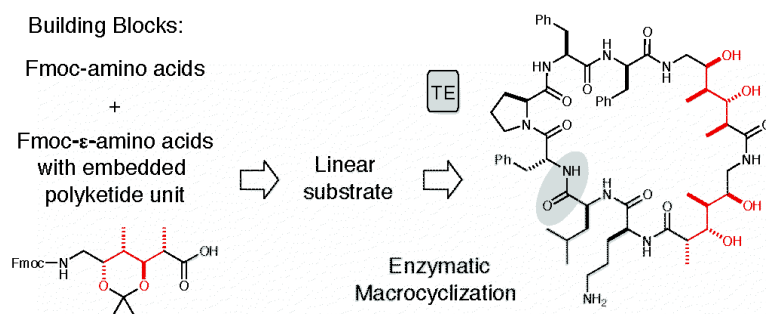# Chemoenzymatic Route to Macrocyclic Hybrid Peptide/Polyketide-like Molecules

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Chemoenzymatic Route to Macrocyclic Hybrid Peptide/Polyketide-like Molecules

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A variety of natural products with therapeutic activities are enabled to populate biologically favorable conformers by macro-cyclization.[1] As with many polyketides (PK) and nonribosomal peptides (NRP), macrocyclic hybrid PK/NRP natural products, such as the immunosupressant rapamycin and the anticancer agent epothilone, have the ability to interact with biological targets or modulate protein−protein interactions.[2] In the biosynthesis of these ring-containing natural products, linear chains constructed by a similar logic are subjected to essentially identical macrocyclization machinery, a thioesterase (TE) domain at the C-terminal end of the multimodular enzymatic assembly lines.[1,2a,3] We have demonstrated that the TE domains excised from the NRPS assembly lines for tyrocidine **1**, gramicidin, and surfactin, as well as the epothilone hybrid synthetase, retain macrocyclization activity when excised and studied as autonomous catalytic domains.[4] We have shown that the excised tyrocidine TE domain, TycC TE, is permissive for alterations in most side chains or the length of a peptidyl thioester substrate.[4,5] Further, the TE will recognize peptidyl oxoesters attached to PEGA beads, so that solid-phase peptide synthesis (SPPS) can be used in a chemoenzymatic approach to production of libraries of cyclic peptides, although previously the functional diversity of PK and hybrid molecules remained inaccessible by this method.[6]

We present here a chemoenzymatic strategy to access the stereochemical and functional diversity of macrocyclic PK/NRP natural products in a manner amenable to efficient library synthesis. Specifically, we have made use of small building blocks presenting embedded PK units in the form of ϵ-amino acids that allow for the construction of linear hybrid peptide/polyketide-like chains through iterative amide bond formation. Further, to increase the potential for attaining the biologically active structures seen in many macrocyclic natural products, we demonstrate the use of the NRPS-derived enzyme, TycC TE, to effect macrocyclization of these linear, hybrid-like substrates.

In PK biosynthesis, following chain elongation by the formation of tethered β-keto-acyl intermediates by ketosynthase domains, coordinated action of ketoreductase domains within the same PKS module typically convert the initial 3-keto condensation product to the 3-OH form. A subsequent iteration would then yield a 3,5-diol with 2,4-dimethyl, monomethyl, or methylene groups, depending on the selection of methylmalonyl or malonyl building blocks. The erythromycin skeleton contains such four-carbon signature epitopes, for example, in the part of structure **2** spanning C_2-C_5 (Figure 1), where glycosylation of the 5-OH in this system is crucial for the gain of antibiotic activity.[7] Using a laboratory analogue of this biosynthetic pathway, developed by Evans and co-workers for
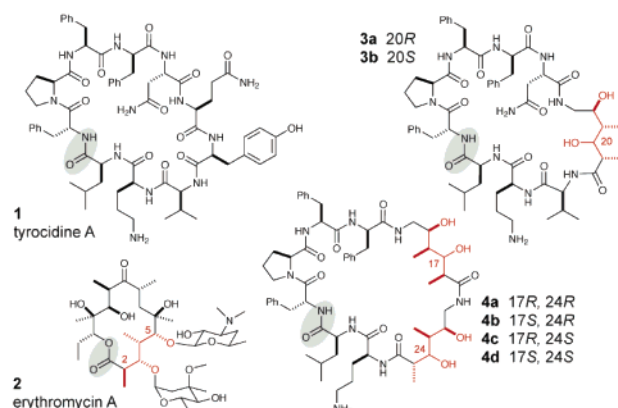
Figure 1. Macrocyclic natural products and chemoenzymatically synthesized hybrid-like products. Polyketide epitopes are shown in red, with bonds formed on macrocyclization highlighted by shading.

Scheme 1 [a]
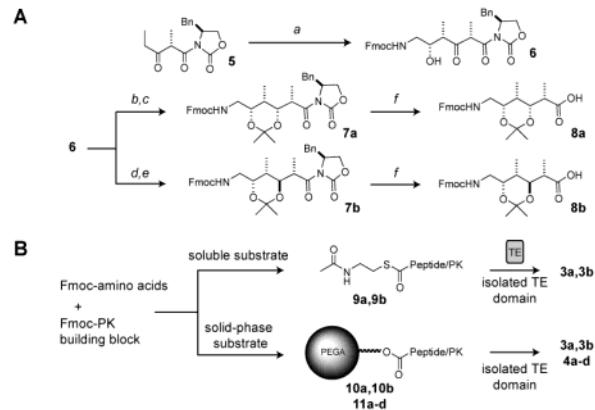
[a] Conditions: (a) $TiCl_4$, DIPEA, *N*-Fmoc-glycinal, then H_2O_2-MeOH, 81%, dr 7.5:1; (b) $Zn(BH_4)_2$; (c) 2,2-dimethoxypropane, TsOH, 61% over two steps; (d) $Me_4N(OAc)_3BH$; (e) 2,2-dimethoxypropane, PPTS, 42% over two steps; (f) LiOOH, $THF-H_2O$, 60−61%.

the efficient construction of polypropionate systems,[8] we developed a four-step route to ϵ-amino acids compatible with SPPS with PK functionality spanning $C_\beta-C_\epsilon$ (Scheme 1A). Specifically, condensation of the *Z* trichlorotitanium enolate derived from the β-keto imide **5** with Fmoc-glycinal[9] provided the all-syn aldol adduct **6**.[10] Subsequent syn or anti diastereoselective ketone reduction, diol ketalization, and lithium hydroperoxide-mediated hydrolysis of the chiral auxiliary afforded the desired Fmoc-protected ϵ-amino acids **8a** and **8b**.[11] Modifications of this synthetic scheme may provide access to significant diversity in the PK building block, in the form of other diastereomers, altered oxidation states, and non-hydrogen substituents at C_α.

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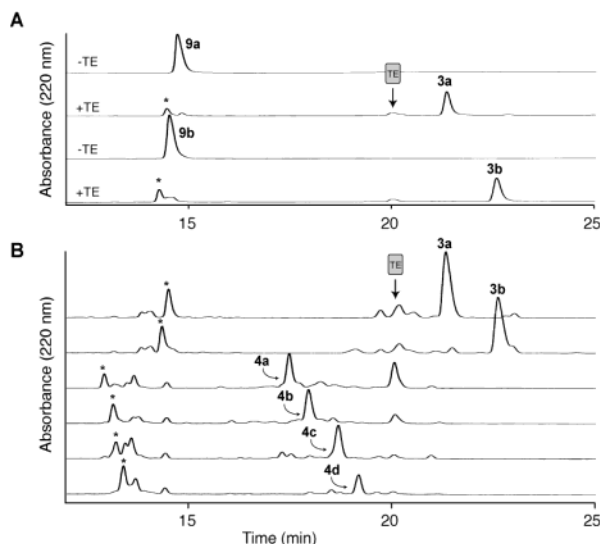
Figure 2. HPLC traces showing chemoenzymatic synthesis products. (A) Soluble thioesters **9a** and **9b** are converted to cyclic products **3a** and **3b** with minor flux to hydrolysis product (denoted by *). (B) Enzymatic reaction products released into solution upon incubation of substrates tethered to the solid phase. Single (**3a** and **3b**) and tandem (**4a**–**d**) insertion products are observed. Products were confirmed by MS/MS fragmentation. Minor products eluting from 13 to 15 min are hydrolysis product (*) and truncated products from SPPS. Peaks eluting at 20 min correspond to enzyme, with variable amounts of catalyst observed.

It was unclear at the outset if a TE domain derived from a strictly NRP synthetase would be capable of macrocyclizing a hybrid-like substrate. As a first test of the ability of TE domains to cyclize peptide backbones with polyketide substructures, we constructed peptide chains by SPPS containing the building blocks **8a** and **8b**, which were subsequently converted to soluble peptide thioester substrate **9a** and **9b** (Scheme 1B). The building blocks were viewed as replacements for a dipeptide in **1**, the natural tyrocidine A peptide scaffold, and targeted to a region known from prior studies to be tolerant to alteration.[5b] Both diastereomeric substrates were smoothly macrocyclized upon reaction with TycC TE to generate the macrolactams **3a** and **3b**, with minor flux to hydrolysis (Figure 2A).[12] Mass spectroscopy fragmentation confirmed the expected head-to-tail cyclization linkage. Cyclization kinetics of the soluble substrates could be measured (**9a**, k_{cat} 37 min^{-1}, K_M 68 μM; **9b**, 36 min^{-1}, 77 μM), revealing that catalytic efficiency is only modestly decreased with these substrates relative to that of the natural substrate (60 min^{-1}, 3 μM).[4a]

Using ϵ-amino acid building blocks to introduce polyketide units into polypeptide structures offers potential for synthesizing diverse libraries of hybrid PK/NRP-like macrocycles. As a demonstration of the feasibility of this aim, we next turned to substrates tethered to a solid-phase resin (Scheme 1B). This scheme is made to resemble the physiologic situation as linear substrates are constructed while tethered to PEGA resin via a linker mimicking pantetheine. TycC TE subsequently catalyzes both release of the substrate from its oxoester linkage to the solid-phase as well as macrocyclization.[6] To verify our methodology, the linear solid-phase substrates **10a** and **10b** corresponding to soluble **9a** and **9b** were synthesized. Incubation with the purified TE domain resulted in the generation of cyclization products **3a** and **3b** (Figure 2B). To generate diversity and increase the PK functionality in the

macrocycle, we next carried out tandem insertion of our ϵ-amino acid building blocks in all four possible permutations, yielding **11a**–**d**, replacing residues 5–8 in tyrocidine A. Remarkably, upon incubation with TycC TE, the hybrid-like macrocycle was the predominant product observed in each reaction.[12] Macrocycles **4a**–**d** contain eight stereogenic carbons in an eleven-atom stretch. That all four combinations were cyclized with similar efficiency suggests our building block approach should allow for rapid introduction of multiple PK epitopes into a diversified library. Further, that PK functionality can be introduced into a macrocycle via an NRPS-derived TE domain suggests that TE tolerance could have facilitated the evolution of hybrid synthetases in nature.

We have demonstrated a method for accessing the diversity of PK/NRP natural products via a remarkably versatile macrocyclization catalyst and a building-block approach to introducing diversity. It remains to be seen whether any of these polyketide epitopes will suffice on their own for binding to biological targets of polyketides. It will be intriguing to investigate if the advantages endowed to hybrid natural products in their functional diversity, improved cell permeability, or modification by tailoring enzymes (as in the glycosylation of erythromycin C_5-hydroxyl) will translate to our hybrid-like molecules. We anticipate the synthesis of diverse natural product-like libraries tailored for such particular assays or for screening for novel biological activities.

Supporting Information Available: Experimental procedures and characterization of compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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