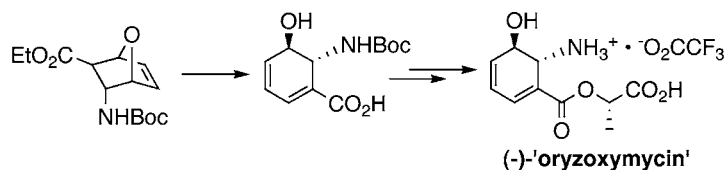


Asymmetric Synthesis of the Putative  
Structure of (–)-OryzoxymycinMark E. Bunnage,<sup>†</sup> Thota Ganesh,<sup>‡</sup> Ishmael B. Masesane,<sup>‡</sup> Darren Orton,<sup>‡</sup> and  
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## ABSTRACT



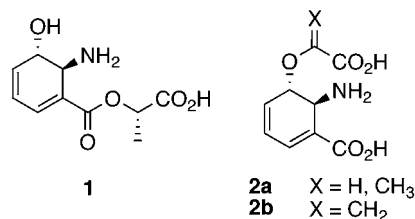
A short asymmetric synthesis of (2′S,5R,6R)-2′-[6-amino-5-hydroxy-1,3-cyclohexadiene-1-carboxyloxy]propionic acid, the enantiomer of the reported structure of (+)-oryzoxymycin is described. The reported spectral data does not match that obtained for synthetic “oryzoxymycin”.

In 1968, Hashimoto et al. reported the isolation of a novel metabolite, oryzoxymycin, from a soil sample of a *Streptomyces* strain, exhibiting moderate in vitro activity against *Xanthomonas oryzae*.<sup>1,2</sup> The structure **1** was subsequently determined to be that shown in Figure 1 by a combination of spectroscopic, analytical, and degradation studies.<sup>3</sup> As such, the structure is closely related to intermediates in the biosynthesis of anthranilate, e.g., **2**.<sup>4,5,6</sup> The principal difference in the compounds appears in the position of the lactate or enoylpyruvate moiety, which is more commonly coupled through the C-5 hydroxyl group.

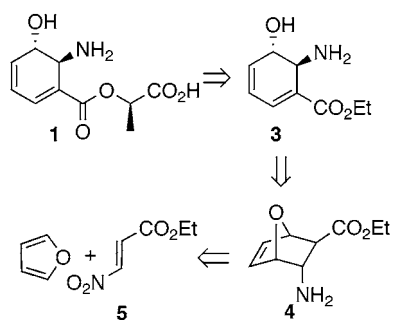
As part of a broader synthetic program, we have been exploring the use of substituted nitroacrylates as versatile synthetic reagents to provide access to a range of conformationally restricted functionalized  $\beta$ -amino acids.<sup>7</sup> Within

this context, the interesting structure of **1** represented an attractive target and prompted us to pursue its synthesis. Reflecting the importance of these biosynthetic pathways, the syntheses of a number of related structures have been published.<sup>8</sup> However, to date, neither a synthetic approach to oryzoxymycin nor an asymmetric synthesis of the dihydroanthranilate core has been reported.<sup>9</sup> In this paper, we describe our approach to these two goals.

Our retrosynthetic analysis for oryzoxymycin disconnected the lactate unit, leaving the dihydroanthranilate ester **3**, Figure 2. This could be generated from the base induced fragmentation of the bicyclic amino ester **4**, derived from a Diels–Alder reaction between furan and a  $\beta$ -nitroacrylate **5**.<sup>10</sup>

<sup>†</sup> Pfizer Global Research and Development.<sup>‡</sup> University of Durham.(1) Hashimoto, T.; Kondo, S.; Takita, T.; Hamada, M.; Takeuchi, T.; Okami, Y.; Umezawa, H. *J. Antibiot.* **1968**, *21*, 653–658.(2) Hashimoto, T.; Takahashi, S.; Naganawa, H.; Takita, T.; Maeda, K.; Umezawa, H. *J. Antibiot.* **1972**, *25*, 350–355.(3) Hashimoto, T.; Kondo, S.; Naganawa, H.; Takita, T.; Maeda, K.; Umezawa, H. *J. Antibiot.* **1974**, *27*, 86–87.(4) Walsh, C. T.; Erion, M. D.; Walts, A. E.; Delany, J. J.; Berchtold, G. A. *Biochemistry* **1987**, *26*, 4734–4745.(5) Morollo, A. A.; Finn, M. G.; Bauerle, R. *J. Am. Chem. Soc.* **1993**, *115*, 5, 816–817.(6) Anderson, K. S.; Kati, W. M.; Ye, C. Z.; Liu, J.; Walsh, C. T.; Benesi, A. J.; Johnson, K. A. *J. Am. Chem. Soc.* **1991**, *113*, 3198–3200.

**Figure 1.** Reported structure of oryzoxymycin and related compounds.



**Figure 2.** Retrosynthetic analysis for oryzoxymycin.

Efficient access to **5** was achieved through a modification of the McMurry method<sup>11,12</sup> involving the reaction of an acrylate with  $\text{N}_2\text{O}_4$  and  $\text{I}_2$  followed by careful elimination of HI with Hunig's base in ether. With this sequence, we can routinely generate 10–20 g batches of this versatile reagent in 70–80% overall yield, Scheme 1. Following literature precedents,<sup>13</sup> the furan Diels–Alder reaction with nitroacrylate **5** occurred rapidly at room temperature to give a mixture of cycloadducts favoring the required endo nitro isomer **8**. Enhanced selectivity could be obtained by running the reaction in  $\text{CHCl}_3$  at  $-20^\circ\text{C}$  to give a separable 4:1 mixture of the two isomers in >90% yield. Attempts to further improve this selectivity with Lewis acids were not successful affording, instead, the substituted furan **11** in moderate yields. Subsequent selective conversion to the protected aminoester **9** was then achieved in a single pot by reduction with  $\text{Zn}/\text{HCl}$  followed by addition of a large excess of  $i\text{Pr}_2\text{NEt}$  and  $\text{Boc}_2\text{O}$ .

With this intermediate in hand, our attention turned to the key fragmentation reaction. Related base promoted transformations have been reported in the literature and our initial experiments followed these precedents.<sup>14</sup> With a variety of lithium bases the reaction appeared, by TLC, to be extremely rapid giving complete conversion of starting material.

(7) For a recent review of this field, see: Fülöp, F. *Chem. Rev.* **2001**, *101*, 2181–2204.

(8) See, for example: (a) Teng, C. Y. P.; Ganem, B.; Doktor, S. Z.; Nichols, B. P.; Bhatnagar, R. K.; Vining, L. C. *J. Am. Chem. Soc.* **1985**, *107*, 5008–5009. (b) Teng, C. Y. P.; Ganem, B. *J. Am. Chem. Soc.* **1984**, *106*, 2463–2464. (c) Policastro, P. P.; Au, K. G.; Walsh, C. T.; Berchtold, G. A. *J. Am. Chem. Soc.* **1984**, *106*, 2443–2444. (d) Kozlowski, M. C.; Tom, N. J.; Seto, C. T.; Seifler, A. M.; Bartlett, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2128–2140.

(9) Enantiomerically pure DHAA has been isolated from a fermentation culture (McCormick, J. R. D.; Reichenthal, J.; Hirsch, U.; Sjolander, N. O. *J. Am. Chem. Soc.* **1962**, *84*, 3711) and subsequently used in asymmetric syntheses of related structures; see ref 8b. For an alternative approach to DHAA, see: Fukuyama, T.; Nakatsuka, S.; Kishi, Y. *Tetrahedron* **1981**, *37*, 2045–2078 references therein.

(10) Just, G.; Martel, A. *Tetrahedron Lett.* **1973**, 1517–1520.

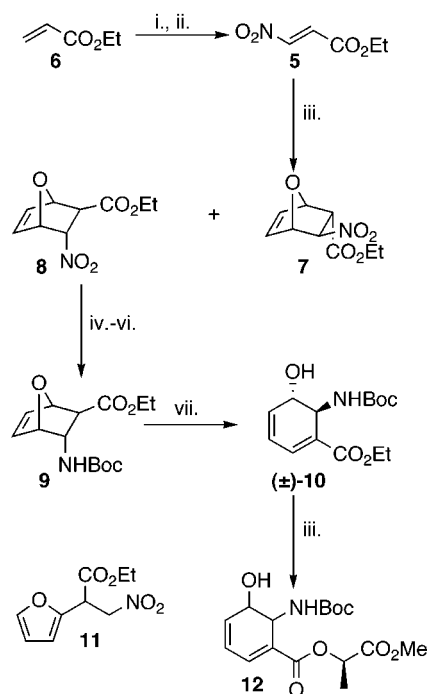
(11) McMurry, J. E.; Musser, J. H. *Org. Synth.* **1977**, *56*, 65–68.

(12) Jew, S. S.; Kim, H. D.; Cho, Y. S.; Cook, C. H. *Chem. Lett.* **1986**, 1747–1748.

(13) Itoh, K.; Kitoh, K.; Sera, A. *Heterocycles* **1999**, *51*, 243–248.

(14) (a) Brion, F. *Tetrahedron Lett.* **1982**, *23*, 5299–5302. (b) Rajapaksa, D.; Keay, B. A.; Rodrigo, R. *Can. J. Chem.* **1984**, *62*, 826–827. (c) Campbell, M. M.; Sainsbury, M.; Searle, P. A. *Synthesis* **1993**, 179–193. (d) Evans, D. A.; Barnes, D. M. *Tetrahedron Lett.* **1997**, *38*, 57–58 and references therein.

**Scheme 1.** Preparation of Dihydroanthranilate Core **10**<sup>a</sup>



<sup>a</sup> Reagents: (i)  $\text{I}_2$ ,  $\text{N}_2\text{O}_4$ ,  $\text{Et}_2\text{O}$ , 91%; (ii)  $i\text{Pr}_2\text{NEt}$ ,  $\text{Et}_2\text{O}$ , 84%; (iii) furan,  $\text{CHCl}_3$ ,  $-20^\circ\text{C}$ , 120 h, 90% **8/7** 81:19; (iv) chromatography; (v)  $\text{Zn}$ ,  $\text{HCl}$ ,  $\text{EtOH}$ ; (vi)  $i\text{Pr}_2\text{NEt}$ ,  $(\text{Boc})_2\text{O}$ , 77%; (vii)  $\text{KHMDS}$ ,  $\text{THF}$ ,  $-50$  to  $+25^\circ\text{C}$ , 71%; (viii)  $\text{KOH}$ ,  $\text{THF}$ ,  $\text{H}_2\text{O}$ , 68%; (ix)  $\text{CsF}$ ,  $(S)\text{-MsOCH}(\text{CH}_3)\text{CO}_2\text{Me}$  **13**,  $\text{DMF}$ ,  $50^\circ\text{C}$ , 83%.

However, on workup considerable amounts of the starting ester were recovered.<sup>15</sup> Attempts to enhance the fragmentation by various rapid, mildly acidic, inverse quenches were partially successful albeit only on very small scale. Finally, the use of a less coordinating potassium counterion ( $\text{KHMDS}$ ) allowed the isolation of the ester **10** in a reproducible 70% yield together with variable amounts of ethyl 3-hydroxybenzoate.

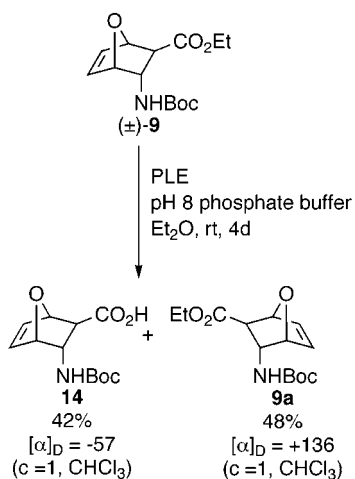
Preliminary attempts to protect the 5-hydroxyl group as a silyl ether proved difficult and although this has subsequently been achieved in high yield, the resultant ether is not particularly stable to acidic or basic conditions undergoing ready aromatization to give 3-hydroxybenzoate esters.<sup>16</sup> Consequently, following routine hydrolysis of the ester group we explored selective coupling of the resultant acid with various lactate derivatives. Initial attempts to achieve this transformation using a large number of classical coupling reagents resulted in extensive decomposition. Believing this to be due to a problem of steric hindrance to nucleophilic attack at the activated carbonyl group we considered other approaches involving nucleophilic displacement of an acti-

(15) Similar observations have been noted elsewhere. See, for example, ref 8d and: Couche, E.; Deschatrete, R.; Poumellec, K.; Bortolussi, M.; Mandville, G.; Bloch, R. *Synlett* **1999**, 87–89.

(16) The relatively high stability of the free hydroxy compound relative to substituted analogues has been noted in similar systems and can be attributed to a conformational effect that places both heteroatom substituent in a different plane to both the diene p-system and the neighboring H atoms. For similar observations, see ref 8d.

vated lactate derivative by a carboxylate anion. In this context, Otera has reported that lactyl esters can be prepared by  $S_N2$  displacement of the corresponding mesylate with carboxylates in the presence of CsF.<sup>17</sup> Consequently, anthranilate ester **10** was hydrolyzed with KOH and the resultant acid treated with CsF and mesylate **13** in DMF to give the desired lactate coupled product **12** as a mixture of two diastereoisomers. Separation of the diastereoisomers proved impossible, and we therefore sought an enantioselective preparation of ester **10**.

Preliminary attempts to prepare and use chiral nitroacrylates did not prove to be viable and consequently we considered resolution.<sup>18</sup> A considerable body of literature has reported on enzymatic hydrolysis of bicyclic esters related to **9**.<sup>19</sup> Following these precedents, we screened a number of different enzyme systems for selectivity. Ultimately, the use of PLE in pH 8 phosphate buffer/ether for 4 days afforded a very effective kinetic resolution, Figure 3. At the



**Figure 3.** Enzymatic resolution of  $(\pm)\text{-9}$ .

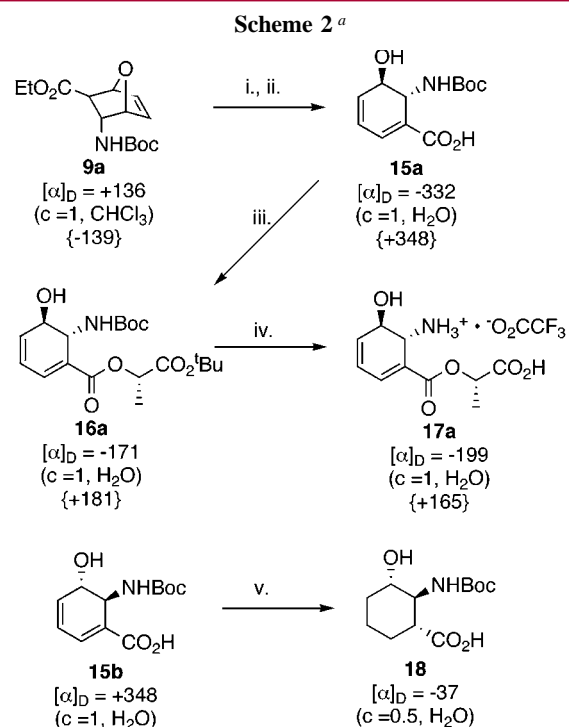
same time, a preparative chiral HPLC method (Chiralpak AD, heptane/ethanol 95:5) was found to be equally effective giving both enantiomers of the bicyclic ester with good recovery. With the chiral esters in hand, a similar sequence to that described above was followed, Scheme 2.

Repeating the fragmentation–coupling sequence starting from enantiomer **9a**, using the mesylate **19**, derived from *tert*-butyl (*R*)-lactate, afforded protected “*ent*-oryzoxymycin” **16a** as a single diastereoisomer as ascertained by high-field NMR. With this substrate complete, deprotection could be

(17) Otera, J.; Nakazawa, K.; Sekoguchi, K.; Orita, A. *Tetrahedron* **1997**, *53*, 13633–13640.

(18) Nitroacrylates bearing chiral auxiliaries have been successfully prepared and used in asymmetric Diels–Alder reactions. See: Clive, D. L. J.; Bo, Y. X.; Selvakumar, N.; McDonald, R.; Santarsiero, B. D. *Tetrahedron* **1999**, *55*, 3277–3290.

(19) See, for example: Bloch, R.; Guibejampel, E.; Girard, C. *Tetrahedron Lett.* **1985**, *26*, 4087–4090. Schueller, C. M.; Manning, D. D.; Kiessling, L. L. *Tetrahedron Lett.* **1996**, *37*, 8853–8856 and references therein.

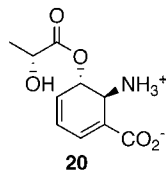


<sup>a</sup> Reagents: (i) KHMDS, THF,  $-50$  to  $+25$  °C, 71%; (ii) KOH, THF,  $\text{H}_2\text{O}$ , 68%; (iii) CsF, (*R*)- $\text{MsOCH}(\text{CH}_3)\text{CO}_2\text{tBu}$  **19**, DMF,  $50$  °C, 83%; (iv) TFA, DCM, 89%; (v)  $\text{H}_2$ , Pd–C, MeOH, 98% ( $[\alpha]_D$  values in parentheses are derived from the enantiomeric bicyclic ester **9b**).

achieved with TFA to provide the enantiomer of natural “oryzoxymycin” in good yield as the TFA salt **17a**. In an identical fashion, treatment of enantiomeric bicyclic ester **9b** led to the C5,C6 diastereoisomer **17b**. The absolute stereochemistry of **15** and hence synthetic “oryzoxymycin” **17a** was confirmed by reproducing the literature reduction of **15b** to known (1*R*,2*S*,3*S*)-2-*tert*-butoxycarbonylamino-3-hydroxycyclohexanecarboxylic acid **18**  $[\alpha]_D^{21} -37$  ( $c = 1, \text{H}_2\text{O}$ ) (lit.<sup>9</sup>  $[\alpha]_D^{21} -35$  ( $c = 0.5, \text{H}_2\text{O}$ )). Interestingly, unlike the free natural product, these “oryzoxymycin” salts show appreciable stability when stored at  $0$  °C, and this has allowed us to obtain satisfactory spectroscopic and analytical data to support our assignment. In particular, HMBC correlations between the cyclohexadienylcarboxylate carbonyl carbon and H-2' and H-2 confirmed the correct point of attachment of the lactate unit. At this stage several differences between our observed data and that reported in the original isolation of oryzoxymycin became apparent. Namely, the optical rotation [**17a**  $[\alpha]_D^{21} -199$  ( $c = 1, \text{H}_2\text{O}$ ); lit.<sup>2</sup>  $[\alpha]_D^{21} +349$  ( $c = 1, \text{H}_2\text{O}$ )] and significant differences in the infrared spectrum both in the carbonyl region and characteristic bands in the fingerprint region. This suggests that the correct structure for oryzoxymycin may be the isomeric C-5 lactate ester **20**.<sup>20</sup> Efforts to prepare this compound and verify this

(20) In this context, it is relevant to note that corresponding C-5 acetate derivative of ester **10** has C=O stretches at 1737, 1717, and  $1679$   $\text{cm}^{-1}$ .

hypothesis are in progress. These results will be reported in detail in due course.



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**Supporting Information Available:** Experimental section containing procedures and characterization of key compounds **10a**, **16a,b**, and **17a,b**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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