Synthesis of Alaremycin

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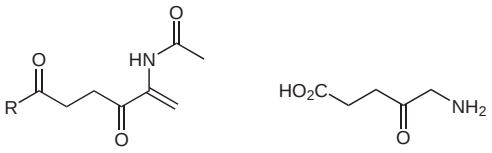
Abstract: Methyl 5-azido-4-oxohexanoate was synthesized from 5-hexenoic acid in six steps and converted to the title compound by $NaReO_4$- and CF_3SO_3H-catalyzed reaction in Ac_2O/CCl_4 followed by hydrolysis of the methyl ester moiety.

Key words: amides, antibiotics, azides, palladium, rhenium

One of the authors (M.W.) has developed a new screening system for finding inhibitors of bacterial chromosome partitioning.[1] Using this assay system, alaremycin (**1**) was isolated from the culture broth of an actinomycete strain, and the producing strain was identified as *Streptomyces sp.* A012304.[2] While structurally similar primocarcin (**2**), known as an antitumor,[3] shows low antibacterial activity, **1** does antibacterial effect on *E. coli* cells. Furthermore, the effect is enhanced by 5-aminolevulinic acid (ALA, **3**), a precursor of heme biosynthesis, that is known to act as a prooxidant both in vivo and in vitro.[4] The structural similarity between compounds **1**–**3** (Figure 1) suggests that alaremycin (**1**) exerts its property through a mechanistic pathway similar to those of **2** and **3**. To continue investigation of **1** along this line we needed a fairly large amount of **1** by organic synthesis since the biological technology to obtain **1** seemed difficult. Herein, we report a synthesis of alaremycin (**1**).

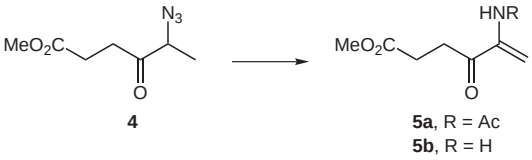


alaremycin (**1**), R = OH
primocarcin (**2**), R = NH_2

5-aminolevulinic acid (ALA) (**3**)

Figure 1

The literature survey revealed that the α-(acetamido)vinyl ketone moiety of primocarcin (**2**) as well as the analogous amides was constructed by aldol reaction of the α-acetamido-β-keto ester with formaldehyde and subsequent decarboxylative elimination of the hydroxyl group.[5] In an attempted sequence of reactions leading to **2**, alaremycin was also synthesized. However, the key step suffers from low yields, and the starting material could be traced back incompletely. Thus, we felt it necessary to exploit a synthesis of **1** from a compound that is available easily. We chose the α-(azido)ethyl ketone **4** as a precursor of the α-(acetamido)vinyl ketone (**5a**) or the amino derivative **5b** (Equation 1) on the basis of our previous work[6] as well as those reported in the literature.[7,8]



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5a, R = Ac
5b, R = H

Equation 1

A sequence of reactions to produce **4** and the conversion of **4** to **1** is shown in Scheme 1.[9] Simple bromination of 5-hexenoic acid (**6**)[10,11] with Br_2 followed by reaction with hot KOH in H_2O[12,13] produced methyl acetylene **7** in 80% yield. The possible intermediates, i.e., terminal acetylene and/or vinyl bromide(s), were not detected by ^1H NMR spectroscopy. Palladium-catalyzed reaction of **7** was carried out with $PdCl_2(PhCN)_2$ in refluxing MeCN according to the literature procedure[14] to produce enol γ-lactone **8**[15] and a small amount of the δ-lactone **10** (Figure 2). Being different from the literature, the reaction proceeded stereoselectively in our case. Bromination of lactone **8** followed by treatment with Et_3N in MeOH afforded the α-bromo ketone **9**, which was converted to the key intermediate **4** with NaN_3 in DMF in good yield.

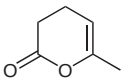

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Figure 2

In order to obtain (α-acetamido)vinyl ketone (**5a**), we first examined the following two-step conversion on the basis of the previous success with similar compounds by us[6] and others:[7] (1) base-catalyzed reaction to afford (α-amino)vinyl ketone (**5b**); (2) acetylation of **5b**. Thus, azide **4** was exposed to ROLi/ROH (R = Me, Et, *t*-Bu) at room temperature in CCl_4 or in ROH, and the crude product was treated with AcCl in the presence of Et_3N. However, a trace amount of **5a** was detected by TLC in the product

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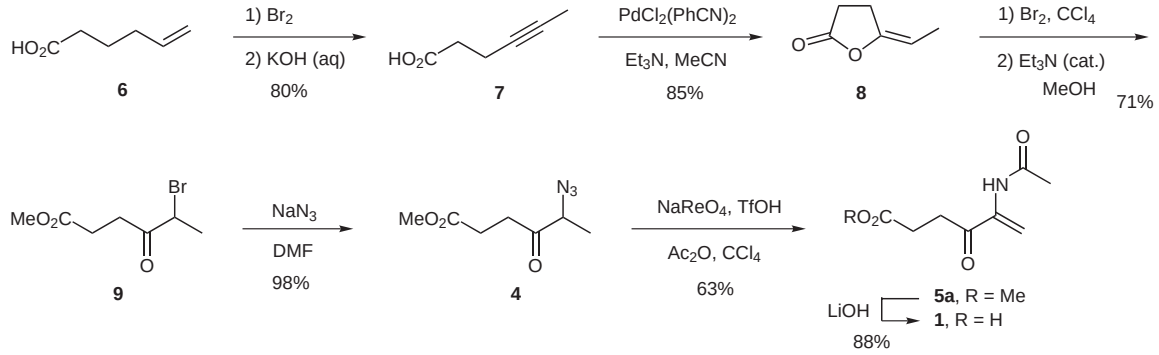

Scheme 1 Synthesis of alaremycin (**1**)

mass. Unidentified products were also obtained when submitted to RONa in ROH (R = Me, Et), the conditions previously applied by us to an α-azido-γ-lactone (R = Et).[6] On the other hand, reaction of **4** with Ac_2O at 120 °C in AcOH for 6 hours afforded **5a** directly, but only in 25% yield with unreacted **4** and other by-products, while longer reaction resulted in formation of the by-products in larger quantity. We then examined $NaReO_4$-catalyzed reaction in Ac_2O under acidic conditions.[8] Thus, reaction of **4** in Ac_2O in the presence of $NaReO_4$ (1 mol%) and CF_3SO_3H (1 mol%) at 50 °C afforded **1** in 46% yield. To improve the yield, the reaction was repeated in Ac_2O and a co-solvent such as Et_2O, CH_2Cl_2, THF, MeCN, CCl_4 in a 1:1 proportion at 50 °C for 8 hours. Among the co-solvents, CCl_4 was found to be most effective to afford **5a** in 63%.

Finally, hydrolysis of methyl ester **5a** with LiOH in aqueous MeOH produced **1** in 88% yield. The ^1H NMR and ^{13}C NMR spectra in DMSO-d_6 and in MeOH-d_4, respectively, were identical with those reported.[2]

In summary, we have established synthesis of alaremycin (**1**) starting with 5-hexenoic acid (**6**) in 26% total yield in 8 steps. The quantity of **1** synthesized is enough for biological study, and the following procedure will be helpful to repeat the synthesis for further study.

Brief Procedure Leading to Compound 1

To a solution of acid **6** (3.70 g, 32.4 mmol) in CH_2Cl_2 (15 mL) at –60 °C was added Br_2 (1.67 mL, 32.4 mmol) in CH_2Cl_2 (5 mL) dropwise. After 1.5 h at –60 °C to –50 °C, the solution was poured into sat. $Na_2S_2O_3$ with vigorous stirring. The mixture was extracted with CH_2Cl_2 to afford crude bromine adduct (8.53 g), which was used for the next reaction without further purification.

A mixture of the above compound and KOH (17.47 g, 311.4 mmol) in H_2O (30 mL) was refluxed for 10 h, diluted with H_2O (30 mL), and acidified to pH 2 by addition of 12 N HCl. The mixture was extracted with Et_2O three times to afford crude **7**, which was purified by distillation (3.02 g, 83%); bp 130 °C (10 Torr).

A solution of acid **7** (1.60 g, 14.3 mmol), $PdCl_2(PhCN)_2$ (54 mg, 0.14 mmol), and Et_3N (60 μL, 0.43 mmol) in MeCN (25 mL) was refluxed for 5 h and concentrated. The residue was purified by chromatography (hexane–EtOAc) to afford enol lactone **8** (1.36 g, 85%).

To an ice-cold solution of lactone **8** (850 mg, 7.58 mmol) in CCl_4 (10 mL) was added Br_2 (0.39 mL, 7.57 mmol) in CCl_4 (2 mL) dropwise. After 30 min at 0 °C, the solution was concentrated to give a residue, which was dissolved in MeOH (10 mL) containing a few drops of Et_3N. After 1 h at r.t., the solution was concentrated, and the crude product was purified by chromatography (hexane–EtOAc) to furnish α-bromo ketone **9** (1.20 g, 71%). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.77 (d, *J* = 7.0 Hz, 3 H), 2.49–2.78 (m, 2 H), 2.91 (ddd, *J* = 18.0, 7.5, 6.0 Hz, 1 H), 3.14 (ddd, *J* = 18.0, 6.5, 6.0 Hz, 1 H), 3.69 (s, 3 H), 4.49 (q, *J* = 7 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.2, 28.4, 33.6, 47.5, 52.0, 172.8, 202.9.

A mixture of α-bromo ketone **9** (1.20 g, 5.38 mmol) and NaN_3 (699 mg, 10.8 mmol) in DMF (5 mL) was stirred at r.t. for 1 h, and diluted with H_2O. The resulting mixture was extracted with Et_2O three times to give a residue, which was purified by chromatography (hexane–EtOAc) to furnish azide **4** (976 mg, 98%). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.41 (d, *J* = 7.0 Hz, 3 H), 2.56–2.62 (m, 2 H), 2.77–2.83 (m, 2 H), 3.63 (s, 3 H), 3.97 (q, *J* = 7.0 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 15.8, 27.6, 33.7, 51.9, 63.2, 172.6, 205.8.

To a solution of $NaReO_4$ (1 mg, 0.0037 mmol) and CF_3SO_3H (1 μL, 0.011 mmol) in Ac_2O (0.5 mL) and CCl_4 (0.5 mL) was added azide **4** (50 mg, 0.27 mmol). The mixture was heated to 50 °C for 8 h and concentrated to produce a residue, which was purified by chromatography (hexane–EtOAc) to afford amide **5a** (34 mg, 63%). ^1H NMR (300 MHz, $CDCl_3$): δ = 2.12 (s, 3 H), 2.68 (t, *J* = 6.5 Hz, 2 H), 3.11 (t, *J* = 6.5 Hz, 2 H), 3.70 (s, 3 H), 5.84 (t, *J* = 1 Hz, 1 H), 6.93 (d, *J* = 1 Hz, 1 H), 7.99 (br s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.8, 28.1, 30.8, 52.0, 109.5, 137.7, 169.0, 172.7, 194.9.

To a solution of amide **5a** (180 mg, 0.904 mmol) in MeOH (1.1 mL) and H_2O (1.1 mL) was added 1 N LiOH (1.2 mL, 1.2 mmol). The mixture was stirred at 0 °C for 30 min, acidified to pH 3–4 with 10% tartaric acid, and extracted with EtOAc three times. The crude acid **1** thus obtained was purified by chromatography (hexane–EtOAc) to afford acid **1** (148 mg, 88%); mp 132–135 °C (recrystallized from MeOH–EtOAc–hexane).

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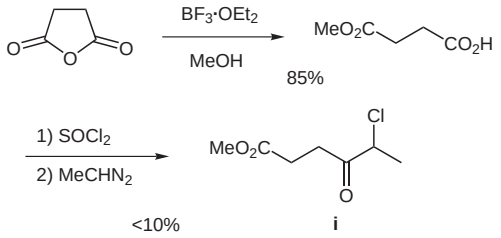
(9) Another sequence shown below produced (α-chloro)ethyl ketone **i** in low yield (Scheme 2).



Scheme 2

(10) Acid **6** is commercially available, but was conveniently prepared from cyclohexanone in 67% yield by oxidative ring cleavage with H_2O_2, $FeSO_4$, and $CuSO_4$.[11]
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