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An unusual intramolecular *trans*-amidation

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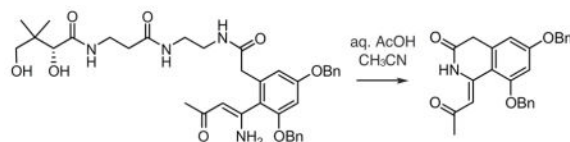
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Abstract

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.

Graphical Abstract



Keywords

Trans-amidation; Polyketide; Cyclization; Intramolecular reactions; Reaction mechanisms

1. Introduction

The mechanisms guiding polyketide biosynthesis have served as inspiration for both total synthesis¹ 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

[†]These authors contributed equally to the work

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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

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).

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).

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).

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. CH₃CN⁸ 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 CH₃CN) 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 Supporting 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 C₂₆H₂₃NO₄, indicating that **10** contained all of the remaining hydrogen, carbon, nitrogen and oxygen atoms of **8** (C₃₇H₄₆N₄O₈) after the elimination of **9** (C₁₁H₂₃N₃O₄).

Following purification of **10**, 1D NMR and 2D NMR analysis allowed complete assignment of the ¹H and ¹³C spectra (see Supporting Information). As summarized on the left of Fig. 6, the ¹³C data was suggestive of the structure of 1,4-dihydroisoquinolin-3(2H)-one motif bearing an exocyclic α,β-unsaturated ketone. Specifically, the data provided strong support for the exocyclic α,β-unsaturated ketone due to the presence of the chemical shift for the carbonyl at 200.7 ppm, the alpha-carbon at 146.4 ppm, and beta-carbon at 102.5 ppm. Further evidence for the 1,4-dihydroisoquinolin-3(2H)-one core was also obtained by the ¹³C peaks at 36.9 and 168.1 ppm. These assignments were further supported by HMBC correlations, as shown at the right of Fig. 6.

3. Conclusion

Ongoing efforts have recently led to the development of stabilized probes for studying intermediate processing within polyketide biosynthesis. While some atom replaced motifs provide sufficient stability (Fig. 4), others due to their access to rapid intramolecular cyclizations still lack sufficient stability (Scheme 1). Here, we report on a rather rare example of an intramolecular *trans*-amidation reaction. As illustrated in Scheme 1, the conversion of **8** to **10** occurred within 4 h at 23 °C under mildly acidic conditions. This suggests a strong driving force intrinsically built within the cyclic framework within atom-replacement probe **8**. While an uncommon event, the *trans*-amide formation observed herein (Scheme 1) not only demonstrates a unique reactivity but also provides a further means to synthetically-divert polyketide biosynthetic methods to enable access to new motifs, such as **10**. This finding suggests another alternative pathway that, while not accessed by the synthase, outlines the inherent non-enzyme catalyzed reactivity within the highly-reactive intermediates in polyketide biosynthesis. Mining these reactions not only expands the molecular diversity available through biosynthetic processes but also offers access to new structural motifs, a key step in expanding the utility of natural products. Overall, this study supports the importance of understanding polyketide biosynthesis not only by offering a means to comprehend and optimize natural product biosynthesis, but also by providing a platform for the advance of biosynthetically-derived natural product-like scaffolds.¹⁰

4. Experimental section

4.1. General

Unless otherwise noted, all reagents and chemical compounds were purchased from Alfa Aesar, Strem Chemicals, Sigma–Aldrich, Cambridge Isotopes or TCI and used without further purification. Flash chromatography was carried out on 40–63 mesh Geduran Silica Gel 60 (EMD Millipore). Thin layer chromatography (TLC) was conducted on 250 μm Silica Gel 60 F254 glass plates (EMD Millipore). NMR spectra were recorded on a Mercury Plus 400 MHz (Varian), a ECA 500 MHz (Jeol), a DMX 400 MHz (Bruker), a DMX 500 MHz (Bruker) or a VX 500 MHz equipped with XSens cold probe (Varian) spectrometer. FID files were processed using MestRenova version 10.0.1 (MestreLab Research). Mass spectrometric analyses were conducted on the following instruments: a LCQ Deca (ThermoFinnigan), MAT900XL (ThermoFinnigan), LTQ Orbitrap XL (ThermoScientific), or a LCT Premier (Waters) mass spectrometer. Reactions were conducted under Ar atmosphere in a round bottom flask or vial capped with a rubber septa and were stirred using a Teflon coated stir bar. All mixtures are provided as v:v ratios.

4.2. Experimental procedures and data of synthetic intermediates

4.2.1. Isoxazole 7—PMB-protected isoxazole **6** (54.0 mg, 0.0683 mmol) was dissolved in 1 mL of 4:1 AcOH:H₂O. The solution was stirred for 4 h, after which starting material was no longer present by TLC analysis using 1:9 MeOH/CH₂Cl₂. Solvent was azeotropically removed by rotary evaporation with addition of toluene (3 \times 5 mL). Flash chromatography (CH₂Cl₂ to 1:9 MeOH:CH₂Cl₂) provided 43.6 mg (95%) of isoxazole **7**; TLC R_f=0.5 (silica gel, MeOH:CH₂Cl₂ 1:9); ¹H NMR (500 MHz CDCl₃) 7.45–7.26 (m, 10H), 6.64 (d, *J* = 2.3 Hz, 1H), 6.63 (d, *J* = 2.3 Hz, 1H), 6.14 (d, *J* = 1.0 Hz, 1H), 5.08 (s, 2H), 5.06 (s, 2H), 4.64 (bs, 1H), 3.88 (s, 1H), 3.64 (dddd, *J* = 3.7, 6.9, 6.9, 13.8 Hz, 2H), 3.54–3.35 (m, 5H), 3.16 (ddd, *J* = 2.6, 5.8, 14.1 Hz, 2H), 3.13 (ddd, *J* = 3.3, 8.2, 14.0 Hz, 2H), 2.47 (d, *J* = 1.0 Hz, 3H), 2.35 (m, 2H), 1.02 (s, 3H), 0.88 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) 174.0, 172.2, 172.1, * 172.0, * 169.1, 160.9, 159.6, 158.5, 136.7, 136.3, 136.3, 128.8, 128.7, 128.4, 128.2, 127.8, 127.0, 111.8, 108.7, 104.9, 100.4, 77.9, 71.1, 70.6, 70.4, 42.6, 39.8, 39.7, * 39.5, 39.3, 39.2, * 36.1, 35.3, 22.4, 20.4, 12.4; HRMS (*m/z*): [M+H]⁺ calcd for C₃₇H₄₄N₄O₈+H⁺ 673.3232; found 673.3230. Multiple confirmations of **7** were observed during NMR analysis with duplicate NMR signatures indicated above by an asterix.

4.2.2. Enaminone 8—Isoxazole **7** (40.0 mg, 0.0595 mmol) was dissolved in 2 mL of 3:1 MeCN:H₂O in 10 mL Teflon tube (Nalgene Oak Ridge Centrifuge Tubes). Solid Mo(CO)₆ (15.7 mg, 0.060 mmol) was added and the tube was flushed with Ar, capped, and warmed to 85 °C. TLC analysis using 1:9 MeOH:DCM indicated that consumption of starting material was complete within 6 h. The crude reaction mixture was transferred to a 50 mL round bottom flask and evaporated to dryness. Flash chromatography (CH₂Cl₂ to 1:9 MeOH:CH₂Cl₂) provided 36.1 mg (90% yield) of enaminone **8**; R_f= 0.45 (silica gel, 1:9 MeOH:CH₂Cl₂); ¹H NMR (500 MHz CDCl₃) 7.39–7.24 (m, 10H), 6.99 (t, *J* = 5.9 Hz, 1H), 6.75 (t, *J* = 6.0 Hz, 1H), 6.55 (d, *J* = 2.3 Hz, 1H), 6.54 (d, *J* = 2.3 Hz, 1H), 5.08 (s, 1H), 5.05 (d, *J* = 2.3 Hz, 1H), 5.02 (s, 2H), 3.79 (bs, 2H), 3.54–3.38 (m, 7H), 3.28 (m, 2H), 3.12 (dddd, *J* = 5.3, 5.3, 5.3, 10.5 Hz, 2H), 2.30 (ddd, *J* = 4.3, 7.4, 15.3 Hz, 1H), 2.20 (m, 1H), 2.06 (s,

3H), 0.95 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3) 197.5, 174.9, 172.4, 172.1, 160.1, 159.8, 157.0, 136.6, 136.5, 135.0, 128.8, 128.7, 128.4, 128.0, 127.8, 127.0, 120.8, 108.4, 100.2, 98.1, 77.9, 71.0, 70.6, 70.3, 41.0, 40.0, 39.6, 39.3, 36.2, 35.3, 29.7, 21.7, 20.6; HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{37}\text{H}_{46}\text{N}_4\text{O}_8+\text{H}^+$ 675.3388; found 675.3389.

4.2.3. Cyclized adduct 10—Enaminone **8** (20.0 mg, 0.0296 mmol) was dissolved in 2:2:1 AcOH:H₂O:MeCN and stirred at rt. TLC analysis using 1:9 MeOH:CH₂Cl₂ indicated consumption of starting material after 4 h. The crude reaction mixture was evaporated to dryness azeotropically with toluene (3×5 mL). Flash chromatography (CH₂Cl₂ to 1:5 CH₂Cl₂:MeOH) provided 8.6 mg (70% yield) of **10**; ^1H NMR (500 MHz CDCl_3) 12.69 (bs, 1H), 7.51–7.34 (m, 10H), 6.89 (s, 1H), 6.60 (d, $J=2.4$ Hz, 1H), 6.42 (d, $J=2.4$ Hz, 1H), 2.01 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3) 200.7, 168.1, 161.7, 159.9, 146.4, 138.5, 135.9, 135.5, 128.9, 128.8, 128.6, 128.1, 127.7, 107.9, 106.2, 102.5, 100.2, 71.6, 70.5, 36.9, 31.4; HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{23}\text{NO}_4+\text{H}^+$ 414.1700; found 414.1701.

4.3. NMR analysis of the conversion of enaminone 8 to cyclized adduct 10

Enaminone **8** (5.2 mg) was dissolved in 0.8 mL of 2:2:1 (AcOD:D₂O:CD₃CN) at rt. The reaction was monitored over the course of 24 h by ^1H NMR analysis on a VX 500 MHz equipped with XSens cold probe (Varian) spectrometer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and notes

1. A selection of recent synthetic efforts that explore biomimetic approaches to polyketide total synthesis: Barrett TN, Barrett AG. *J Am Chem Soc.* 2014; 136:17013–17015. [PubMed: 25423048] Hager A, Mazunin D, Mayer P, Trauner D. *Org Lett.* 2011; 13:1386–1389. [PubMed: 21323383] Norris MD, Perkins MV, Sorensen EJ. *Org Lett.* 2015; 17:668–671. [PubMed: 25621375]
2. See the following articles for examples of biosynthetically-inspired method development Aquino C, Sarkar M, Chalmers MJ, Mendes K, Kodadek T, Micalizio GC. *Nat Chem.* 2011; 4:99–104. [PubMed: 22270625] Pan Y, Kee CW, Jiang Z, Ma T, Zhao Y, Yang Y, Xue H, Tan CH. *Chemistry.* 2011; 17:8363–8370. [PubMed: 21678511] Zheng K, Xie C, Hong R. *Front Chem.* 2015; 3:32. [PubMed: 26052510]
3. Kim HJ, Choi SH, Jeon BS, Kim N, Pongdee R, Wu Q, Liu HW. *Angew Chem Int Ed Engl.* 2014; 53:13553–13557. [PubMed: 25287333]
4. (a) Nguyen C, Haushalter RW, Lee DJ, Markwick PR, Bruegger J, Caldara-Festin G, Finzel K, Jackson DR, Ishikawa F, O'Dowd B, McCammon JA, Opella SJ, Tsai SC, Burkart MD. *Nature.* 2014; 505:427–431. [PubMed: 24362570] (b) Teufel R, Miyanaga A, Michaudel Q, Stull F, Louie G, Noel JP, Baran PS, Palfey B, Moore BS. *Nature.* 2013; 503:552–556. [PubMed: 24162851] (c) Piasecki SK, Zheng J, Axelrod AJ, Detelich ME, Keatinge-Clay AT. *Proteins.* 2014; 82:2067–2077. [PubMed: 24634061] (d) Bretschneider T, Heim JB, Heine D, Winkler R, Busch B, Kusebauch B, Stehle T, Zocher G, Hertweck C. *Nature.* 2013; 502:124–128. [PubMed: 24048471] (e) Mori T, Yang D, Matsui T, Hashimoto M, Morita H, Fujii I, Abe I. *J Biol Chem.* 2015; 290:5214–5225.

- [PubMed: 25564614] (f) Lee MY, Ames BD, Tsai SC. *Biochemistry*. 2012; 51:3079–3091. [PubMed: 22432862]
5. For studies that address the limits of polyketone stability see: Fouché M, Rooney L, Barrett AG. *J Org Chem*. 2012; 77:3060–3070. [PubMed: 22401367] Calo F, Richardson J, Barrett AG. *Org Lett*. 2009; 11:4910–4913. [PubMed: 19799447] Navarro I, Basset JF, Hebbe S, Major SM, Werner T, Howsham C, Bräckow J, Barrett AG. *J Am Chem Soc*. 2008; 130:10293–10298. [PubMed: 18611011] Harris TM, Murray TP, Harris CM, Gumulka M. *J Chem Soc Chem Comm*. 1974:362–363. Harris TM, Harris CM. *Tetrahedron*. 1977; 33:2159–2185.
 6. While multiple intermediates can be drawn at each step of this process including different enol/ketol states, we presented this route based on evidence developed by the Malpartida and Hopwood laboratories. For further information, see: Fernández-Moreno MA, Martínez E, Caballero JL, Ichinose K, Hopwood DA, Malpartida F. *J Biol Chem*. 1994; 269:24854–24863. [PubMed: 7929165] Kendrew SG, Hopwood DA, Marsh ENG. *J Bacteriol*. 1997; 179:4305–4310. [PubMed: 9209048]
 7. Shakya G, Rivera H Jr, Lee DJ, Jaremko MJ, La Clair JJ, Fox DT, Haushalter RW, Schaub AJ, Bruegger J, Barajas JF, White AR, Kaur P, Gwozdzowski ER, Wong F, Tsai SC, Burkart MD. *J Am Chem Soc*. 2014; 136:16792–16799. [PubMed: 25406716]
 8. (a) Nitta M, Kobayashi T. *J Chem Soc, Chem Comm*. 1982:877–878. (b) Anderson-McKay J, Savage GP, Simpson GW. *Aust J Chem*. 1996; 46:163–166.
 9. Compound 9 was not isolated or characterized from this reaction. It was however detected in the NMR monitor of the conversion of 8 to 10, as shown in Supporting Figures S6–S8
 10. (a) Jürjens G, Kirschning A, Candito DA. *Nat Prod Rep*. 2015; 32:723–737. [PubMed: 25728458] (b) Xu Y, Zhou T, Zhang S, Espinosa-Artiles P, Wang L, Zhang W, Lin M, Gunatilaka AA, Zhan J, Molnár I. *Proc Natl Acad Sci U S A*. 2014; 111:12354–12359. [PubMed: 25049383] (c) Walsh CT. *Acc Chem Res*. 2008; 41:4–10. [PubMed: 17506516]

Appendix A. Supplementary data

Supplementary data related to this article can be found at: <http://>

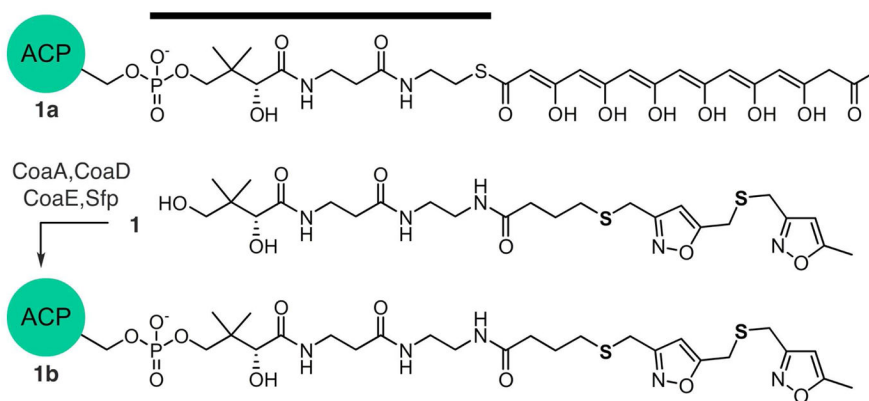


Fig. 1. Exemplary ketide acyl-carrier protein (ACP) tethered intermediate **1a** along with the corresponding stabilized atom replacement probe **1** and probe-loaded ACP **1b**. The black bar denotes the 4'-phosphopantetheinyl arm.

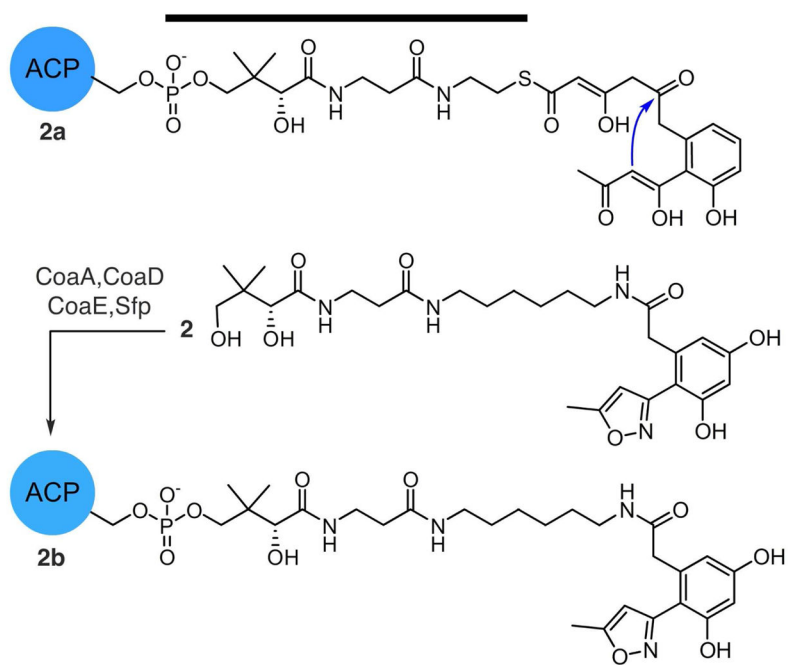


Fig. 2. Exemplary cyclized intermediate **2a** along with its associated atom replacement probe **2** and probe loaded ACP **2b**. The black bar denotes the 4'-phosphopantetheinyl arm.

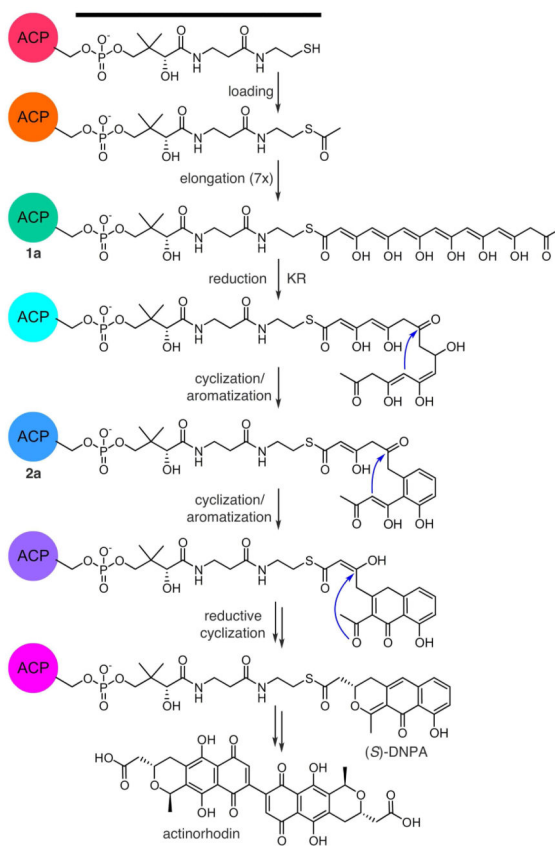


Fig. 3. Proposed biosynthesis of actinorhodin from *holo*-actACP.⁶ One of the enol/ketol tautomerization states has been depicted. In solution, multiple tautomers exist. The processing of substrates on the actACP has been depicted by a color change of the sphere representing the ACP from red to violet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

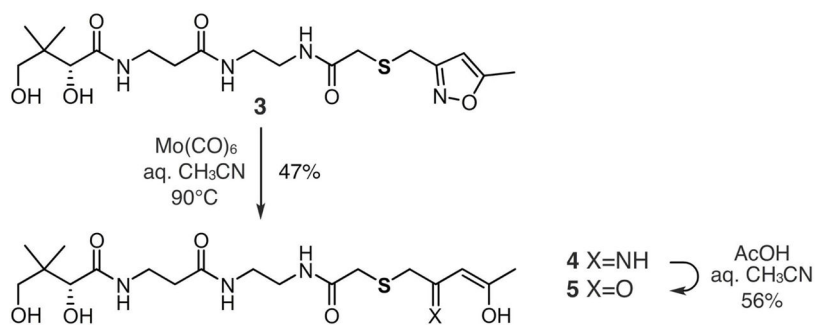


Fig. 4. Isoxazole cleavage. Ring opening of isoxazole **3** results in enaminone **4**, which in turn is hydrolytically-converted to ketide **5**.

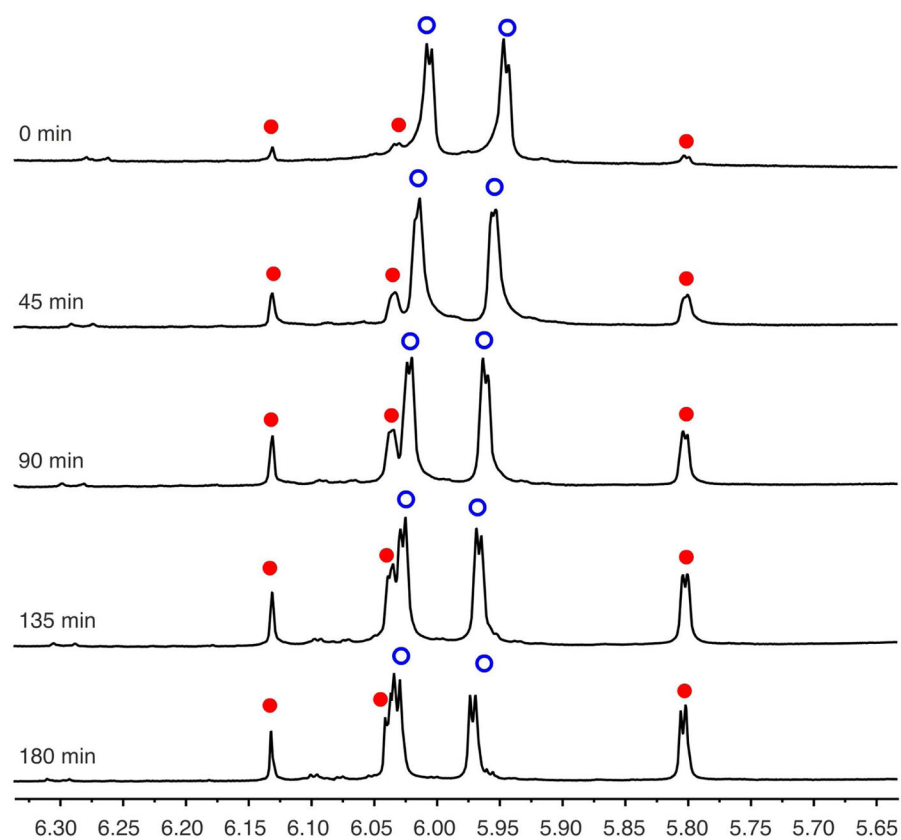


Fig. 5. ¹H NMR time course analysis depicting the conversion of **8** (blue circles) to **10** (red spheres) in 2:2:1 CD₃OOD:D₂O:CD₃OD. An expansion of the region between 5.60–6.35 ppm has been provided. Full spectral data has been provided in Supporting Figures S6–S8.

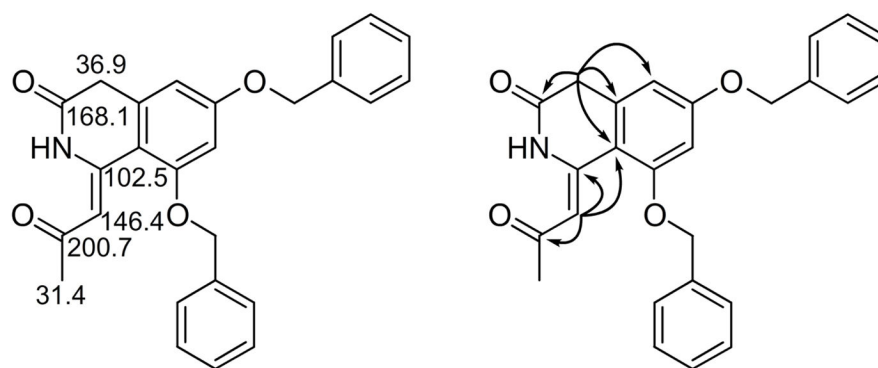
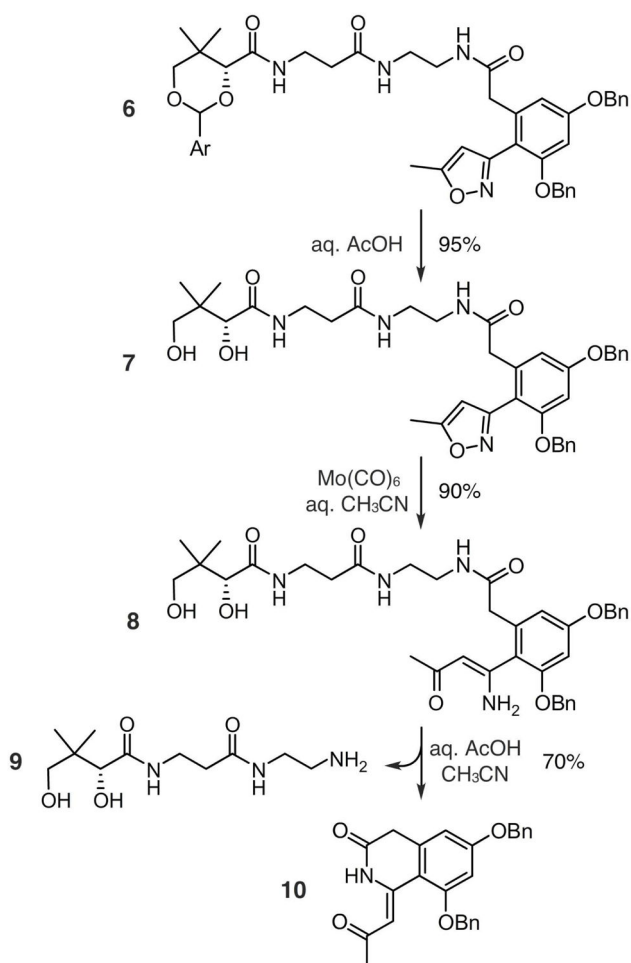


Fig. 6. NMR data confirming the assignment of **10**. (left) Select ^{13}C NMR assignments and (right) select ^1H , ^{13}C HMBC correlations.

**Scheme 1.**

Advance of cyclic mimetic **6**. Samples of **8** can be prepared two steps and in excellent yield from **6**. While stable to heat, compound **8** readily undergoes intramolecular *trans*-amidation resulting the formation of adduct **10** at rt under mildly acidic conditions.