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Employing Modular Polyketide Synthase Ketoreductases as Biocatalysts in the Preparative Chemoenzymatic Syntheses of Diketide Chiral Building Blocks

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SUMMARY

Chiral building blocks are valuable intermediates in the syntheses of natural products and pharmaceuticals. A scalable chemoenzymatic route to chiral diketides has been developed that includes the general synthesis of α-substituted, β-ketoacyl N-acetylcysteamine thioesters followed by a biocatalytic cycle in which a glucose-fueled NADPH-regeneration system drives reductions catalyzed by isolated modular polyketide synthase (PKS) ketoreductases (KRs). To identify KRs that operate as active, stereospecific biocatalysts, 11 isolated KRs were incubated with 5 diketides and their products were analyzed by chiral chromatography. KRs that naturally reduce small polyketide intermediates were the most active and stereospecific toward the panel of diketides. Several biocatalytic reactions were scaled up to yield more than 100 mg of product. These syntheses demonstrate the ability of PKS enzymes to economically and greenly generate diverse chiral building blocks on a preparative scale.

INTRODUCTION

Modular polyketide synthases (PKSs) are enzymatic assembly lines that control the substitution and stereochemistry of complex polyketides in the biosyntheses of such compounds as the antibacterial erythromycin, the antifungal amphotericin, and the immunosuppressant rapamycin (Staunton and Weissman, 2001; Khosla et al., 2007; Smith and Tsai, 2007; Sherman and Smith, 2006). Many synthetic organic chemists seek to emulate the reactions they catalyze and obtain the products they produce; however, if PKS enzymes, themselves, were harnessed as biocatalysts, coveted chiral building blocks and biologically active molecules would be more readily accessed.

Libraries of chiral building blocks are highly desired by synthetic organic chemists to accelerate the synthesis of natural products and pharmaceuticals (Hilterhaus and Liese, 2007; Patel, 2008). Such compounds would be particularly helpful in the syntheses of complex polyketides because the required synthetic fragments often harbor multiple stereocenters and are challenging to prepare. The complexity of synthesizing reduced polyketides can be appreciated by examining the synthetic routes established for 6-deoxyerythronolide B (6-dEB), the precursor of erythromycin antibiotics (Masamune et al., 1981; Myles et al., 1990; Evans et al., 1998; Crimmins and Slade, 2006; Stang and White, 2009). Although several groups over the last 30 years have worked on the synthesis of this archetypal macrolide core, the shortest is 22 steps with an overall yield of 7.8% (Stang and White, 2009).

The soil bacterium *Saccharopolyspora erythraea* synthesizes 6-dEB with DEBS (6-dEB synthase), a PKS that contains diverse catalytic activities: acyltransferases (ATs) catalyze the *trans*-esterification of small organic extender units from acyl-CoAs onto the PKS, ketosynthases (KSs) catalyze decarboxylative Claisen condensations that create carbon-carbon bonds between these extender units and the growing polyketide chain, ketoreductases (KRs) catalyze stereospecific, NADPH-coupled reductions of β-keto groups formed through condensation, a dehydratase (DH) catalyzes an elimination reaction to yield an olefin, and an enoylreductase (ER) catalyzes the stereospecific, NADPH-coupled reduction of that olefin (Figure 1A) (Khosla et al., 2007). Growing polyketides are passed along the PKS assembly line through *trans*-thioesterification reactions between acyl carrier proteins (ACPs) and KSs to a thioesterase (TE) that catalyzes the cyclization of a heptaketide to yield 6-dEB. Thus, enzymes in the PKS cooperate to transform seven molecules of propionate into one molecule of 6-dEB under ambient, aqueous conditions. As the current syntheses of 6-dEB and related natural products rely on organic solvents, chiral auxiliaries, and metals that can be both expensive and environmentally harmful, employing PKS enzymes in such syntheses would be advantageous.

Aldol reactions employed in organic syntheses to form carbon-carbon bonds and set stereocenters in the same reaction sometimes lack the desired level of stereocontrol. The equivalent process in PKSs is carefully regulated by two enzymes: KSs that mediate carbon-carbon bond formation and KRs that guide the setting of stereocenters. The combination of stereocenters produced is dependent on the KR type. KRs that perform reductions yielding L-β-hydroxyl groups are referred to as "A-type," and KRs that perform reductions

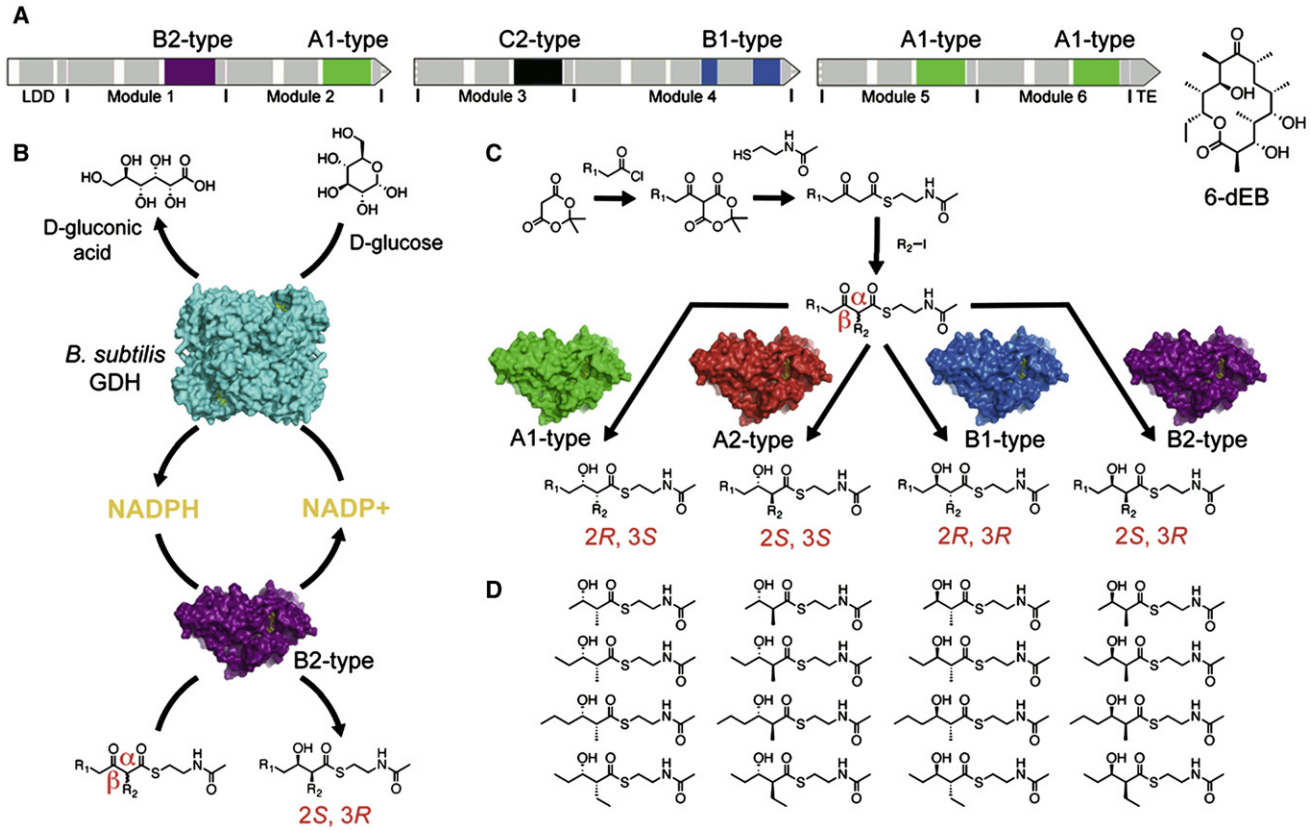

Figure 1. Employing PKS KRs to Chemoenzymatically Synthesize a Library of Chiral Building Blocks

(A) The DEBS assembly line synthesizes 6-dEB; 9/10 stereocenters are set by KRs that are classified by the combination of stereocenters they produce. LDD, loading didomain.

(B) GDH helps drive the reduction of diketide-SNACs, regenerating NADPH through the oxidation of D-glucose.

(C) A general synthesis of α-substituted, β-keto diketides was developed. Stereospecific KRs can convert these common intermediates into chiral diketide-SNACs.

(D) Representatives of the library of diketide chiral building blocks that can be accessed through KR biocatalytic reactions.

See also Figure S1 and Table S1.

yielding D-β-hydroxyl groups are referred to as "B-type" (reductase-incompetent KRs are referred to as "C-type") (Reid et al., 2003; Caffrey, 2003; Keatinge-Clay, 2007; Valenzano et al., 2009). KRs that reduce D-α-substituted intermediates are denoted with a "1," whereas KRs that reduce L-α-substituted intermediates are denoted with a "2" (Keatinge-Clay, 2007). Thus, an α-substituted, β-keto diketide intermediate can be reduced to the "2*R*,3*S*" product by an A1-type KR, the "2*S*,3*S*" product by an A2-type KR, the "2*R*,3*R*" product by a B1-type KR, or the "2*S*,3*R*" product by a B2-type KR (Keatinge-Clay and Stroud, 2006; Keatinge-Clay, 2007; Zheng et al., 2010).

Because KRs can exert control over two stereocenters in one reduction reaction and, as a family, operate on diverse organic substrates, they possess extraordinary potential as biocatalysts (Patel, 2008). At least two studies of KRs as biocatalysts have been reported; however, the substrates examined were not very similar to those naturally encountered and the stereochemical purities of the reaction products were not investigated (Bali et al., 2006; Bali and Weissman, 2006). Analytical-scale enzymological studies have focused on the substrate analog (2*RS*)-methyl-3-oxopentanoyl-S-N-acetylcysteamine (-SNAC), **3**. Reductions of **3** mediated by EryKR1, EryKR2, EryKR5, EryKR6, MycKRA, MycKRB, and TylKR1 revealed that EryKR1 (B2-type) and TylKR1 (B1-type) were completely stereospecific, whereas the other KRs produced a mixture of diastereomers (isolated domains are referred to by their PKS and module of origin, except the mycolactone KRs, which are identified by KR type) (Siskos et al., 2005; Bali and Weissman, 2006). That from the isolated KRs examined for their stereospecificity two KRs were identified that catalyze stereospecific reduction suggested that other KRs that perform stereocontrolled reductions isolated from their PKSs could be discovered. If all KR types were available as biocatalysts, then the full stereochemical diversity naturally achieved by KRs could be accessed.

Thus, to demonstrate the utility of PKS enzymes in natural product and pharmaceutical synthesis, we aimed to develop KRs as biocatalysts and access a library of diketide chiral building blocks (Figure 1D). Required were both a facile synthetic route to α-substituted, β-keto diketide thioester analogs of the polyketide intermediates naturally reduced by KRs as well as a scalable, economic biocatalytic process that does not require stoichiometric NADPH for KR-mediated reduction.

A general synthesis of α-substituted, β-ketoacyl N-acetylcysteamine thioesters was developed and used to generate diketide substrates **1**–**5**. An NADPH-regeneration system in which KR-mediated reductions are driven by glucose was designed. Eleven isolated KRs were incubated with **1**–**5** in biocatalytic reactions and the products were analyzed by chiral chromatography. Robust A1-type and A2-type KR biocatalysts were identified. Several biocatalytic reactions were scaled up, both with purified KRs as well as KR-containing lysate, to readily provide 100 mg yields of stereopure chiral building blocks. In addition to illustrating that building blocks for the synthesis of natural products and pharmaceuticals can be accessed by PKS enzymes, the described biocatalytic systems provide PKS enzymologists access to polyketide substrates and enable experiments on PKS enzymes to be performed on a more readily analyzable scale.

RESULTS AND DISCUSSION

Chemoenzymatic Design

NADPH-Regeneration Scheme

To drive KR-mediated reduction reactions, an NADPH-regeneration scheme was employed (Figure 1B). Glucose dehydrogenase (GDH) cloned from *Bacillus subtilis* was used to couple the oxidation of glucose to the reduction of diketides. GDH generates NADPH from $NADP^+$ as it oxidizes glucose to gluconolactone (Wong et al., 1985; Kataoka et al., 2003). A high concentration of glucose establishes a large NADPH:$NADP^+$ ratio that drives the reduction reaction toward completion. At the pH of the biocatalytic reactions (pH 7.5), most of the gluconolactone produced is hydrolyzed to gluconic acid, which deprotonates to gluconate. Because decreases in pH result in GDH and KRs precipitating out of solution, excess buffering strength is supplied.

General Synthetic Route to α-Substituted, β-Keto N-Acetylcysteamine Thioesters

The route most commonly employed to generate **3** requires a costly chiral auxiliary to generate an α-substituted, β-ketoimide that is subsequently reacted with excess N-acetylcysteamine (even more expensive than the chiral auxiliary) (Gilbert et al., 1995). Instead, we access α-substituted, β-keto N-acetylcysteamine thioesters through an efficient and cost-effective extension of a described route to β-keto N-acetylcysteamine thioesters (Figure 1C) (Gilbert et al., 1995). After the desired β-keto N-acetylcysteamine thioester has been obtained from Meldrum's acid, an acyl chloride, and N-acetylcysteamine (generated from cysteamine and acetic anhydride), an α substituent is installed via an alkylation reaction. Thus, the γ substituent of a diketide-SNAC is selected through the acyl chloride, and the α substituent is selected through the alkyl halide. That the syntheses yield racemic mixtures of α-substituted, β-ketoacyl-SNACs is a nonissue because the enantiomers rapidly interconvert in the aqueous environment of the subsequent biocatalytic reaction.

For isolated KRs to be considered robust biocatalysts, they should catalyze the reduction of a variety of substrates. Thus, a panel of five diketide substrates was synthesized to test the KRs: 3-oxopentanoyl-SNAC (**1**), (2*RS*)-methyl-3-oxobutanoyl-SNAC (**2**), (2*RS*)-methyl-3-oxopentanoyl-SNAC (**3**), (2*RS*)-methyl-3-oxohexanoyl-SNAC (**4**), and (2*RS*)-ethyl-3-oxopentanoyl-SNAC (**5**) (Figure 2). Compared to **3**, each of the other diketides is one methylene shorter or longer at the α or γ position.

Selection of KRs

To identify KRs capable of stereospecifically reducing a range of diketide substrates, 11 diverse reductase-competent KRs were selected (see "Cloning" in Experimental Procedures). They were isolated from modules that do not possess DHs or ERs because KRs that cooperate with these enzymes are usually B1-type (Valenzano et al., 2010). MycKRA (A-type), TylKR1 (B1-type), and EryKR1 (B2-type) were chosen, in part, to serve as positive controls, as how they process **3** has already been determined (Siskos et al., 2005; Bali and Weissman, 2006). The selection of the other eight KRs was biased toward A-type KRs both because isolated A1- or A2-type KRs that stereospecifically reduce β-ketoacyl-SNAC substrates had previously not been identified and because A1-type KRs are the most common in modules without DHs or ERs. All but two of the KRs selected naturally operate on α-substituted intermediates because we aimed to set two stereocenters through a single KR-mediated reduction reaction. KRs were chosen from diverse PKSs (amphotericin, erythromycin, mycolactone, oleandomycin, pikromycin, spinosyn, and tylosin) and from varying locations within PKS assembly lines (see Figure S1 available online). Thus, the selection of reductase-competent KRs includes two A-type KRs that naturally reduce α-unsubstituted intermediates, four A1-type KRs, two A2-type KRs, one B1-type KR, and two B2-type KRs. A reductase-incompetent C1-type KR was also selected as a negative control (Figure S2).

Chiral Chromatography and Establishing Elution Orders

To determine the stereocontrol exerted by KRs when reducing **3**, Leadlay and coworkers synthesized standards that established the **3a**–**3d** elution order from a chiral column (Holzbaur et al., 1999). We sought to establish the elution order of **2a**–**2d** and **4a**–**4d** on the same chiral column to identify the reduced diketides produced by the 11 KRs in the biocatalytic reactions. Thus, well-established diastereospecific aldol reactions were performed to obtain synthetic "2*R*,3*R*," "2*R*,3*S*," and "2*S*,3*R*" diketide-SNACs and the elution orders of **2a**–**2d** and **4a**–**4d** were determined (Evans et al., 1981; Raimundo and Heathcock, 1995) (Figure 3). The products of **2**, **3**, and **4** elute in the same order—first the two *anti* products, then the two *syn* products: "2*R*,3*R*" (B1-type product), "2*S*,3*S*" (A2-type product), "2*R*,3*S*" (A1-type product), and "2*S*,3*R*" (B2-type product) (Holzbaur et al., 1999).

Syntheses of reduced diketide-SNACs were initiated through aldol reactions between (4*S*)-4-benzyl-3-propionyl-2-oxazolidinone and the appropriate aldehyde (Figure 3A). In keeping with the classic Evans *syn*-selective reaction protocol, one equivalent of dibutylboron triflate was used to generate the "2*S*,3*R*" product (Evans et al., 1981). Heathcock and coworkers demonstrated that if two equivalents of dibutylboron triflate are supplied to this reaction, the *anti* product with the α stereocenter opposite in configuration to the Evans *syn* product as well as some quantity of anti-Evans *syn* product are generated (Raimundo and Heathcock, 1995). We utilized this protocol to generate a mixture of "2*R*,3*R*" and "2*R*,3*S*" products. Chemistry detailed by Boddy and coworkers was applied to each of the

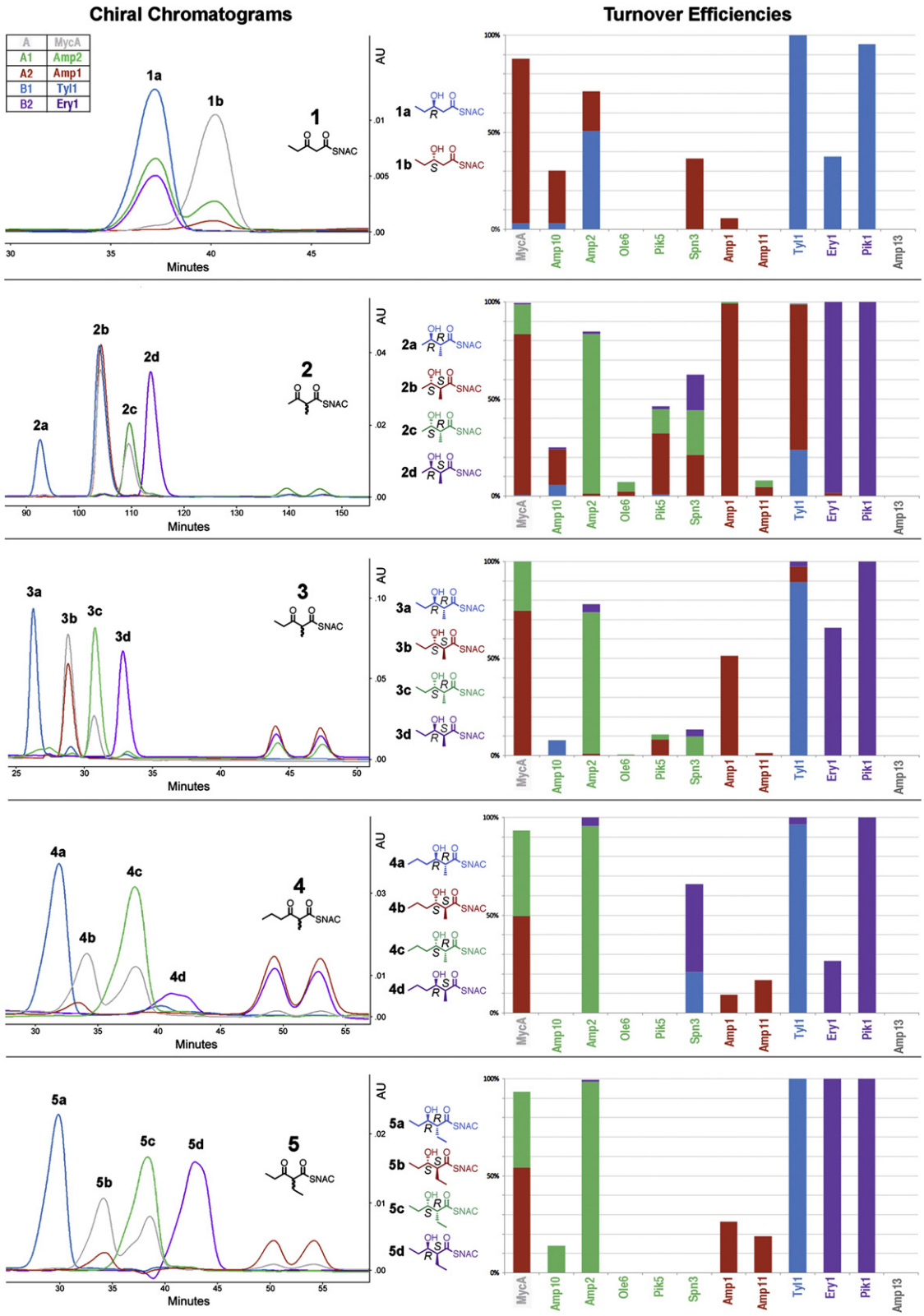

Figure 2. KR Biocatalytic Assays

Representative chromatograms show the reactions of five of the most active KRs with each of the diketide substrates **1–5** on the left (representative chromatograms of the other KRs are illustrated in Figure S2). Chiral building block products are displayed in the middle. The turnover efficiencies of each KR for each substrate are graphed on the right ("turnover efficiency" is defined as the quantity of a product divided by quantities of the products and remaining substrate). The

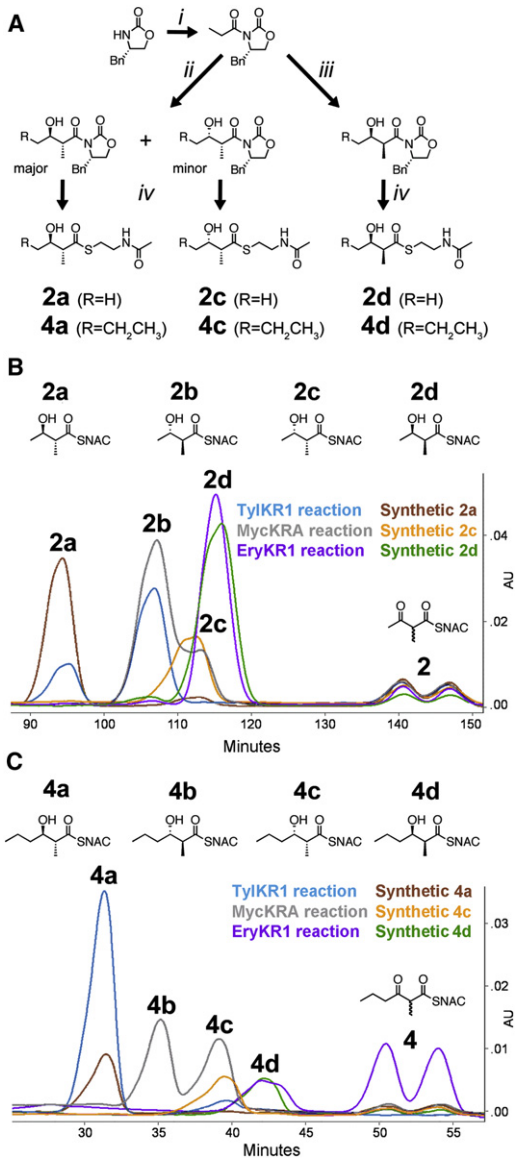

Figure 3. Determining Elution Orders

(A) Aldol reactions helped synthesize chiral chromatography standards. After (*i*) the propionyl group was added to the chiral auxiliary, then either (*ii*) the Heathcock route using two equivalents of dibutylboron triflate or (*iii*) the standard Evans route using one equivalent yielded diketide oxazolidinones that were (*iv*) hydrolyzed and coupled to NAC.

(B and C) Standards were compared to products from MycKRA (A-type), TylKR1 (B1-type), and EryKR1 (B2-type) to establish the elution order of products from KR reactions with **2** and **4**. See also Table S3.

diketide-oxazolidinones to hydrolyze the diketides from their chiral auxiliaries and couple them to NAC (Sharma and Boddy, 2007). The "2*R*,3*R*" and "2*R*,3*S*" diketide-SNACs were separated by reverse-phase HPLC.

Analyzing KR Reactions

After reactions in which each of the 11 reductase-competent KRs was paired with each of **1**–**5** had been incubated for 1 day at 22°C, they were extracted with ethyl acetate and analyzed by chiral chromatography (Figure 2; Figure S2). Some reactions may not have gone to completion due to substrate incompatibility, slow kinetics, protein stability, or many other factors. Because the overall fitness of KRs as biocatalysts was of interest, a metric of "turnover efficiency" was constructed, defined as the quantity of a product formed divided by the quantities of all products and unreacted substrate. Relative quantities were measured by integrating peaks from the 235 nm absorbance traces of chiral chromatography runs. Because all reactions were performed in duplicate, the reported turnover efficiencies are averages from two runs (in no case was there a deviation larger than 10%) (Table S2). As **1** could not be accurately quantified (it migrates as an extremely broad peak), the turnover efficiencies of **1a** and **1b** were calculated relative to the TylKR1 reaction, which produced the largest product peak. The elution order for **1a** and **1b** was assigned as first the "3*R*" product then the "3*S*" product because reactions of active B-type KRs generated the first peak and reactions of all of the active A-type KRs (with the exception of AmpKR2) generated the second peak. The elution order of **5a**–**5d** was assigned in the same order as the products of **2**–**4**. Added validation for this assignment comes from the observation that the *syn* and *anti* products of **2**–**4** have different maximal absorbances (the *anti* products maximally absorb at ~232 nm, whereas the *syn* products maximally absorb at ~233 nm): the first two biocatalytic products of **5** that elute from the chiral column absorb as *anti* products, whereas the last two absorb as *syn* products.

KR Biocatalysis Results

KRs Usually Maintain Natural Stereocontrol

Active KRs usually maintained the stereocontrol anticipated from their KR types (Figure 2). Most of these KRs even maintained stereocontrol toward substrate **5**, which harbors an uncommon α-ethyl group. AmpKR2 was identified as a general A1-type KR biocatalyst, and AmpKR1 was identified as a general A2-type KR biocatalyst. Because TylKR1 proved to be a general B1-type KR biocatalyst and EryKR1 proved to be a general B2-type KR biocatalyst (PikKR1 is even more robust), the desired spectrum of KR activities was realized. That the KR reactions are as stereocontrolled as they were determined to be is noteworthy, because the natural substrates of these enzymes are polyketide intermediates bound to the pantetheinyl arm prosthetic group of holo-ACPs.

KRs that Naturally Reduce Smaller Intermediates Are Most Active toward Diketide-SNACs

In general, KRs that reduce small polyketides within their native PKSs, such as AmpKR1, AmpKR2, EryKR1, PikKR1, and TylKR1, were most active on the panel of diketides. KRs that reduce larger polyketides within their native PKSs, such as PikKR5, OleKR6, AmpKR10, and AmpKR11, were nearly inactive

heights of the bars can be interpreted as relative activities, whereas the ratio of colors within a bar can be interpreted as a measure of the stereocontrol exerted by a KR on a substrate. Diverse KR types were used in these assays: two A types that naturally operate on α-unsubstituted intermediates, four A1 types, two A2 types, one B1 type, two B2 types, and one C1 type (as a negative control). See also Figure S2 and Table S2.

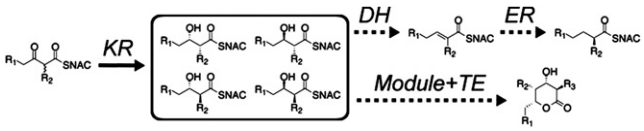

Figure 4. Accessing Diverse Building Block Libraries through PKS Biocatalysis

Biocatalytic reactions using DHs, ERs, and Module+TEs could enable the generation of libraries of other building blocks useful in the syntheses of natural products, such as α-substituted diketides and triketide lactones containing up to four contiguous stereocenters (representative molecules are shown).

toward **1**–**5**. This could indicate that KRs that naturally reduce short-chain polyketide intermediates within their native PKSs obtain binding energy for catalysis from interactions with the polyketide.

MycKRA was one of the most active KRs in these studies. Three modules of the mycolactone PKS contain 99% identical MycKRA enzymes as a result of duplication events; thus, within the synthase MycKRA enzymes reduce a diketide, a triketide, and a hexaketide (Stinear et al., 2004). That both A1-type and A2-type products were produced when MycKRA was incubated with α-substituted diketides **2**–**5** is understandable in that this enzyme naturally operates on α-unsubstituted intermediates. MycKRA did not produce B-type products. Broad in its substrate selectivity, MycKRA is a robust, stereocontrolled A-type KR biocatalyst.

Deviations from Expected

A few KRs were sensitive to slight differences within the panel of diketides. TylKR1, which is in general a robust, active B1-type KR, produced mainly the A2-type product **2b** when incubated with **2**, which is one methylene shorter than its natural substrate. AmpKR2, an A1-type KR, converted the α-unsubstituted diketide **1** mainly to a B-type product. Research is ongoing to determine whether KRs are more active and/or stereocontrolled toward pantetheine-bound diketides than NAC-bound diketides.

Scaled-Up Reactions

Because KR biocatalysts representing each KR type were identified that could generate the desired library of diketide chiral building blocks, we sought to demonstrate that useful quantities of chiral building blocks could be obtained. AmpKR2 (A1-type), AmpKR1 (A2-type), TylKR1 (B1-type), and EryKR1 (B2-type) were each incubated in scaled-up reactions with **3**. Over 100 mg of each of **3a**, **3b**, **3c**, and **3d** was obtained from these scaled-up reactions. Because the reactions with lower turnover efficiencies required significant KR concentrations and the nickel column purification step only captures a small percentage of KR in the lysate from overexpressing cells, reactions were also attempted using lysate. Reactions using the lysate were complete within 6 hr and also provided more than 100 mg of chiral product. Both for reactions using pure KRs and KR-containing lysate, KR reactions were as stereocontrolled as in the smaller-scale reactions.

Building Block Libraries, PKS Biocatalysis, and PKS Enzymology

As well as containing valuable combinations of stereocenters, the described building blocks harbor a synthetic handle in the form of a thioester. This moiety can be converted to a carboxylic acid or an aldehyde or used to couple the diketide to other fragments through an ester, amide, or ketone linkage, making thioester-containing building blocks very versatile in the syntheses of natural products (Bruice et al., 1963; Tokuyama et al., 1998). The installation of a second synthetic handle on the other end of the diketide chain is also desirable. Because the biocatalytic assays reported here revealed that variations at the γ position of the diketide substrates are tolerated by many KRs, it may be possible to incorporate synthetic handles such as a terminal chlorine, alcohol, double bond, or triple bond.

Additional building block libraries may be accessed using other PKS enzymes as biocatalysts. Because many DHs form *trans* double bonds from (2*R*,3*R*)-α-alkyl-β-hydroxy intermediates, preparative quantities of α-substituted, α/β-unsaturated diketides may be accessed by incubating α-substituted, β-keto-diketide-SNACs with appropriate KRs and DHs (Keatinge-Clay, 2008). As ERs stereospecifically reduce such double bonds, α-substituted, β-keto-diketide-SNACs could be converted, through the driving force of an NADPH-regeneration system, to chiral α-substituted, β-methylene-diketide-SNACs by employing a combination of KR, DH, and ER biocatalysts (Kwan et al., 2008). Diketide-SNACs can also be enzymatically converted to triketide lactones containing four contiguous stereocenters (Figure 4). Such molecules have been generated by Module+TEs (PKS modules C-terminally fused to EryTE) in quantities analyzable by radio-TLC or GC-MS as readout of enzymological studies (Chen et al., 2006). Recently, we have developed an economical route to supply extender units accepted by ATs to in vitro PKS reactions (Hughes and Keatinge-Clay, 2011). Thus, the scaled-up incubation of reduced, chiral diketide-SNACs with diverse Module+TEs in the presence of NADPH may yield libraries of triketide building blocks.

Currently, most experiments on modular PKS enzymes are performed either in vivo, by expressing PKSs heterologously and extracting polyketides from the growth medium, or in vitro, by combining the appropriate enzymes, cofactors, and substrates and then analyzing the reaction products by radio-TLC, GC-MS, or LC-MS (reduced polyketides are usually invisible on UV absorbance traces). An experimental platform in which PKS enzymes can perform catalysis in a controlled setting to produce quantities of polyketides analyzable by NMR or crystallography is highly desired (the stereocenters of products would be much more readily determined, for example). Expense has been the principal deterrent of larger in vitro reactions because polyketide extender units such as methylmalonyl-CoA and cofactors like NADPH are costly. However, such biocatalytic processes as the MatB-catalyzed production of extender units and GDH-catalyzed regeneration of NADPH should enable PKS enzymologists to perform in vitro reactions more affordably under controlled conditions, generating multimilligram quantities of polyketide products.

SIGNIFICANCE

The scalable production of reduced diketide-SNACs (-S-N-acetylcysteamines) has been realized through the described general chemoenzymatic route. A screen of 11 diverse,

isolated ketoreductases (KRs) toward diketides 1–5 revealed that KRs isolated from modules that naturally reduce shorter polyketide intermediates are most active on diketide-SNACs. AmpKR2 and AmpKR1 were identified as robust A1- and A2-type KR biocatalysts, and TylKR1 and EryKR1 were shown to be robust B1- and B2-type KR biocatalysts. More than 100 mg of each diketide 3a–3d was produced to demonstrate that quantities of chiral diketide building blocks useful for natural product and pharmaceutical syntheses could be generated economically and greenly. Other polyketide synthase (PKS) enzymes such as dehydratases, enoylreductases, and PKS modules C-terminally fused to EryTE can now be investigated as biocatalysts in the generation of an even greater diversity of diketide and triketide building blocks. The described biocatalysis will also benefit PKS enzymology because it is no longer cost-prohibitive to perform in vitro reactions that yield significant quantities of polyketide products as readout.

EXPERIMENTAL PROCEDURES

Cloning

All KRs, with the exception of EryKR1, and GDH were cloned from genomic DNA (gDNA) (Table S1). Regions of DNA encoding KRs from amphotericin (Amp), oleandomycin (Ole), pikromycin (Pik), spinosyn (Spn), and tylosin (Tyl) were amplified from *Streptomyces nodosus*, *Streptomyces antibioticus*, *Streptomyces venezuelae*, *Saccharopolyspora spinosa*, and *Streptomyces fradiae* gDNA, respectively. N- and C-terminal boundaries were chosen using the boundaries determined from the structures of EryKR1 and TylKR1 (Keatinge-Clay and Stroud, 2006; Keatinge-Clay, 2007). The region encoding the KR from the mycolactone (Myc) PKS was amplified from a bacterial artificial chromosome containing DNA from pMUM001 (Stinear et al., 2004). The regions encoding the KRs from the erythromycin (Ery) PKS were amplified from a synthetic gene (Kodumal et al., 2004). GDH was cloned from *B. subtilis* gDNA. NdeI and EcoRI restriction sites were designed to flank the amplicons encoding AmpKR2, AmpKR10, AmpKR11, AmpKR13, EryKR2, OleKR6, PikKR1, PikKR5, and SpnKR3; NdeI and XhoI restriction sites were designed to flank the amplicons encoding AmpKR1, EryKR1, TylKR1, and GDH; NheI and NotI restriction sites were designed to flank the amplicon encoding MycKRA. All restriction sites were engineered so that histidine tags encoded by pET28b (Novagen) would be N-terminally fused to the proteins, facilitating purification.

Protein Expression and Purification

All plasmids were transformed into *Escherichia coli* BL21(DE3). The cells were grown in Luria broth with 25 mg/l kanamycin at 37°C until they reached an OD_{600} of 0.5. They were then cooled to 15°C, induced with 1 mM IPTG, and grown for an additional 16 hr. Cells were spun down, resuspended in lysis buffer (10% [w/v] glycerol, 0.5 M NaCl, 30 mM Tris [pH 7.5]), and lysed by sonication. After centrifugation at 30,000 × g for 30 min, lysate was poured over nickel-NTA resin (QIAGEN) equilibrated with lysis buffer. Bound protein was washed with 15 mM imidazole in lysis buffer and eluted with 150 mM imidazole in lysis buffer. Proteins were then polished using a Superdex 200 gel filtration column that was equilibrated with 10% (w/v) glycerol, 150 mM NaCl, 10 mM Tris (pH 7.5) and concentrated to 200 μM. Protein purity was evaluated by Coomassie-stained SDS-PAGE gels (Figure S1).

KR Reductions

Biocatalytic Screen

Reactions were performed on 10 mM **1**–**5** in 10% (w/v) glycerol, 100 mM NaCl, 150 mM HEPES (pH 7.5), 200 mM D-glucose, 100 μM $NADP^+$, 1 μM GDH, and 5 μM KR in a total volume of 200 μl. Following incubation at 22°C for 1 day, reactions were extracted with 2 × 500 μl ethyl acetate and evaporated. The extracts were resuspended in ethanol prior to analysis by chiral chromatography.

Scaled-Up Reactions

Reactions were performed on 50 mM **3** in 10% (w/v) glycerol, 300 mM HEPES (pH 7.5), 100 mM NaCl, 300 mM D-glucose, 100 μM $NADP^+$, 1 μM GDH, 50 μM purified KR in a total volume of 15 ml. All reactions were incubated at 22°C for 1 day. Each was determined to be complete by reverse-phase HPLC. After EtOAc extraction and silica gel chromatography (EtOAc), yields of ~75% were obtained. Reactions were also performed using lysate (50 ml from 6 l culture) that was not poured over a nickel column but rather dialyzed overnight at 4°C in 10 kDa MWCO cellophane dialysis tubing against 10% (w/v) glycerol, 250 mM NaCl, 30 mM HEPES (pH 7.5). In these reactions, 50 mM **3** was incubated in 10% (w/v) glycerol, 300 mM HEPES (pH 7.5), 100 mM NaCl, 300 mM D-glucose, 100 μM $NADP^+$, 1 μM GDH in a total volume of 15 ml, but with 5 ml of the reaction volume being supplied from dialyzed lysate. All reactions were incubated at 22°C for 6 hr. Each was determined to be complete by reverse-phase HPLC. After EtOAc extraction and silica gel chromatography (EtOAc), yields of ~85% were obtained. Background thioester hydrolysis occurs with a half-time of ~3 days for both reactions with purified KRs and reactions with KR-containing lysate.

Chiral Chromatography

Extracts of reactions from the biocatalytic screens were separated using a ChiralCel OC-H column (250 × 4.6 mm) on a Beckman Coulter HPLC system with a 20 μl loop. Solvent systems and flow rates were optimized for each set of reactions with a particular diketide-SNAC (Table S3). Substrates and products were observed at a wavelength of 235 nm.

Synthetic Protocols

General Synthetic Route to α-Substituted, β-Keto Diketide-SNAC Substrates

N-Acetylcysteamine. Cysteamine hydrochloride (3.05 g, 53.6 mmol, 1 eq.), potassium hydroxide (1.50 g, 53.6 mmol, 1 eq.), and sodium bicarbonate (6.75 g, 160.8 mmol, 3 eq.) were added to 50 ml H_2O. Acetic anhydride (2.28 ml, 48.2 mmol, 0.9 eq.) was then added dropwise over 5 min, and the solution was allowed to stir at 22°C for 15 min. The reaction was quenched with 1 M HCl, and the product was extracted with ethyl acetate (3 × 50 ml), dried with $MgSO_4$, filtered, and concentrated in vacuo, yielding a clear, viscous liquid.

Meldrum's Acid Derivatives. Pyridine (2 eq.) was added to Meldrum's acid (1 eq.) in 40 ml dry dichloromethane (DCM) at 0°C. Next, the acyl chloride (1 eq.) was added dropwise over 15 min, turning the solution a dark orange color, and the reaction was stirred overnight at 22°C. The reaction was washed with 0.1 M HCl (3 × 50 ml) and the organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo.

α-Unsubstituted Diketide-SNACs. The Meldrum's acid derivative (1 eq.) and N-acetylcysteamine (1 eq.) were refluxed in dry toluene for 5 hr. The resulting solution was then concentrated in vacuo.

α-Substituted Diketide-SNACs. The α-unsubstituted diketide-SNAC (1 eq.) was dissolved in 50 ml dry THF at 0°C. Potassium *tert*-butoxide (1.2 eq.) was added. After the alkyl iodide (5 eq.) was injected, the reaction was allowed to warm to 22°C and stir overnight. After the reaction was quenched with 0.1 M HCl, the product was extracted with ethyl acetate (3 × 50 ml), dried with $MgSO_4$, filtered, and concentrated in vacuo, resulting in a clear, viscous liquid.

Syntheses of α-Methyl, β-Hydroxyl Diketide-SNAC Standards

(4S)-4-Benzyl-3-Propionyl-2-Oxazolidinone (**6**). (4*S*)-benzyl-2-oxazolidinone (250 mg, 1.41 mmol, 1 eq.) was dissolved in 5 ml dry THF at −78°C under nitrogen. *n*-butyllithium (0.64 ml of a 2.5 M stock in hexanes, 1.59 mmol, 1.13 eq.) was added dropwise to the cooled solution by syringe and stirred for 5 min. Propionyl chloride (0.158 ml, 1.81 mmol, 1.28 eq.) was added dropwise by syringe, and the solution was stirred at −78°C for 30 min. The reaction was warmed to room temperature and stirred for an additional 30 min before being quenched with 3 ml saturated NH_4Cl (aq.). The product was extracted with DCM (2 × 20 ml), washed with 10 ml saturated $NaHCO_3$ (aq.) and then 10 ml brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to produce a clear oil (260 mg, 80%). R_f 0.40 (20% EtOAc in hexanes).

(4S,2′S,3′R)-3-(3′-Hydroxy-2′-Methylbutanoyl)-4-Benzyl-2-Oxazolidinone (**7**). (4*S*)-4-benzyl-3-propionyl-2-oxazolidinone (**6**) (560 mg, 2.40 mmol, 1 eq.) was added to 10 ml dry DCM under nitrogen and cooled to 0°C. Dibutylboron triflate

(Bu_2BOTf) (2.83 ml of a 1 M solution in DCM, 2.83 mmol, 1.18 eq.) was supplied via syringe. Diisopropylethylamine (0.56 ml, 3.19 mmol, 1.33 eq.) was then added dropwise over 2 min, and the solution was cooled to −78°C. After acetaldehyde (0.151 ml, 270 mmol, 1.12 eq.) was added, the solution was stirred at −78°C for 30 min, allowed to warm to 0°C, and further stirred for 2 hr. A solution of 3.3 ml Na_2HPO_4 (1 M, pH 7.4) and 8.3 ml MeOH was added to the reaction, followed by the dropwise addition of 8.5 ml 2:1 MeOH:30% H_2O_2 (aq.). After 1.5 hr, organic solvents were evaporated in vacuo and the remaining material was resuspended in 10 ml 5% $NaHCO_3$ (aq.). The product was extracted in DCM (3 × 20 ml), washed with 20 ml 5% $NaHCO_3$ (aq.) and then 20 ml brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to yield a clear oil (350 mg, 53% after flash chromatography). Flash chromatography was performed with a 15%–30% EtOAc in hexanes gradient. R_f 0.38 (50% EtOAc in hexanes).

(4S,2′S,3′R)-3-(3′-Hydroxy-2′-Methylhexanoyl)-4-Benzyl-2-Oxazolidinone (**8**). The synthesis for **7** was followed with the exception that butyrylaldehyde (0.213 ml, 2.41 mmol, 1.12 eq.) was substituted for acetaldehyde. Flash chromatography was performed in the same manner, yielding 241 mg (33%) of the desired product. R_f 0.67 (50% EtOAc in hexanes).

(4S,2′R,3′R)-3-(3′-Hydroxy-2′-Methylbutanoyl)-4-Benzyl-2-Oxazolidinone (**9**) *and (4S,2′R,3′S)-3-(3′-Hydroxy-2′-Methylbutanoyl)-4-Benzyl-2-Oxazolidinone* (**10**). Synthesis followed that of **7**, with the exception that two equivalents of Bu_2BOTf were used (2.4 ml of a 1 M solution in DCM, 2.4 mmol, 2 eq.). R_f 0.56 and 0.60 (50% EtOAc in hexanes).

(4S,2′R,3′R)-3-(3′-Hydroxy-2′-Methylhexanoyl)-4-Benzyl-2-Oxazolidinone (**11**) *and (4S,2′R,3′S)-3-(3′-Hydroxy-2′-Methylhexanoyl)-4-Benzyl-2-Oxazolidinone* (**12**). Synthesis followed that of **8**, with the exception that two equivalents of Bu_2BOTf was added (4.32 ml of a 1 M solution in DCM, 4.32 mmol, 2 eq.). R_f 0.73 and 0.77 (50% EtOAc in hexanes).

(2S,3R)-3-Hydroxy-2-Methylbutanoyl-N-Acetylcysteamine Thioester (**13**). Aldol product **7** (187 mg, 0.68 mmol) was dissolved in 3 ml of a 4:1 mixture of THF:H_2O and cooled to 0°C. Slowly, 3 ml of 30% H_2O_2 was added, followed by 1 ml saturated LiOH (0.5 g LiOH in 1 ml H_2O). The reaction was left to stir at 0°C for 2 hr, at which point Na_2SO_3 was added until H_2O_2 was quenched (bubbling ceased). The aqueous phase was washed with DCM (2 × 15 ml), and the DCM layers were back-extracted with H_2O (1 × 15 ml). The combined aqueous layers were cooled to 0°C and the pH was lowered to 1 with 6 M HCl. The aldol acid was extracted into EtOAc (3 × 20 ml), dried with $MgSO_4$, filtered, and concentrated in vacuo (as the aldol acid is somewhat volatile, the solution was only concentrated to 1 ml). 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (211.1 mg, 1.36 mmol, 2 eq.) was added to the solution under nitrogen at 0°C. NAC (96.8 mg, 0.81 mmol, 1.2 eq.) and a few crystals of 4-dimethylaminopyridine were added. After 4 hr at 0°C, the reaction was quenched with 5 ml H_2O. Product was extracted into EtOAc (3 × 15 ml), washed with 5 ml brine, dried with $MgSO_4$, and filtered. A yellowish oil was produced after concentration in vacuo (50 mg, 47%). R_f 0.20 (EtOAc).

(2S,3R)-3-Hydroxy-2-Methylhexanoyl-N-Acetylcysteamine Thioester (**14**). **14** was synthesized in the same manner as **13** from **8** (81%). R_f 0.35 (in EtOAc).

(2R,3R)-3-Hydroxy-2-Methylbutanoyl-N-Acetylcysteamine Thioester (**15**) *and (2R,3S)-3-Hydroxy-2-Methylbutanoyl-N-Acetylcysteamine Thioester* (**16**). **15** and **16** were synthesized in the same manner as **13** from a mixture of **9** and **10** (33%, **15** + **16**). They were then separated by reverse-phase HPLC on a semipreparative C_{18} column (BondClone, 300 × 7.8 mm; Phenomenex) connected to a Waters 1525 HPLC system through a gradient of 15%–100% B over 30 min, where the mobile phases were (A) H_2O with 0.1% TFA and (B) MeOH with 0.1% TFA, pumped at a flow rate of 2 ml/min. R_f 0.15 and 0.20 (in EtOAc).

(2R,3R)-3-Hydroxy-2-Methylhexanoyl-N-Acetylcysteamine Thioester (**17**) *and (2R,3S)-3-Hydroxy-2-Methylhexanoyl-N-Acetylcysteamine Thioester* (**18**). **17** and **18** were synthesized in the same manner as **13** from a mixture of **11** and **12** (85%, **17** + **18**). They were then separated as described for **15** and **16**. R_f 0.30 and 0.35 (in EtOAc).

Characterization via NMR and LC-MS

NMR was performed either on a Varian Mercury 400 MHz or a Varian INOVA 500 MHz instrument. LC-MS was performed on an Agilent Technologies 1200 Series HPLC with a Gemini C_{18} column (5 μm, 2 × 50 mm; Phenomenex) coupled to an Agilent Technologies 6130 quadrupole mass spectrometer system equipped with an electrospray-ionization source. A 5%–95% B gradient over 12 min at a flow rate of 0.7 ml/min was run in which the mobile phases were (A) H_2O with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid.

3-Oxopentanoyl-N-Acetylcysteamine Thioester (**1**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (t, 3H, J = 7.4 Hz, CH_3), 1.96 (s, 3H, CH_3-C=O), 2.55 (q, 2H, J = 7.4 Hz C-CH_2-C=O), 3.00–3.10 (m, 2H, S-CH_2), 3.4–3.5 (m, 2H, N-CH_2), 3.7 (s, 2H, O=C-CH_2-C=O), 5.88 (br s, 1H, NH). ESI-MS expected mass: 218.3; observed mass: 218.2.

3-Oxobutanoyl-N-Acetylcysteamine Thioester. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 (s, 3H, CH_3-C(=O)-N), 2.20 (s, 3H, CH_3-C=O), 2.93–3.16 (m, 2H, S-CH_2), 3.36–3.42 (m, 2H, N-CH_2), 3.62 (s, 2H, O=C-CH_2-C=O), 5.99 (br s, 1H, NH).

(2RS)-Methyl-3-Oxobutanoyl-N-Acetylcysteamine Thioester (**2**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (d, 3H, J = 7.5 Hz, CH_3-C-$(C=O)_2$), 1.90 (s, 3H, CH_3-C(=O)-N), 2.19 (s, 3H, CH_3-C=O), 2.95–3.08 (m, 2H, S-CH_2), 3.30–3.46 (m, 2H, N-CH_2), 3.71 (q, 1H, J = 7.5 Hz, O=C-CH-C=O), 5.87 (br s, 1H, NH). ESI-MS expected mass: 218.3; observed mass: 218.4.

(2RS)-Methyl-3-Oxopentanoyl-N-Acetylcysteamine Thioester (**3**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (t, 3H, J = 7.5 Hz, CH_3), 1.38 (d, 3H, J = 7.5 Hz, CH_3-C-$(C=O)_2$), 1.96 (s, 3H, CH_3-C=O), 2.48–2.68 (m, 2H, C-CH_2-C=O), 3.00–3.14 (m, 2H, S-CH_2), 3.36–3.52 (m, 2H, N-CH_2), 3.79 (q, 1H, J = 7.5 Hz, O=C-CH-C=O), 5.92 (br s, 1H, NH). ESI-MS expected mass: 232.3; observed mass: 232.4.

3-Oxohexanoyl-N-Acetylcysteamine Thioester. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90–1.00 (m, 3H, CH_3), 1.55–1.70 (m, 2H, C-CH_2-C), 1.96 (s, 3H, CH_3-C=O), 2.48–2.60 (m, 2H, C-CH_2-C=O), 3.00–3.14 (m, 2H, S-CH_2), 3.40–3.52 (m, 2H, N-CH_2), 3.70 (s, 2H, O=C-CH_2-C=O), 5.92 (br s, 1H, NH).

(2RS)-Methyl-3-Oxohexanoyl-N-Acetylcysteamine Thioester (**4**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 7.6 Hz, CH_3), 1.33 (d, 3H, J = 7.9 Hz, CH_3-C-$(C=O)_2$), 1.50–1.60 (m, 2H, C-CH_2-C), 1.98 (s, 3H, CH_3-C=O), 2.45 (m, 2H, C-CH_2-C=O), 2.95–3.12 (m, 2H, S-CH_2), 3.34–3.52 (m, 2H, N-CH_2), 3.74 (q, 1H, J = 7.9 Hz, O=C-CH-C=O), 5.92 (br s, 1H, NH). ESI-MS expected mass: 246.3; observed mass: 246.4.

(2RS)-Ethyl-3-Oxopentanoyl-N-Acetylcysteamine Thioester (**5**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (t, 3H, J = 7.3 Hz, CH_3), 1.17 (t, 3H, J = 8.4 Hz, CH_3-C-C=O), 1.88–1.98 (m, 2H, C-CH_2-C-$(C=O)_2$), 2.15 (s, 3H, CH_3-C=O), 2.48–2.66 (m, 2H, C-CH_2-C=O), 3.01–3.20 (m, 2H, S-CH_2), 3.41–3.58 (m, 2H, N-CH_2), 3.68–3.74 (m, 1H, O=C-CH-C=O), 6.27 (br s, 1H, NH). ESI-MS expected mass: 246.3; observed mass: 246.4.

(4S)-4-Benzyl-3-Propyl-2-Oxazolidinone (**6**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 3H, J = 8.0 Hz, CH_3), 2.76–2.82 (m, 1H, one of CH_2-Ph), 2.90–3.05 (m, 2H, CH_2-C=O), 3.31 (dd, 1H, J = 15.2 Hz, J = 5.5 Hz, one of CH_2-Ph), 4.16–4.23 (m, 2H, CH_2-O), 4.66–4.70 (m, 1H, CH-N).

(4S,2′S,3′R)-3-(3′-Hydroxy-2′-Methylbutanoyl)-4-Benzyl-2-Oxazolidinone (**7**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (d, 3H, J = 7.4 Hz, CH_3), 1.28 (d, 3H, J = 6.1Hz, CH_3-C-C=O), 2.76–2.82 (m, 1H, one of CH_2-Ph), 2.93 (br s, 1H, OH), 3.26 (dd, 1H, J = 12.3 Hz, J = 3.3 Hz, one of CH_2-Ph), 3.75 (dq, 1H, J = 9.8 Hz, J = 3.1 Hz, CH), 4.16–4.23 (m, 2H, CH_2-O), 4.65–4.69 (m, 1H, CH-N).

(4S,2′S,3′R)-3-(3′-Hydroxy-2′-Methylhexanoyl)-4-Benzyl-2-Oxazolidinone (**8**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (t, 3H, J = 7.2 Hz, CH_3), 1.30 (d, 3H, CH_3-C-C=O), 1.35–1.45 (m, 1H, one of C-CH_2-C), 1.45–1.58 (m, 1H one of C-CH_2-C), 2.75–2.81 (m, 1H, one of CH_2-Ph), 2.84 (d, 1H, OH), 3.26 (dd, 1H, J = 13.7 Hz, J = 3.1 Hz, one of CH_2-Ph), 3.76 (dq, 1H, J = 7.2 Hz, J = 2.6 Hz, CH), 3.95–4.00 (m, 1H, CH-C=O), 4.17–4.27 (m, 2H, CH_2-O), 4.68–4.74 (m, 1H, CH-N).

(4S,2′R,3′R)-3-(3′-Hydroxy-2′-Methylbutanoyl)-4-Benzyl-2-Oxazolidinone (**9**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (d, 3H, J = 5.6 Hz, CH_3), 1.32 (d, 3H, J = 6.2Hz, CH_3-C-C=O), 2.56 (d, 1H, J = 7.4 Hz one of CH_2-Ph), 2.82 (br s, 1H, OH), 3.28–3.35 (m, 1H, one of CH_2-Ph), 3.90–3.95 (m, 1H, CH), 3.96–4.00 (m, 1H, CH-C=O), 4.15–4.25 (m, 2H, CH_2-O), 4.67–4.72 (m, 1H, CH-N).

(4S,2′R,3′R)-3-(3′-Hydroxy-2′-Methylhexanoyl)-4-Benzyl-2-Oxazolidinone (**11**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (t, 3H, CH_3), 1.20 (d, 3H, J = 7.2 Hz, CH_3-C-C=O), 1.35–1.45 (m, 1H, one of C-CH_2-C), 1.45–1.58 (m, 1H one of C-CH_2-C), 2.75–2.82 (m, 1H, one of CH_2-Ph), 2.91 (d, 1H, J = 7.2 Hz, OH), 3.31 (dd, 1H, J = 7.9 Hz, J = 3.1 Hz, one of CH_2-Ph), 3.85 (dq, 1H, J = 7.2 Hz, J = 2.7 Hz, CH), 3.95–4.04 (m, 1H, CH-C=O), 4.15–4.26 (m, 2H, CH_2-O), 4.66–4.74 (m, 1H, CH-N).

(2S,3R)-3-Hydroxy-2-Methylbutanoyl-N-Acetylcysteamine Thioester (**13**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22 (d, 3H, J = 7.6 Hz, CH_3), 1.24 (d, 3H, CH_3-C-C=O), 2.01 (s, 3H, CH_3-C=O), 2.70–2.75 (m, 1H, CH-C=O), 3.00–3.10 (m, 2H, S-CH_2), 3.42–3.53 (m, 2H, N-CH_2), 3.70–3.79 (m, 1H, CH), 5.93 (br s, 1H, NH). ESI-MS expected mass: 220.3; observed mass: 220.2.

(2S,3R)-3-Hydroxy-2-Methylhexanoyl-N-Acetylcysteamine Thioester (**14**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, 3H, J = 7.7 Hz, CH_3), 1.22 (d, 3H, J = 7.7 Hz, CH_3-C-C=O), 1.50–1.60 (m, 1H, one of C-CH_2-C), 1.60–1.70 (m, 1H one of C-CH_2-C), 1.95 (s, 3H, CH_3-C=O), 2.70–2.75 (m, 1H, CH-C=O), 3.00–3.08 (m, 2H, S-CH_2), 3.40–3.52 (m, 2H, N-CH_2), 3.92–3.98 (m, 1H, J = 5.4 Hz, CH), 5.86 (br s, 1H, NH). ESI-MS expected mass: 248.3; observed mass: 248.4.

(2R,3R)-3-Hydroxy-2-Methylbutanoyl-N-Acetylcysteamine Thioester (**15**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22 (d, 3H, J = 7.7 Hz, CH_3), 1.24 (d, 3H, CH_3-C-C=O), 1.96 (s, 3H, CH_3-C=O), 2.70–2.76 (m, 1H, CH-C=O), 3.01–3.14 (m, 2H, S-CH_2), 3.47–3.58 (m, 2H, N-CH_2), 3.79–3.83 (m, 1H, CH), 6.26 (br s, 1H, NH). ESI-MS expected mass: 220.3; observed mass: 220.2.

(2R,3R)-3-Hydroxy-2-Methylhexanoyl-N-Acetylcysteamine Thioester (**17**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, 3H, J = 7.2 Hz, CH_3), 1.22 (d, 3H, J = 6.9 Hz, CH_3-C-C=O), 1.38–1.46 (m, 1H, one of C-CH_2-C), 1.46–1.56 (m, 1H one of C-CH_2-C), 2.01 (s, 3H, CH_3-C=O), 2.72–2.79 (m, 1H, CH-C=O), 3.01–3.10 (m, 2H, S-CH_2), 3.43–3.55 (m, 2H, N-CH_2), 3.72–3.78 (m, 1H, CH), 6.07 (br s, 1H, NH). ESI-MS expected mass: 248.3; observed mass: 248.4.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and three tables and can be found with this article online at doi:10.1016/j.chembiol.2011.07.021.

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