extends a unique substrate (shown in Figure 1). The results of our studies not only provide a better understanding of the substrate recognition features of the

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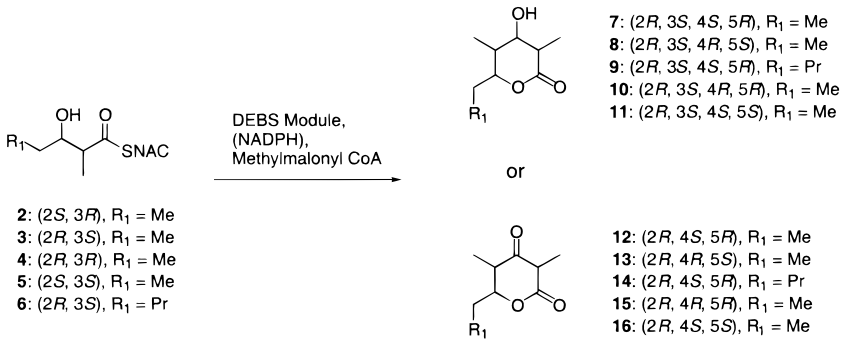

Figure 2. Substrates and products of enzymatic reactions. Products **10**, **11**, **15**, and **16** were not detected by radio-TLC.

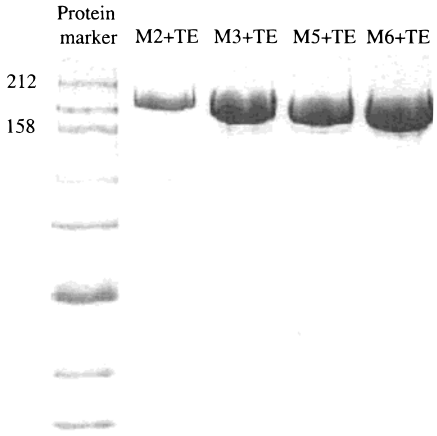

Figure 3. SDS-PAGE gel of purified module 2 + TE, module 3 + TE, module 5 + TE, and module 6 + TE.

individual DEBS modules but also facilitate assessment of the combinatorial biosynthetic potential of modular polyketide synthases.

Results

Protein Purification. Each of the proteins used in this study was expressed and purified as described in the Experimental Section. The purity of these proteins was determined to be >95% by SDS-PAGE (Figure 3). The yields of the purified proteins were approximately 2 mg/L of culture for M2+TE, 3 mg/L of culture for M3+TE and M5+TE, and 4 mg/L of culture for M6+TE.

Assays for Substrate Incorporation. Initially, each module was qualitatively assayed with each substrate using conditions similar to those described in the Experimental Section for the kinetic assays. The results of these assays are tabulated in Figure 4. For all modules, the diketides with anti stereochemistry across their α- and β-positions were not substrates within the limits of detection, whereas diketides with syn stereochemistry were substrates.

For each case where a product was detected in the above-mentioned qualitative assays, the steady-state parameters associated with the reactions shown in Figure 2 were measured. (The normalized v vs [S] plots for these reactions are available as Supporting Information.) From these data, the k_{cat}, K_M, and k_{cat}/K_M values could be calculated; these parameters are tabulated in Figure 5. In each case, k_{cat} and K_M were calculated by fitting the normalized v vs [S] plots to the Michaelis−Menten equation. It should be noted that in some cases, substrate inhibition was observed at high concentrations. In these cases, the reported k_{cat} and K_M values should be regarded as lower limits. However, since the specificity parameter, k_{cat}/K_M, was calculated directly from the slope of the v vs [S] curve at low substrate concentrations, there is a greater degree of precision in the latter values.

In an earlier study describing the construction of these individual modules, the k_{cat} and K_M for each module were measured using **2** as a substrate.[12] Our values for K_M are in good agreement with those reported earlier. However, as a result of improved purification and assay techniques (see Experimental Section), we have been able to reproducibly enhance the k_{cat} values for modules 2, 3, and 6 by approximately an order of magnitude over those reported earlier. Indeed, the highest k_{cat} values reported in Figure 5 are comparable to those reported for intact, self-priming multi-modular PKSs,[23] suggesting that the properties of isolated modules described here actually reflect those observed in multi-modular systems. Although the activity of module 5 in the presence of **2** did not seem to benefit from the improved purification and assay condition, the activity of this enzyme is validated by the relatively high turnover rate of **6**.

Verification of Products of Enzymatic Reactions. Verification of the structure of the triketide lactones **7** and **8** produced by the reaction of diketide **2** with modules 2+TE, 5+TE, and 6+TE, and with module 3+TE, respectively, has been previously reported.[12] Triketide lactone **8** has previously been confirmed as the product of the bimodular DEBS3 alone.[24] To confirm the identity of the products obtained from the incubations of diketides **2** and **3** with modules 2+TE, 5+TE, and 6+TE, the radioactive products were converted to the corresponding 3-dinitrobenzoates and analyzed by reverse-phase HPLC with radiodetection and direct comparison with synthetic standards. Because of the inefficient turnover of diketide **3** by module 5+TE, the radioactivity level of the corresponding **8**-DNB product was too low to be detected by radio-HPLC. The TLC R_f values of the products in 60% EtOAc/hexanes are as follows: **7**, 0.36; **8**, 0.35; **9**, 0.41; **12**, 0.30; **13**, 0.30; and **14**, 0.32

Discussion

We have previously studied the bimodular PKS construct DEBS1+TE(KS1°), a truncated DEBS mutant in which KS1 (and thereby module 1) has been inactivated by site-directed mutagenesis. Incubation of DEBS1+TE(KS1°) with diketide **2** in the presence of methylmalonyl-CoA and NADPH gave the natural triketide lactone **7**. By carrying out incubations with diketide substrate analogues of varying chain length and substitution pattern, we obtained the relative k_{cat}/K_M values for each substrate, which in turn provides a measure of the substrate specificity of module 2 fused to its natural module 1 partner.[19]

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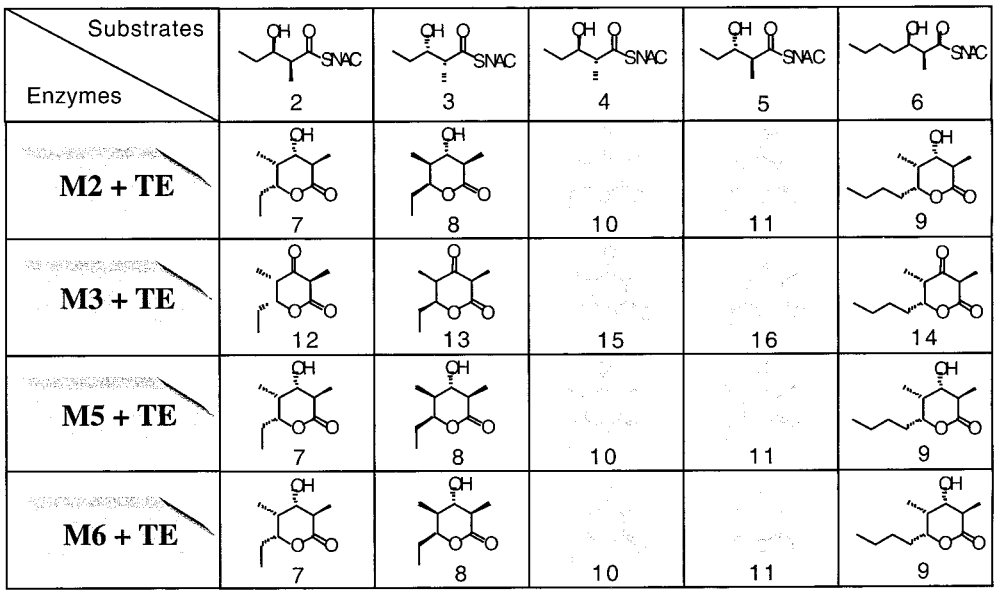

Figure 4. Expected products and qualitative results of in vitro enzymatic reactions with compounds **2**−**6**. Products that were not detected by radio-TLC are shown in faded print, while products that were detected by radio-TLC are shown in normal print.

	kcat (1/min)	KM (mM)	kcat/KM (1/min/mM)
Compound **2**			
M2+TE	>4.58±0.561	>3.21±1.59	0.753±.0975
M3+TE	>1.12±0.0626	>1.98±0.406	0.316±0.00283
M5+TE	0.24±0.0131	16.1±1.99	0.0161±0.00166
M6+TE	>17.1±2.92	>9.94±5.34	1.07±0.124
Compound **3**			
M2+TE	0.249±0.0183	32.5±5.50	0.00756±0.000582
M3+TE	0.194±0.0187	14.7±3.66	0.0094±0.000943
M5+TE	0.0165±0.000988	11.4±1.80	0.00106±0.000141
M6+TE	2.39±0.220	50.4±9.16	0.058±0.00565
Compound **6**			
M2+TE	1.86±0.0512	0.521±0.0614	2.26±0.138
M3+TE	>0.569±0.0927	>0.349±0.309	0.632±0.034
M5+TE	1.92±0.123	3.96±0.739	0.346±0.069
M6+TE	>9.71±1.42	>1.68±0.953	3.03±0.0778

Figure 5. Summary of kinetic parameters for module 2 + TE, module 3 + TE, module 5 + TE, and module 6 + TE with compounds **2**, **3**, and **6**.

A similar study describing the qualitative properties of "wild-type" DEBS1+TE has also been reported.[25]

In this paper, we have probed the specificity and tolerance of individual modules of a modular polyketide synthase. The constructs included in this study are DEBS modules 2, 3, 5, and 6, each of which is fused to the DEBS TE domain (whose molecular recognition features have been probed elsewhere).[26] The substrates against which we tested our enzymes include the four diastereomers of the natural diketide **2**, as well as the homologous diketide **6**, which has two extra carbons at the end of the carbon backbone. The specificities of the enzymes were assessed by steady-state kinetic analysis.

Qualitatively, there are two distinct categories of substrates (Figure 4). The first category includes those diketides that are substrates for all the enzymes; and the second category includes those diketides that are not substrates for any of the enzymes. Quite notably, the defining feature that determines whether a compound is accepted as a substrate seems to be the *relative* configuration of the α- and *β*-substituents. All compounds with syn stereochemistry (i.e., **2**, **3**, and **6**) across their α- and *β*-positions are substrates, while all the compounds with anti stereochemistry (**4** and **5**) are not substrates. This observation has two significant implications.

First, the fact that all the DEBS modules that were tested have similar substrate preferences suggests that these modules have common recognition features, even though their natural substrates differ widely in chain length, oxidation level, substitution pattern, and stereochemistry. Perhaps this commonality in substrate preference reinforces the notion that multi-modular PKSs arose through duplication of a common ancestral module. Indeed, sequence analysis of the KS domains of the erythromycin (6 modules), rapamycin (14 modules), tylosin (7 modules), rifamycin (10 modules), and avermectin (12 modules) reveals that the KS domains from the same PKS share significantly closer evolutionary relationships to each other than to the KS domains from other PKSs (unpublished observation). This distinct partitioning of KS domains therefore suggests that each synthase arose independently of the others via gene duplication.

The second significant implication of the distinct preference of DEBS modules for syn diketides over anti diketides is that the enzymes distinguish between the relative configurations, rather than the absolute configurations, of the α- and *β*-substituents of the substrates. This sensitivity toward relative stereochemistry is quite unusual in the context of enzyme stereose-

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(25) Weissman et al. (ref 16) subsequently reported a study in which a variety of diketide analogues of varying stereochemistry and chain length were incubated with the "wild-type" DEBS1+TE, and substrates were evaluated by whether the expected product was detected by GC-MS analysis of a 45 h incubation. Although steady-state kinetic parameters were not reported for any substrate, where the same substrates were examined, the results were in qualitative agreement with our earlier study (ref 19) as well as with those of the current study with one exception. Weissman et al. were unable to observe turnover of diketide **3** by their DEBS1+TE preparation. In the absence of quantitative kinetic data for their enzyme preparation, the reasons for this discrepancy are unclear, although the low k_{cat} and k_{cat}/K_m values for **3** require enzyme preparations of high specific activity. **Note Added in Proof**: Bycroft et al. (Bycroft, M.; Weissman, K. J.; Staunton, J.; Leadlay, P. F. *Eur. J. Biochem.*, **2000**, *267*, 520) recently reported kinetic data for a truncated PKS DEBS 1+TE. They attributed their lower k_{cat} value of 0.84 min^{-1} for the synthesis of (3*S*,5*R*)-dihydroxy-(2*R*,4*R*)-dimethyl-*n*-heptanoic acid-*δ*-lactone to the hybrid ACP2 domain of their engineered protein.

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lectivity. In addition, it may imply that the substrate binding pocket of the enzyme has sufficient flexibility to allow a syn diketide to bind in two different mirror image conformations.

Quantitative analysis of the steady-state kinetics parameters of the four DEBS modules (Figure 5) also reveals several interesting features. The focus of these data is on the k_{cat}/K_M parameters, since these values provide the most informative measure of the substrate specificity. As with the qualitative data, the order of preference of substrates is consistent across all the tested modules and is as follows: **6** > **2** > **3**. The k_{cat}/K_M preference for **2** over **3** for all enzymes ranges from 20- to 100-fold. On the other hand, the preference for **6** over **2** is modest (2- to 3-fold) in the cases of modules 2, 3, and 6, but substantial (>25-fold) in the case of module 5. (It should be noted that although M5+TE turns over **2** and **3** at rather low rates, the rate of product formation with **6** is comparable to that by the other modules, suggesting that the intrinsic enzymatic activity of module 5 has not been attenuated by protein engineering or purification.) The general preference of all the enzymes for longer chain substrates over shorter chain substrates has been previously observed using multi-modular proteins,[19,27] and suggests that extended chain length and/or hydrophobicity are desirable characteristics of good substrates.

Perhaps the most intriguing observation in this study is the universal preference of all four modules tested for **2** over **3**. Considering that the natural substrates (shown in Figure 1) of modules 3 and 6 have the same α- and *β*-configuration as **3**, this observation suggests that there is not a strong correlation between the optimal substrate for these modules and their natural substrates. Several hypotheses can be invoked to rationalize this perplexing conclusion.

For example, it is possible that our synthetic substrates do not incorporate the complex structural features of the natural substrates for modules 3 and 6 which are important for recognition. This explanation is unlikely for at least some modules, since the k_{cat} values of these modules (especially module 6, which ordinarily handles the most complex substrate) with the simple diketide substrates are comparable to the highest reported k_{cat} of multi-modular systems, suggesting that complex recognition features are not essential for optimal activity.

In addition, the suggestion that the observed substrate specificity could merely be a reflection of the substrate specificity of the TE domain at the C-termini of the modules can be ruled out, since k_{cat}/K_M is a function of only those kinetic steps up to and including the first irreversible step. Therefore, in all likelihood, the relative k_{cat}/K_M values for each module reflect the intrinsic substrate preference of the corresponding KS domains and are unlikely to be influenced by downstream events such as KR-catalyzed *β*-keto reduction or TE-catalyzed lactonization. Furthermore, it should also be noted that **3** is in fact a better substrate than **2** for direct TE-catalyzed hydrolysis.[26]

Yet another hypothesis to rationalize the universal preference of the four modules for **2** over **3** is that the substrate specificity observed in these studies is not representative of what is going on in a multi-modular system. According to this hypothesis, other factors such as protein−protein interactions between modules play a role in the overall activity of multi-modular systems.[12] However, there is at least some evidence that suggests that the results reported here on isolated modules are consistent with the properties of multi-modular systems. For example, construction and in vivo analysis of engineered bimodular derivatives of DEBS has shown that whereas modules 2, 3, and 6 are able to efficiently accept and extend the diketide product of module 1 (which is analogous to substrate **2**),[12] a module 1−module 5−TE bimodular system is incapable of producing isolable quantities of the expected triketide (S. Tsuji and C. Khosla, unpublished observations). This disparity is in agreement with our observations (Figure 5) that modules 2, 3, and 6 are significantly better catalysts for the elongation of **2** than is module 5.

Finally, the lack of correlation between the optimal substrates for the modules in this study and their natural substrates can be rationalized by the postulation that each module in a polyketide synthase has evolved to optimize the biological activity of the resulting products rather than to optimize the rate of processing of the incoming intermediate. While the enzymes might have an inherent preference for certain substrates, they are capable of catalyzing reactions using their less optimal, natural substrate to obtain a final biosynthetic product with the desired biological activity. Although further studies will be required to definitively address this intriguing question, our bias toward this final hypothesis is based on the surprising data reported here as well as the extraordinary diversity of substrates handled by naturally occurring PKSs.

The modularity of DEBS offers exciting possibilities for the combinatorial biosynthesis of complex polyketide products through engineering of individual modules and domains. But before such potential can be realized, it is important to determine the tolerance and specificity of the modules for unnatural substrates. In this study, we have shown that while there is not a strict correlation between the in vitro substrate preference of the individual modules and the absolute configuration of their natural substrates, the modules do retain a strict discrimination against substrates with anti stereochemistry at the α- and *β*-positions. Since the corresponding anti configurations are found in numerous polyketides, probing the intrinsic preference of the responsible modules is an important goal for the future.

Experimental Section

Synthesis of Substrates. The diketide substrates used in this study (**2**−**6**) were synthesized using established procedures and were determined to be spectroscopically equivalent to the published compounds.[18,22] Each was determined to have de > 99% by NMR.

Construction of Plasmids. The *sfp* plasmid as well as plasmids pRSG64, pRSG34, pRSG46, and pRSG54 (encoding for module 2 + TE, module 3 + TE, module 5 + TE, module 6 + TE, respectively) were constructed as previously described.[12]

Protein Purification. Buffer A is composed of 100 mM NaH_2PO_4, 1 mM EDTA, 2.5 mM DTT, and 20% glycerol. Buffer B (for the butyl sepharose column) is composed of 100 mM NaH_2PO_4, 1 M $(NH_4)_2SO_4$, 1 mM EDTA, 2.5 mM DTT, and 20% glycerol. Buffer C (Resource Q column) is composed of 100 mM NaH_2PO_4, 0.5 M NaCl, 1 mM EDTA, 2.5 mM DTT, and 20% glycerol. The proteins were purified on a 30 mL butyl sepharose column (4 Fast Flow resin from Amersham Pharmacia Biotech AB) followed by a 6 mL Resource Q from Amersham Pharmacia Biotech AB. The proteins were quantitated by Lowry assay from Sigma. PD-10 desalting columns were purchased from Amersham Pharmacia Biotech AB.

Plasmids pRSG64, pRSG34, pRSG46, and pRSG54 were coexpressed in *Escherichia coli* with an *sfp* plasmid to ensure complete pantetheinylation of the ACP domains. The cells were grown in LB at 37 °C until $OD_{600} = 0.6-0.7$. At this point, the cultures were induced with 1 mM final concentration of IPTG, and then pRSG34, pRSG46, and pRSG54 grown at 30 °C overnight while pRSG64 was grown at 22 °C overnight. After spinning down the cells, the pellet was washed with a buffer consisting of 50 mM Tris-HCl/1 mM EDTA, pH 8. The washed cells were then resuspended in disruption buffer and lysed with a French Press at 1000 psi. After centrifugation, the supernatant was treated with a 0.1% PEI precipitation followed by a 50% $(NH_4)_2SO_4$

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precipitation for 2 h. The resulting precipitate was resuspended in Buffer A and the protein was filtered through a PD-10 column, eluting with Buffer B. The protein was purified on a butyl sepharose column, using a gradient from 100% buffer B to 100% buffer A. The proteins eluted between 200 mM $(NH_4)_2SO_4$ and 0 mM $(NH_4)_2SO_4$. The appropriate fractions were then collected (and diluted with buffer A if necessary) and then applied to a Resource Q column. Using a gradient from 100% buffer A to 100% buffer C, the protein eluted at approximately 150 mM NaCl.

Kinetic Assays. [2-^{14}C]-Methylmalonyl Coenzyme A (54 mCi/mmol) was purchased from American Radiolabeled Chemicals, Inc. The enzymatically generated labeled triketide lactone products were separated on Baker Si250F silica gel TLC plates, and the radioactivity of the individual components was quantitated using a Packard PhosphoImager.

The kinetic assays were performed in a buffered solution of 400 mM NaH_2PO_4, 1 mM EDTA, 2.5 mM DTT, 5 mM NaCl, 20% glyercol, and 1.5% DMSO (to improve solubility of the substrate), pH 7.2, in the presence of 800 μM [2-^{14}C]-methylmalonyl CoA and 4 mM NADPH (for modules 2, 5, and 6) (Figure 2). After 5−50 turnovers at 30 °C, the reactions were quenched by the addition of ethyl acetate and subsequent vortexing. Extraction twice with ethyl acetate removed the triketide lactone product from the aqueous layer, and the product yield was quantitated by radio-TLC. Each assay was performed at least two times on two different days to ensure reproducibility. Reproducibility across protein preparations was also verified through kinetic analysis on two different batches of module 6+TE.

Analysis of Enzymatically Generated Triketide Lactones. The incubation reaction was carried out with 1 μM protein, 20 mM diketide substrate, 4 mM NADPH, 200 μM methylmalonyl CoA, 600−800 mM sodium phosphate buffer (pH 7.2), 20% glycerol, 2.5 mM DTT, and 1 mM EDT in 100 μL total volume at 30 °C for 1.5−16 h. After extraction of the enzyme reaction products and evaporation, the triketide lactone was converted to the corresponding 3,4-dinitrobenzoate derivative by reaction with 5 mg of 3,5-dinitrobenzoyl chloride and 5 mg of DMAP in CH_2Cl_2 at room temperature for 1 h. The derivative was prepurified by preparative TLC (R_f ca. 0.3, 1:1 EtOAc/hexane), then dissolved in 200 μL of CH_3CN. A portion (20 μL) of the solution was analyzed by reverse-phase HPLC on a Rainin Dynamax HPLC system using a C18 column (Microsorb, 4.6 mm i.d. × 250 mm, Rainin) with a mobile phase of 60% CH_3CN in deionized water at a flow rate of 1 mL/min. The elution was monitored by UV at 249 nm and by detection of ^{14}C (Packard Radiomatic Flo-One\Beta), with a Ultima-Flo M scintillation cocktail being mixed downstream with the eluant (2 mL/min). The retention time was compared directly with authentic samples of each triketide lactone derivative. (2*R*,3*S*,4*S*,5*R*)-3-(3′,5′-Dinitrobenzoyl)-2,4-dimethyl-3,5-dihydroxy-*n*-heptanoic acid δ-lactone: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (t, J = 7.2 Hz, 3H, H-7), 1.09 (d, J = 7.0 Hz, 3H, 4-CH_3), 1.44 (d, J = 7.1 Hz, 3H, 2-CH_3), 1.64 and 1.89 (m, 2H, H-6), 2.58 (ddq, J = 2.3, 4.4, and 7.0 Hz, 1H, H-4), 2.96 (dq, J = 7.1 and 10.6 Hz, 1H, H-2), 4.33 (ddd, J = 2.3, 6.0, and 8.1 Hz, 1H, H-5), 5.29 (dd, J = 4.4 and 10.6 Hz, 1H, H-3), 9.17 (d, J = 2.0 Hz, 2H, H-2′,H-6′), 9.29 (d, J = 2.0 Hz, 1H, H-4′); λ_{max} 249 nm, ϵ_{max} 8000. (2*R*,3*S*,4*R*,5*S*)-3-(3′,5′-Dinitrobenzoyl)-2,4-dimethyl-3,5-dihydroxy-*n*-heptanoic acid δ-lactone: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (t, J = 7.4 Hz, 3H, H-7), 1.15 (d, J = 7.4 Hz, 3H, 4-CH_3), 1.40 (d, J = 6.9 Hz, 3H, 2-CH_3), 1.59 and 1.85 (m, 2H, H-6), 2.20 (ddq, J = 2.2 and 7.4 Hz, 1H, H-4), 3.03 (dq, J = 6.9 Hz, 1H, H-2), 4.54 (ddd, J = 2.4, 5.0, and 8.1 Hz, 1H, H-5), 4.83 (dd, J = 2.2 and 7.8 Hz, 1H, H-3), 9.17 (d, J = 2.0 Hz, 2H, Ph), 9.29 (d, J = 2.0 Hz, 1H, Ph); λ_{max} 249 nm, ϵ_{max} 8000.

Supporting Information Available: Normalized v vs [S] plots for the reactions of compounds **2**, **3**, and **6** with module 2 + TE, module 3 + TE, module 5 + TE, and module 6 + TE (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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