Tetrahedron xxx (2016) 1–4

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

An unusual intramolecular *trans*-amidation

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ARTICLE INFO

Article history:
Received 25 August 2015
Received in revised form 27 January 2016
Accepted 30 January 2016
Available online xxx

Keywords:
trans-Amidation
Polyketide
Cyclization
Intramolecular reactions
Reaction mechanisms

ABSTRACT

Polyketide biosynthesis engages a series of well-timed biosynthetic operations to generate elaborate natural products from simple building blocks. Mimicry of these processes has offered practical means for total synthesis and provided a foundation for reaction discovery. We now report an unusual intramolecular *trans*-amidation reaction discovered while preparing stabilized probes for the study of actinorhodin biosynthesis. This rapid cyclization event offers insight into the natural cyclization process inherent to the biosynthesis of type II polyketide antibiotics.

1. Introduction

The mechanisms guiding polyketide biosynthesis have served as inspiration for both total synthesis[1] and reaction development efforts.[2] Within recent years, the fusion between synthetic organic chemistry and structural biology has provided a rich forum to further explore the mechanisms that guide each of the discrete operations during a biosynthetic process.[3] The significance of these advances has most recently allowed chemoenzymatic methods to address the total synthesis of complex natural products such as spinosyn A.[4]

One of the key complications encountered when studying intermediate formation within natural product biosynthesis arises from compound instability. This is particularly problematic in type II polyketide biosynthesis, as the formation of the acyl carrier protein (ACP) tethered polyketones such as **1a** (Fig. 1), are highly unstable.[5]

Similar problems with instability also arise after the cyclization begins, as intermediates such as **2a** are prone to uncontrolled aldol-type cyclizations, if not regulated. Our current hypothesis supports the concept that the ACP serves to both protect unstable cargo (**1a**, Fig. 1) as well as guide these species to the enzymes, which will catalyze specific cyclization events (Fig. 2). Interested in further understanding these processes at the structural level, we turned to the preparation of 'atom replacement probes'.[6]

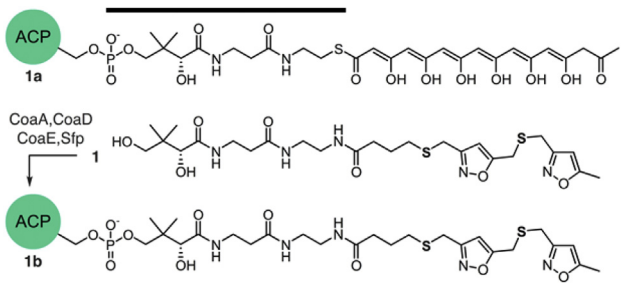

Fig. 1. Exemplary ketide acyl-carrier protein (ACP) tethered intermediate **1a** along with the corresponding stabilized atom replacement probe **1** and probe-loaded ACP **1b**. The black bar denotes the 4′-phosphopantetheinyl arm.

Through these studies, we learned that one could effectively prepare pantetheinylated mimetics of polyketones by the selective replacement of specific carbonyls with heteroatoms. As depicted in **1** (Fig. 1), we prepared probes bearing both thioethers and isoxazoles to represent key carbonyl units within the polyketone **1a**.[6] Similarly, we also developed partially-cyclized intermediates of **2a**, as shown in **2** (Fig. 2).

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http://dx.doi.org/10.1016/j.tet.2016.01.062
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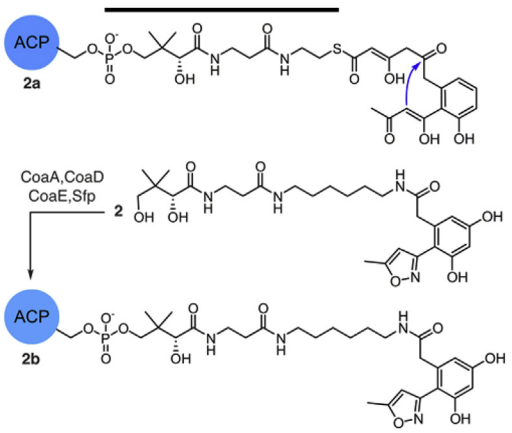

Fig. 2. Exemplary cyclized intermediate **2a** along with its associated atom replacement probe **2** and probe loaded ACP **2b**. The black bar denotes the 4′-phosphopantetheinyl arm.

Using the actinorhodin biosynthetic system as a model (Fig. 3), we were able to demonstrate that probes **1** and **2** can be loaded onto their ascribed ACP as given by the conversion of **1** to **1b** (Fig. 1) and **2** to **2b** (Fig. 2). As part of this study, we were able to apply solution-based protein NMR methods to demonstrate that intermediates **1b** and **2b** did indeed provide viable mimetics of their corresponding naturally-loaded **1a** and **2a**, respectively.[6] Overall, we were able to synthesize stable mimetics of intermediates at two stages of the actinorhodin biosynthetic process (Fig. 3).

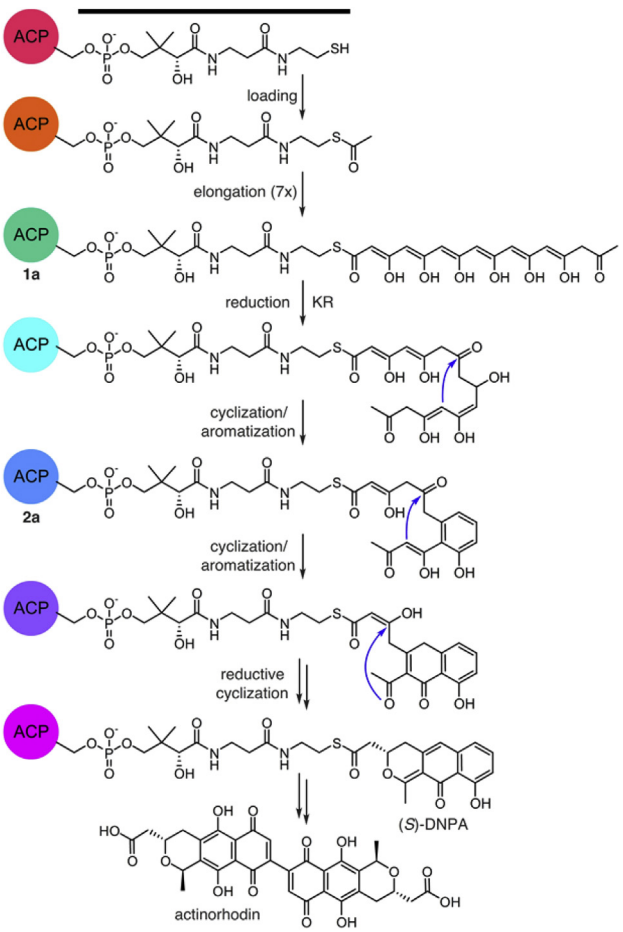

Fig. 3. Proposed biosynthesis of actinorhodin from *holo*-actACP.[6] One of the enol/ketol tautomerization states has been depicted. In solution, multiple tautomers exist. The processing of substrates on the actACP has been depicted by a color change of the sphere representing the ACP from red to violet with states **1a** and **2a** shown in Figs. 1 and 2, respectively.

2. Results and discussion

As part of this effort, we were interested in exploring the stability of ketides arising from opening of the isoxazole motif. Our plan was to use the isoxazole as a tool to mimic 1,3-dicarbonyl units, and hence restrict access to unwanted spontaneous aldol reactions. Our goal was to deliver a series of bench stable mimetics that could be used for structural biological studies. We began by preparing linear and cyclized mimetics and examining their incorporation on actACP by NMR.[7] We then examined what would happen upon opening of the isoxazole. To this end, we were able to open the isoxazole in simpler intermediates such as **3** providing imine **4** and diketide **5** (Fig. 4) in a sequential fashion. While not unexpected, this process was not possible with more complicated materials such as **1** (Fig. 1), which underwent rapid degradation. LC-MS monitoring of the cleavage process using probes such as isoxazole **1** clearly showed the rapid formation of multiple products immediately after treatment with $Mo(CO)_6$, indicating that even the imine-type intermediates were highly-reactive. We then turned to explore if this process was possible on our partially-cyclized materials, such as probe **2** (Fig. 2).

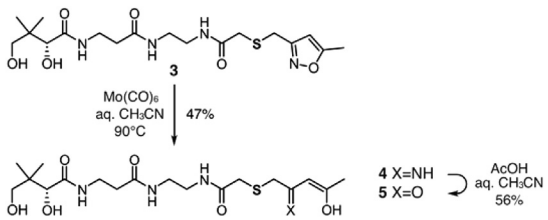

Fig. 4. Isoxazole cleavage. Ring opening of isoxazole **3** results in enaminone **4**, which in turn is hydrolytically-converted to ketide **5**.

2.1. Precursor synthesis

We began by examining materials from our recent studies.[7] Beginning with **6**[7] (Scheme 1), we were able to selectively deprotect the acetal by treatment in aq AcOH at rt to provide mimetic **7**. Treatment of **7** with fresh $Mo(CO)_6$ in refluxing aq CH_3CN[8] resulted in a clean conversion to enaminone **8** in an overall 86% yield from **6**.

2.2. Intramolecular *trans*-amidation of benzyl-protected enaminone 8

While stable thermally, exposure of **8** to mild acidic conditions (aq AcOH in CH_3CN) resulted in a crude product that lacked mass spectral signatures of the desired ketone. NMR monitoring of this reaction (Fig. 5) indicated the formation of two major materials, one of which was pantetheinamine **9**[9] (Scheme 1 and Supplementary Figs. S6–S8). The remaining material was attributed to **10**, as given by the presence of a compound containing two distinct benzylic groups in the crude NMR spectrum. Mass spectral analysis returned a formula of $C_{26}H_{23}NO_4$, indicating that **10** contained all of the remaining hydrogen, carbon, nitrogen and oxygen atoms of **8** ($C_{37}H_{46}N_4O_8$) after the elimination of **9** ($C_{11}H_{23}N_3O_4$).

Following purification of **10**, 1D NMR and 2D NMR analysis allowed complete assignment of the ^1H and ^{13}C spectra (see Supplementary data). As summarized on the left of Fig. 6, the ^{13}C data was suggestive of the structure of 1,4-dihydroisoquinolin-3(2H)-one motif bearing an exocyclic α,β-unsaturated ketone.

Please cite this article in press as: Rivera, H.; Jr.et al., Tetrahedron (2016), http://dx.doi.org/10.1016/j.tet.2016.01.062