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Synthesis of tyrosine derivatives for saframycin MX1 biosynthetic studies☆

Eric W. Schmidt,* James T. Nelson and John P. Fillmore

Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84112, USA

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Abstract—Saframycin MX1 and structural relatives are natural anticancer agents isolated from bacteria and marine invertebrates. For biosynthetic studies and to make a library of modified natural products, a series of tyrosine derivatives were synthesized in a concise manner.

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Saframycin MX1 (**1**)[1] is a member of a group of isoquinoline natural products, including renieramycins,[2] jorumycin,[3] and ecteinascidin 743 (Et-743),[4] that show promise in the treatment of some cancers.[5] Because of the potential clinical efficacy of Et-743, several synthetic and semisynthetic routes to this compound class have been reported.[6–8] In addition, combinatorial strategies have been employed to increase structural diversity of saframycins and their relatives.[9] Combinatorial biosynthesis provides a complimentary method to produce a large number of saframycin analogs, but an understanding of the saframycin biosynthetic pathway is required before such a method can be used. Here, we report on our preliminary efforts to de-convolute the biosynthesis of **1**.

Saframycins are composed of the amino acids glycine, alanine, and tyrosine, or derivatives thereof, as demonstrated by feeding studies (Fig. 1) and further supported by biosynthetic gene sequencing.[10–13] Analysis of the genes by us and by the Shen group[14] revealed that a single adenylation domain is likely responsible for activation and incorporation of two modified Tyr derivatives into **1**. We propose that the known *safA* and *safB* genes probably also catalyze heterocyclization via novel Pictet–Spengler- or Bischler–Napieralski-type condensations (Scheme 1).[15,16] Because of the novel mechanism of heterocycle formation and the possibility of exploiting the biochemistry for drug design, we are exploring the biosynthesis of saframycin MX1 (**1**).

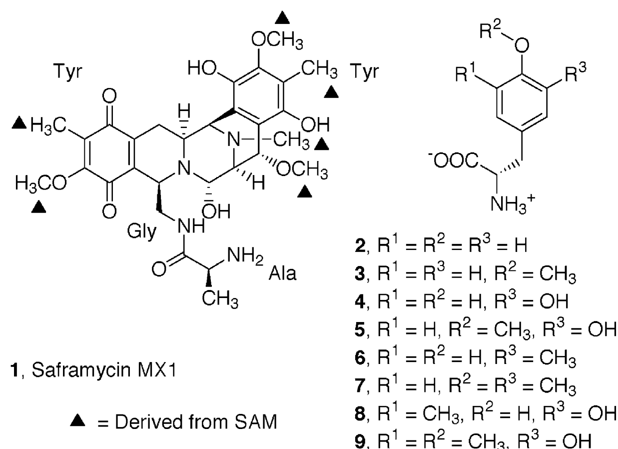

Figure 1. Biosynthesis of saframycin MX1 (**1**) and potentially incorporated Tyr derivatives. Depicted amino acid incorporation is based upon studies with saframycin A.[10]

Eight L-Tyr derivatives, three of which can be purchased, were identified as candidates for incorporation by SafA2 (Fig. 1). The *p*-quinone derivatives of Tyr are not likely precursors because of their instability and because the oxidation state of Tyr in saframycin derivatives is highly variable. We sought to design a simple synthesis that would be flexible enough to provide the other five intermediates using parallel chemistry and that would allow the later synthesis of compound libraries. We chose aromatic iodination and formylation to synthesize intermediates **10**, **12**, and **16**, which could

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* Corresponding author. Tel.: +1-801-585-5234; fax: +1-801-585-9119; e-mail: ews1@utah.edu

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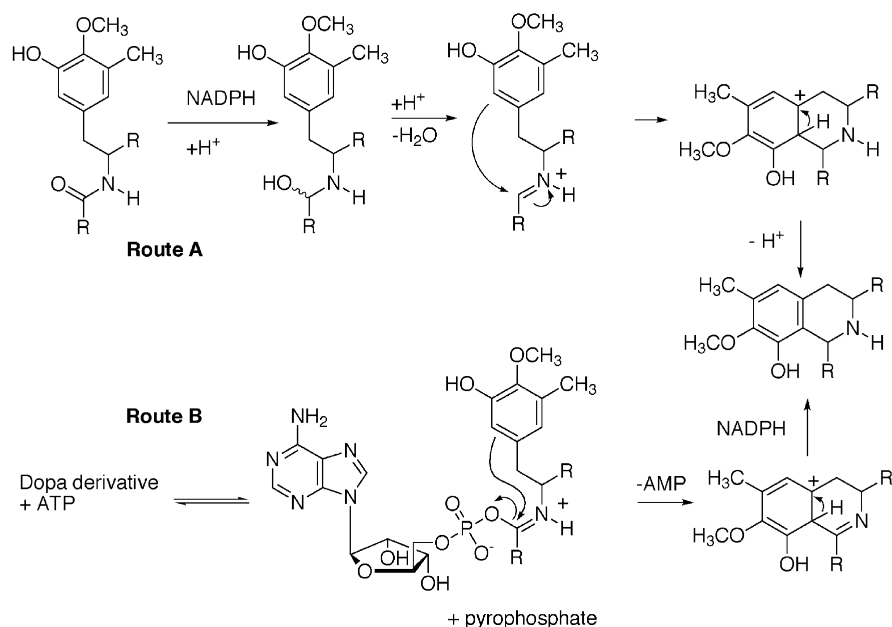


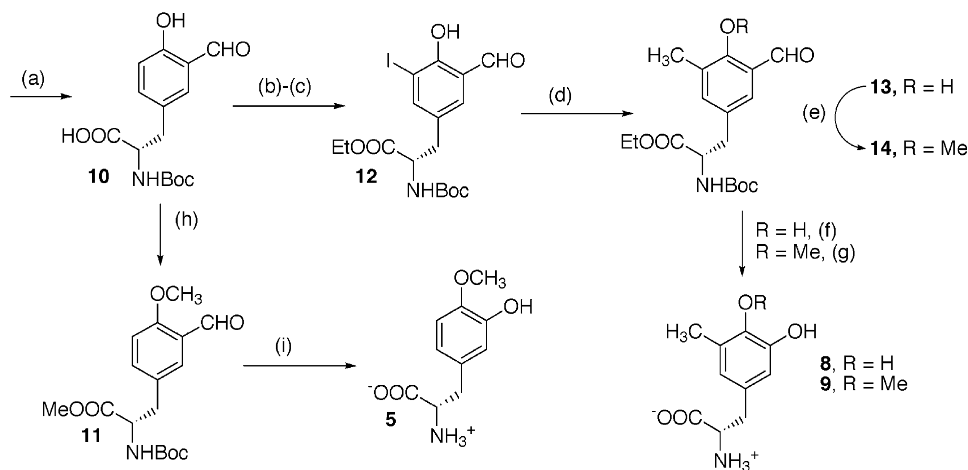
Scheme 1. Proposed biogenesis of saframycin heterocycle. Route A: Pictet–Spengler route; and Route B: Bischler–Napieralski route.

be further elaborated by Stille coupling, oxidation, and etherification. All compounds were prepared from the readily available starting material, *N*-Boc-Tyr.

Key steps in the synthesis of Dopa derivatives **5**, **8**, and **9** were the C-acylation of tyrosine and eventual oxidative cleavage of the acyl group to afford a phenol. A variety of acylation/Baeyer–Villiger oxidation protocols were explored, but ultimately we settled on the method of Jung and Lazarova,[17] which employs a Riemer–Tiemann reaction followed by a modified Dakin oxidation. While the Riemer–Tiemann reaction affords formylated tyrosine **10** in low yield (25–35% in our hands), it is simple to perform and does not racemize the substrate (>95% L-isomer recovered). The resulting formyl group is stable to acid, base, and oxidative conditions, but can be readily reduced by metal hydrides (data not shown) or cleaved by the Dakin reaction.

Using the formylation/oxidation protocol,[17] **5** was synthesized from *N*-Boc-formyl-Tyr **10** in 60% over three steps (Scheme 2). The two methyl groups of **11** were introduced in a single step,[18] but carboxyl-protected Tyr derivatives proved extremely resistant to Dakin or Baeyer–Villiger oxidation. Compound **11** was deprotected with LiOH/DMF/H_2O, oxidized using the Dakin reaction with $H_2O_2/(PhSe)_2$, and treated with 1:1 TFA/DCM to give Dopa derivative **5** in 68% overall yield.

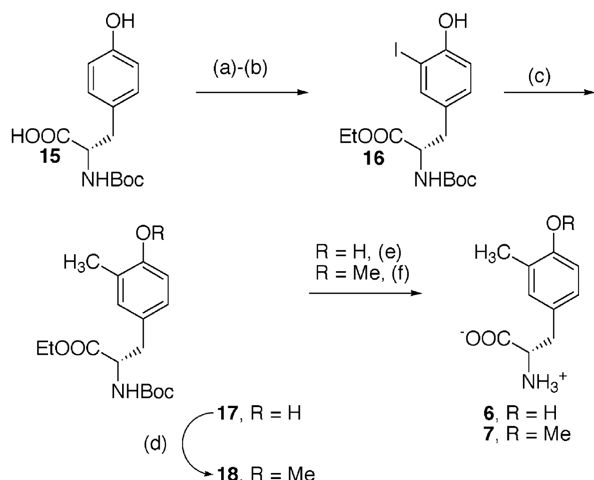
With an efficient route to Dopa derivatives in hand, we investigated the efficiency of iodination and Stille coupling of various Tyr derivatives. Appropriate iodination



Scheme 2. Synthesis of Dopa derivatives. Reagents and conditions: (a) BocTyr/NaOH/$CHCl_3/H_2O$, 65–70 °C, 16 h (25–35%); (b) DCC/DMAP/EtOH, rt, 36 h (quant); (c) I_2/H_2O_2/MeOH, rt, 16 h (77%); (d) $(CH_3)_4Sn$/NMP, $Pd_2dba_2{\cdot}CHCl_3$/TPP/CuI (cat), 70 °C, 1 d (94%); (e) MeI/K_2CO_3/acetone, 50 °C, 16 h (96%); (f) i. LiOH/DMF/H_2O, rt, 16 h, ii. Ph_2Se_2/H_2O_2/DCM, rt, 18–20 h, iii. TFA/DCM, rt, 20 min (77%); (g) i. LiOH/DMF/H_2O, rt, 16 h, ii. Ph_2Se_2/H_2O_2/18-crown-6/K_2HPO_4/DCM, rt, 18–20 h, iii. TFA/DCM, rt, 20 min (50%); (h) as in (e) (86%); (i) as in (f) (53%).

protocols were developed, and Stille reactions were performed following the method of Hudgens and Turnbull.[19] C-Methylated Tyr derivatives were synthesized by coupling tetramethyltin and *N*-Boc-3-iodotyrosine ethyl ester (**16**; Scheme 3).[20] The reaction was slow on this substrate, taking approximately 6 d at 70 °C. By contrast, C-methylated Dopa derivatives were more efficiently produced from key intermediate **12**, providing the *C*-methyl compound **13** in 94% yield in 24 h. O-Methylation and deprotection of these intermediates proceeded in a similar manner to that used for **11**.

Dakin oxidation of **13** followed by Boc deprotection cleanly afforded the Dopa derivative **8** (Scheme 2). Unexpectedly, upon LiOH deprotection the *O*-methyl derivative **14** formed a stable hydrate or acylal (Fig. 2), which was resistant to oxidative cleavage. The hydrate structure was proposed because the CHO proton singlet at $\sim\delta$ 10.3 ppm in the parent compound **14** was replaced by a doublet at $\sim\delta$ 8.0 ppm after hydrolysis, while all other protons exhibited their expected chemical shifts. Treatment of this intermediate with acids or bases transiently yielded the aldehyde compound, but upon workup only acetal was retrieved. Therefore, a tandem dehydration/Dakin oxidation reaction was performed by adding 18-crown-6 and dipotassium phosphate to the standard Dakin conditions. After 72 h and subsequent treatment with TFA, the Dopa product **9** was produced in 52% isolated yield.



Scheme 3. Synthesis of Tyr derivatives. Reagents and conditions: (a) i. NaI/NaOH/MeOH, ii. NaOCl (4% aq), 4 °C, 2 h (quant); (b) DCC/DMAP/EtOH, rt, 1 h (68%); (c) $(CH_3)_4Sn$/NMP, $Pd_2dba_2{\cdot}CHCl_3$/TPP/CuI (cat), 70 °C, 6 d (90%); (d) MeI/K_2CO_3/acetone, 50 °C, 16 h (59%); (e) i. LiOH/DMF/H_2O, rt, 16 h, ii. Ph_2Se_2/H_2O_2/DCM, rt, 18–20 h, iii. TFA/DCM, rt, 20 min (88%); (f) as in (e) (80%).

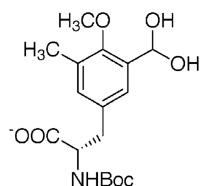


Figure 2. Proposed hydrate structure.

The chiral purity of the amino acid products was assessed by Marfey's method.[21] Surprisingly, despite suggestions that harsh, basic reaction conditions such as those employed by Jung and Lazarova will lead to substantial racemization,[22] only a single isomer was detected for each compound. Based upon HPLC of Marfey's derivatives, Tyr and Dopa compounds **5–9** are at least 90% enantiopure, since only a single peak was detected at the appropriate wavelength and elution time in each HPLC run. By comparison, an authentic standard of D,L-tyrosine gave rise to two sharp HPLC peaks, which eluted after 37.2 and 39.8 min. Because complete inversion of configuration is an unlikely event under our reaction conditions, the derivates all adopt the L-configuration. Chemical shift data for **5–9** are given below.[23]

Supplementary data

Detailed experimental procedures and ^{1}H and ^{13}C NMR data for all compounds.

Acknowledgements

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23. Compound **5**: ^{1}H NMR (D_2O, 400 MHz) δ 6.83 (1H, d, $J = 8.4$ Hz, C6-H), 6.64 (1H, s, C2-H), 6.63 (1H, d, $J = 8.4$ Hz, C5-H), 3.73 (1H, dd, $J = 8.0$, 5.6 Hz, αH), 3.67 (3H, s, OCH_3), 2.97 (1H, dd, $J = 13.8$, 5.6 Hz, βH), 2.81 (1H, dd, $J = 13.8$, 8.0 Hz, βH$'$); ^{13}C NMR (D_2O, CD_3OD int. std., 125 MHz) δ 174.3, 147.0, 145.3, 128.3, 121.7, 116.4, 113.0, 56.1, 54.0, 35.8; Compound **6**: ^{1}H NMR (D_2O, 400 MHz) δ 6.92 (1H, d, $J = 1.2$ Hz, C2-H), 6.84 (1H, dd, $J = 8.0$, 1.2 Hz, C6-H), 6.68 (1H, d, $J = 8.0$ Hz, H-5), 3.74 (1H, dd, $J = 7.0$, 5.6 Hz, αH), 2.99 (1H, dd, $J = 14.8$, 5.6 Hz, βH), 2.83 (1H, dd, $J = 14.8$, 8.0 Hz, βH$'$), 2.02 (3H, s, C–CH_3); ^{13}C NMR (D_2O, CD_3OD int. std., 125 MHz) δ 153.0, 132.0, 128.0, 126.9, 125.5, 115.4, 53.8, 35.6, 15.2. Note: carboxyl C not observed; Compound **7**: ^{1}H NMR (D_2O, 400 MHz) δ 6.91 (1H, d, $J = 9.2$ Hz, C6-H), 6.90 (1H, s, C2-H), 6.79 (1H, d, $J = 9.2$ Hz, C5-H), 3.64 (3H, s, O–CH_3), 3.63 (1H, dd, $J = 7.6$, 3.2 Hz, αH), 2.94 (1H, dd, $J = 14.4$, 3.2 Hz, βH), 2.77 (1H, dd, $J = 14.4$, 7.6 Hz, βH$'$), 1.99 (3H, s, C–CH_3); ^{13}C NMR (D_2O, CD_3OD int. std., 125 MHz) δ 157.5, 132.6, 128.9, 128.8, 128.1, 112.4, 57.3, 54.8, 37.2, 16.3. Note: carboxyl C not observed; Compound **8**: ^{1}H NMR (D_2O, 400 MHz) δ 6.51 (1H, s, ArH), 6.50 (1H, s, ArH), 3.88 (1H, dd, $J = 8.0$, 5.6 Hz, αH), 2.99 (1H, dd, $J = 14.4$, 5.6 Hz, β H), 2.84 (1H, $J = 14.4$, 8.0 Hz, βH$'$), 2.04 (3H, s, C–CH_3); Compound **9**: ^{1}H NMR (D_2O, 400 MHz) δ 6.59 (2H, s, ArH), 3.80 (1H, dd, $J = 8.0$, 5.2 Hz, αH), 3.65 (3H, s, OCH_3), 3.03 (1H, dd, $J = 14.8$, 5.2, βH), 2.85 (1H, dd, $J = 14.8$, 8.0 Hz, βH$'$), 2.13 (3H, s, C–CH_3); ^{13}C NMR (D_2O, CD_3OD int. std., 125 MHz) δ 174.9, 149.7, 145.7, 134.0, 132.7, 124.5, 115.9, 61.3, 56.9, 36.8, 15.9.