

From nature, through chemical synthesis, toward use in agriculture: Oryzoxymycin case study*

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Abstract: The Diels–Alder adducts of ethyl (*E*)-3-nitroacrylate and furan provided a common and versatile template for the stereocontrolled synthesis of an isomer of the natural product oryzoxymycin and polyhydroxylated cyclohexyl β -amino acid derivatives. The strategy for the synthesis of the polyhydroxylated cyclic β -amino acid derivatives involved base-induced fragmentation of the oxanorbornene skeleton and face selective oxidation reactions. A Pd-catalyzed transfer hydrogenation reaction in the presence of organic acids is also described. This reaction is amenable to being enantioselective through use of optical pure chiral organic acids.

Keywords: oryzoxymycin; Diels–Alder reaction; nitroacrylate; epoxidation; transfer hydrogenation.

INTRODUCTION

The chemical pest control paradigm has been a key component of the green revolution that resulted in increases in crop yields during the past 50 years. However, a large number of synthetic pesticides have been banned in a number of countries because of their undesirable attributes such as high and acute toxicity, long degradation periods, accumulation in the food chain, and extension of their power to destroy both useful and harmful pests [1]. To replace the chemicals lost due to the new registration requirements, there is a need to develop natural product-based agrochemicals along with novel chemistry. Natural product-based agrochemicals are generally considered safer than synthetic agrochemicals because of their relatively short environmental half life. This article deals with development of some novel chemistry in the synthesis of oryzoxymycin **1**, a natural product with potential as an agrochemical and hydroxylated cyclohexyl β -amino acids.

Oryzoxymycin **1** was isolated from a soil sample of *Streptomyces venezuelae*, var. *oryzoxymyceticus*, and this compound was shown to exhibit moderate activity against *Xanthomonas oryzae*, gram positive bacteria that attack the leaves of rice plants [2]. On the basis of spectroscopic and degradation studies, the structure of oryzoxymycin was elucidated as a composite of (*R*)-lactic acid and (*5S,6S*)-5,6-dihydroxyanthranilic acid **2** (DHAA), a compound previously isolated from *Streptomyces aureofaciens*, Fig. 1 [3,4]. Oryzoxymycin belongs to a class of compounds called cyclic β -amino acids.

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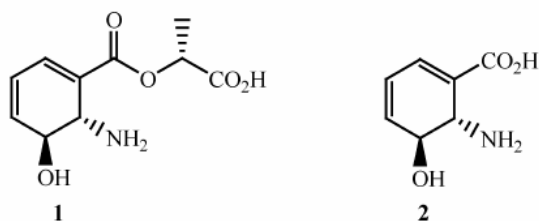


Fig. 1 Reported structure of oryzoxylic acid **1** and dihydro-3-hydroxyanthranilic acid **2**.

Cyclic β -amino acids have received so much attention due to their biological activity and lately because of the properties of their oligopeptides. (1*R*,2*S*)-2-Aminocyclopentanecarboxylic acid (cispentacin), for example, is an antifungal antibiotic possessing potent anti-*Candida* activity [5]. Lately, Gellman has reported that a β -17 oligomer has significant activity against four species of bacteria including vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* [6–10]. All these reports have generated a lot of interest in the synthesis of various cyclic β -amino acids. Consequently, we have been developing synthetic strategies for the preparation of oryzoxylic acid and hydroxylated cyclohexyl β -amino acids as lead compounds to agro-antibiotics against gram positive bacteria.

ASYMMETRIC SYNTHESIS OF (–) ORYZOXYMYCIN

The general strategy for the synthesis of oryzoxylic acid is based on the retrosynthetic analysis shown in Fig. 2. Disconnection at the ester bond furnished the lactic acid unit and dihydroanthranilic acid **2** as key building blocks. Dihydroanthranilic acid **2** could be generated from the base-induced fragmentation of the bicyclic amino ester **3**, which in turn could be derived from a Diels–Alder reaction between β -nitroacrylate **4** and furan. From this retrosynthetic analysis, a plan for a stepwise and stereocontrolled total synthesis of oryzoxylic acid evolved.

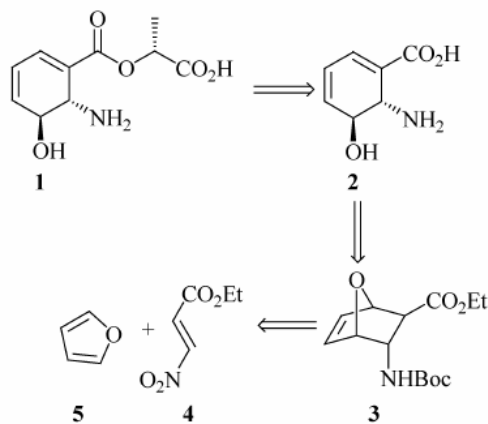
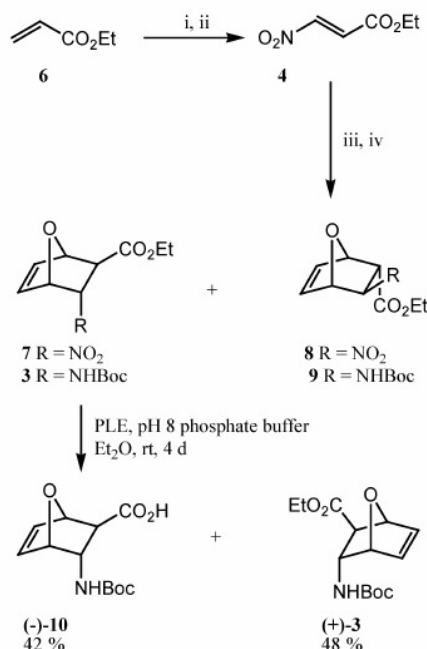


Fig. 2 Retrosynthetic analysis for oryzoxylic acid **1**.

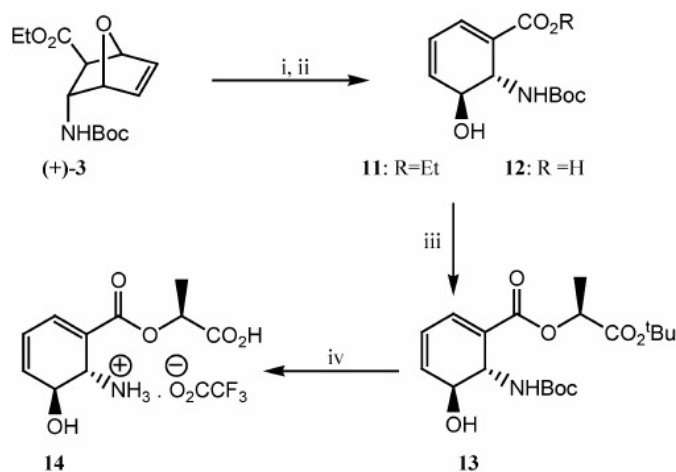
Efficient access to **4** was achieved through a modification of the McMurry protocol [11] involving the reaction of acrylate **6** with N_2O_4 and I_2 followed by careful elimination of HI with Hunig's base in ether. With this sequence of reactions, nitroacrylate **4** could be generated in 10–20 g batches, Scheme 1. Subjecting nitroacrylate **4** to cycloaddition reaction with furan in CH_2Cl_2 at $-20\text{ }^\circ\text{C}$ for five days afforded an 80:20 mixture of adducts **7** and **8**, respectively, in 90 % yield. When the temperature of the reaction was increased to room temperature, considerable acceleration of the reaction was observed but was accompanied by a significant reduction in diastereoselectivity (2:1 mixture of **7** and **8**, respectively). The two isomers were easily separated by column chromatography. Subsequent conversion of adduct **7** to the protected aminoester **3** was achieved in a single pot by reduction of the nitro group with Zn/HCl followed by addition of a large excess of $^i\text{Pr}_2\text{NEt}$ and Boc_2O . Pig liver esterase (PLE)-mediated hydrolysis of aminoester **3** in pH 8 phosphate buffer/ether proved very stereoselective and therefore afforded an effective kinetic resolution method. At the same time, a preparative high-performance liquid chromatography (HPLC) method (Chiralpak AD, heptane/ethanol 95:5) was found to be equally effective giving both enantiomers of the aminoester **3**.



Scheme 1 Reagents: (i) I_2 , N_2O_4 , Et_2O , 91 %; (ii) $^i\text{Pr}_2\text{NEt}$, Et_2O , 84 %; (iii) furan, CHCl_3 , $25\text{ }^\circ\text{C}$, (7:8 2:1), 90 %; (iv) Zn, HCl, EtOH then Boc_2O , Et_3N , $25\text{ }^\circ\text{C}$, 3.77 %, 9.75 %.

In the event that the oxygen bridge of oxanorbornene systems could be eliminated, optically pure (+)-**3** could serve as a potential precursor to the cyclohexadiene moiety of oryzoxylicin. Indeed, lithium hexamethyldisilazide (LiHMDS)-induced fragmentations of oxanorbornane systems were first reported by Brion [12]. On the basis of this precedent, the oxygen bridge of adduct (+)-**3** was eliminated using potassium bis(trimethylsilyl)amide (KHMDs), which was found to be more effective than LiHMDS. In the event, treatment of (+)-**3** with KHMDs in tetrahydrofuran (THF) for 15 min afforded

cyclohexadiene **11** together with ethyl 3-hydroxybenzoate as a by-product, Scheme 2. It is instructive to note that the proportion of ethyl 3-hydroxybenzoate increases very quickly and becomes the sole product if the reaction is allowed beyond the 15 min. Subsequent base-mediated hydrolysis of the ester afforded substituted diene (**-12**) in acceptable yields. With intermediate **12** in hand, we explored selective coupling of its acid group 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 activated 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 [13]. In the event, acid (**-12**) was treated with CsF and (*R*)-*t*-butyl *O*-mesyllactate in dimethylformamide (DMF) to afford protected "ent-oryzoxymycin" **13** as a single diastereoisomer as ascertained by high-field and 2D NMR experiments. Deprotection was achieved with trifluoroacetic acid (TFA) to give the enantiomer of the natural "oryzoxymycin" in good yield as the TFA salt **14** [14], Scheme 2. Efforts to prepare other isomers of oryzoxymycin are in progress, and these results will be reported in detail in due course.

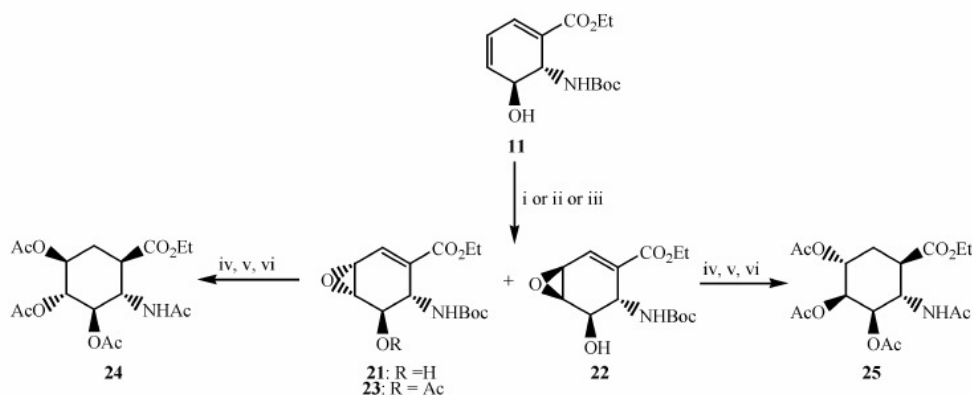


Scheme 2 Reagents: (i) KHMDS, THF, -50 to 25 °C, 71 %; (ii) KOH, THF, H_2O , 68 %; (iii) CsF, (*R*)-MsOCH(CH_3)CO $_2$ t Bu, DMF, 50 °C, 83%; (iv) TFA, DCM, 89 %.

SYNTHESIS OF HYDROXYLATED CYCLOHEXYL β -AMINO ACIDS

During the course of the synthesis of oryzoxymycin, it was recognized that cyclohexadiene **11** represented a useful template for the construction of highly functionalized cyclohexyl β -amino acids. Our strategy toward synthesis of these cyclohexyl β -amino acids identified epoxidation as a central reaction. Thus, treatment of a solution of cyclohexadiene **11** in CH_2Cl_2 with *m*CPBA and $NaHCO_3$ resulted in a highly selective oxidation of the remote π bond to give a separable 9:1 mixture of epoxides **21** and **22**, respectively, Scheme 3. Preliminary acetylation of the hydroxyl group enhanced selectivity, allowing the formation of epoxide **23** as a single isomer. Treatment of epoxide **23** with aqueous perchloric acid led to a single *anti* diols isomer, albeit accompanied by loss of the acid-sensitive *t*-butoxycarbonyl group. NMR analysis supported the hypothesis that preferential nucleophilic attack had occurred at the allylic position. Following peracylation, stereoselective reduction of the enoate double bond occurred

to give the *trans-trans-trans-trans*-trihydroxycyclohexyl β -amino acid derivative **24**, Scheme 3. The stereoselectivity of the reduction of the double bond is consistent with reported observations [15], and the overall stereochemistry was confirmed by 2D NMR experiments, particularly nuclear Overhauser enhancement spectroscopy (NOESY). Important NOESY correlations are shown in Fig. 3.



Scheme 3 Reagents: (i) *m*CPBA, CH_2Cl_2 (**21**:**22** 9:1), 77 %; (ii) Ac_2O , Py then *m*CPBA, CH_2Cl_2 , (**13** only), 65 %; (iii) *m*CPBA, MeCN, NaHCO_3 , (**21**:**22** 1:2), 95 %; (iv) HClO_4 , H_2O /acetone; (v) Ac_2O , pyridine; (vi) H_2 , Pd/C, **24** 72 %, **25** 73 %.

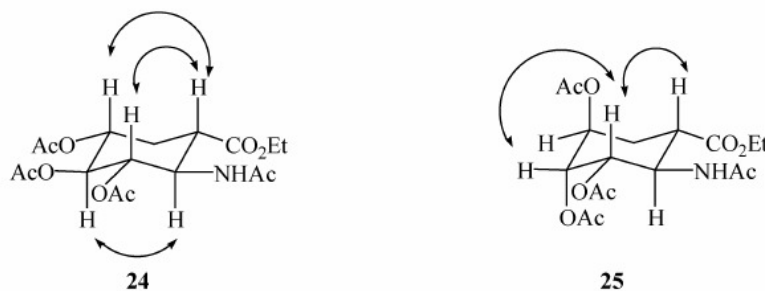
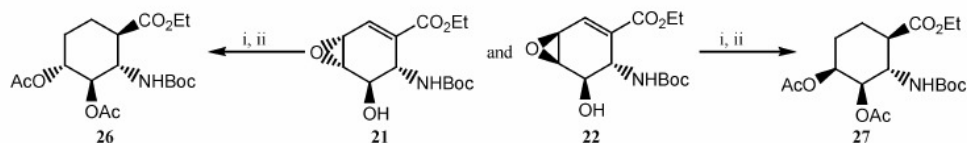


Fig. 3 Selected NOESY correlations for **24** and **25**.

To generate the alternative *anti* diols isomer, we explored methods to override the dominant directing effect of the carbamate group. However, all attempts to utilize the free hydroxyl group as a stereocontrolling element with reagents such as $\text{VO}(\text{acac})_2$ [16,17] were unsuccessful. Speculating that alternative solvents may disrupt the directing effect of the carbamate group, we surveyed a range of polar protic and aprotic solvents. Gratifyingly, when a solution of the cyclohexadiene **11**, *m*-chloro peroxybenzoic acid (*m*CPBA), and NaHCO_3 in CH_3CN was stirred at room temperature for 16 h, a separable 2:1 mixture of isomers favoring the desired *syn* epoxyalcohol **22** was isolated in excellent yield, Scheme 3. Acid-catalyzed opening of the epoxide **22** and reduction of the enoate double bond as before afforded the desired *trans-trans-cis-trans* amino acid derivative **25** [18].

The extension of the above methodology to the preparation of the 3,4-dihydroxylated cyclohexyl β -amino acids was based on reductive opening of epoxide intermediates **21** and **22**. Consequently, exposure of epoxide **21** to the action of Pd-C under a hydrogen atmosphere afforded the *trans-trans-trans* dihydroxycyclohexyl β -amino acid derivative **26** [19] as the only detectable isomer, Scheme 4. The

trans-trans-trans configuration of **26** was confirmed by NOESY experiments, Fig. 4. Reductive opening of vinyl epoxides was described by Danishefsky [20], and on the basis of this precedent, the above results were not surprising. It is noteworthy that the addition of the hydride took place on the more hindered diastereo-face of the alkene probably due to the coordination of the Pd-hydride complex to the carbamate.



Scheme 4 Reagents: (i) H₂, Pd/C, 25 °C; (ii) Ac₂O, pyridine, 25 °C, **26** 88 % from **21**, **27** 84 % from **22**.

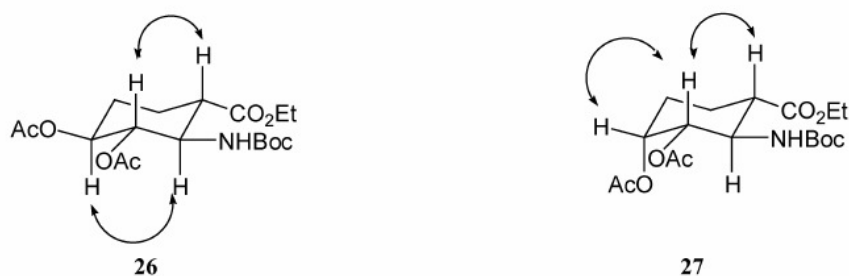


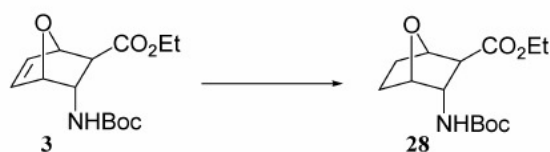
Fig. 4 Selected NOESY correlations for **26** and **27**.

Repeating the reductive epoxide opening reaction on epoxide **22** gave the *trans-trans-cis*-dihydroxycyclohexyl β-amino acid derivative **27** [19] in 84 % yield, Scheme 4. The *trans-trans-cis* configuration of **27** was also confirmed by NOESY experiments, Fig. 4. Tests for antibiotic activity against gram positive bacteria for all the prepared compounds are ongoing, and the results will be reported in due course.

Pd-CATALYZED TRANSFER HYDROGENATION OF ALKENES IN THE PRESENCE OF ORGANIC ACIDS

While studying the Pd-catalyzed reductive ring-opening reactions of oxabicyclic alkenes in the presence of Zn powder and benzoic acid as described by Cheng [21], we noted that oxanorbornene adduct **3** exclusively led to its saturated derivative **28**, Scheme 5. The reaction was also successful with other substrates such as cinnamic acid and the natural product eugenol. Control experiments indicated that no reaction occurred in the absence of Zn powder, benzoic acid, or Pd(II) chloride [22]. Other organic acids such as acetic acid, formic acid, and ammonium acetate were found to be equally effective as hydrogen donors. The Pd-catalyzed transfer hydrogenation reaction was also extended to prochiral alkenes and optical pure chiral organic acids. For the reduction of α-methyl cinnamic acid, high enantioselectivities (90–99 % ee) were observed when (+)-mandelic and (+)-tartaric acids were used as hydrogen donors and acetonitrile as the solvent [23].

The catalytic transfer hydrogenation reaction described above is thought to proceed by first reduction of Pd(II) to Pd(0) by the Zn powder. Oxidative addition of the organic acid to the Pd(0) affords a Pd(II) hydride species which then acts as the reducing agent [22].



Scheme 5 Reagents: cat. PdCl₂, Zn, organic acid, acetonitrile, 25 °C, 80–96 %.

CONCLUSION

The stereoselective syntheses described in this paper reveals the power of the oxanorbornene adduct derived from the Diels–Alder reaction of ethyl (*E*)-3-nitroacrylate and furan in the preparation of the putative structure of (–)-oryzoxymycin and hydroxylated cyclohexyl β-amino acid derivatives. The syntheses of the cyclohexyl β-amino acid derivatives are distinguished by the use of substrate-stereocontrolled processes to secure stereochemical relationships around the cyclohexane rings. The enforced reliance of the syntheses on simple classic reactions at ambient conditions is another positive attribute of the strategy worth mentioning. A facile Pd-catalyzed transfer hydrogenation procedure in the presence of organic acids has also been described.

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