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FORMAL SYNTHESIS OF ANISOMYCIN

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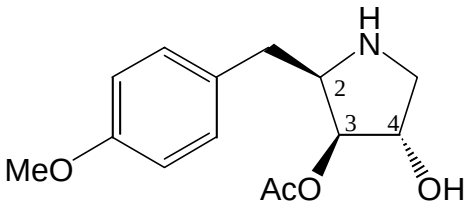
Abstract—A straightforward formal synthesis of anisomycin has been established starting from *trans*-4-hydroxyproline.

1. INTRODUCTION

The antibiotic anisomycin was first isolated from the fermentation broths of *Streptomyces griseolus* and *Streptomyces roseochromogenes* by Sobin and Tanner at Pfizer, Inc. in 1954.[1] Subsequently, it was found in two related strains, *Streptomyces* sp. No. 638 and *SA* 3097, by groups in Japan.[2-3] This alkaloid's absolute stereochemistry has been firmly established as 2*R*,3*S*,4*S* by correlation of chemical structure with L-tyrosine,[4] and its relative stereochemistry was elucidated by chemical, spectroscopic and X-Ray crystallographic means.[5-7] Anisomycin is a valuable tool in molecular biology. It possesses strong and selective activity against pathogenic protozoa and several strains of fungi[8-9] and has been used successfully in the clinics for the treatment of trichomonas vaginitis[10-11] and amoebic dysentery.[12]



anisomycin (**1**)

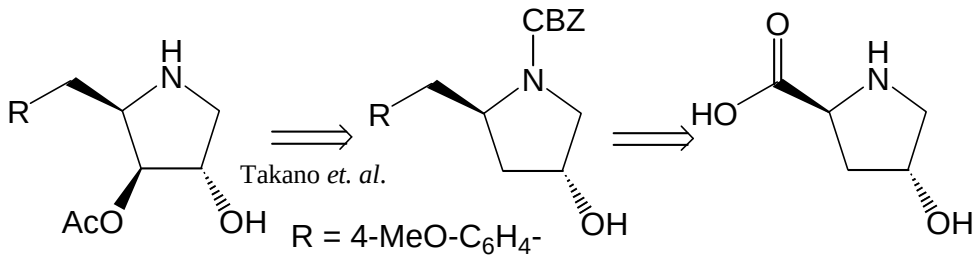
Recently, Kameyama, *et al.*, reported that anisomycin and its derivatives (3097-B1, 3097-B2 and 3097-C2) were employed as fungicides and to inhibit other pathogenic fungi in plants.[3] Its structure-activity relationship has been reported.[3] Anisomycin also found additional uses to inhibit effectively peptide bond formation on eukaryotic ribosomes.[14] To date, the preparation of anisomycin has almost invariably relied on a pool of chiral starting materials and has often proceeded in modest yield.[15-16] Therefore, there is a continuing need for other chiral materials to synthesize anisomycin. Here we report a straightforward synthesis of anisomycin using *trans*-4-hydroxyproline as a new chiral material.

2. RESULTS AND DISCUSSION

2.1 Retrosynthetic analysis of anisomycin (1)

Our approach to target molecule (**1**) is shown in Scheme 1. In 1989, Takano and his co-workers[16] described a synthesis of anisomycin (**1**) in which compound (**2**) was the important intermediate. To increase total yield and to shorten the steps, we developed a straightforward result using *trans*-4-hydroxyproline (**3**), a commercial available starting material.

Scheme 1. Retrosynthetic analysis of anisomycin (**1**)



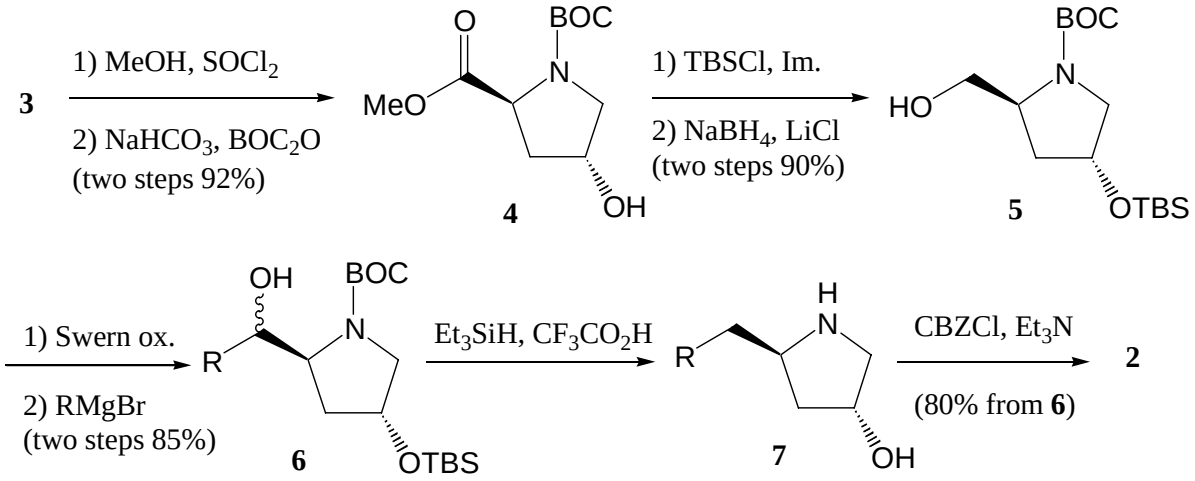
anisomycin (**1**) **2** **3**

2.2. Formal synthesis of anisomycin (1)

The formal synthesis of anisomycin (**1**), as shown in Scheme 2, uses a facile strategy from *trans*-hydroxyproline (**3**). *N*-BOC-proline methyl ester (**4**) was readily obtained from **3** in two-step reactions of methylation with thionyl chloride and methanol in an ice bath and *t*-Boc-protection with di-*t*-butyl dicarbonate and sodium bicarbonate in a mixture of water and dioxane at room temperature. *N*-BOC-proline methyl ester (**4**) was silylated with *t*-butyldimethylsilyl chloride (TBSCl) and imidazole to give the resulting *O*-TBS-proline ester. Reduction of *O*-TBS-proline ester with sodium borohydride

($NaBH_4$) in the presence of lithium chloride[17] furnished alcohol (**5**). **5** was transformed into the aldehyde by Swern oxidation.[18] Chain extension with the 4-methoxyphenyl anion derived by Grignard reaction of 4-bromoanisole and magnesium in anhydrous tetrahydrofuran provided the alcohol (**6**) as a mixture in the ratio of 1:1. Exposure of **6** to triethylsilane in trifluoroacetic acid caused simultaneous desilylation, debutoxycarbonylation, and reduction of the benzylic hydroxyl group, providing the pyrrolidine (**7**), which was *N*-protected with benzyloxycarbonyl chloride (CBZCl) to give the hydroxypyrrolidine (**2**).

Scheme 2. Formal synthesis of anisomycin (**1**)



3. CONCLUSION

In summary, the straightforward formal synthesis of anisomycin (**1**) from *trans*-4-hydroxyproline (**3**) to alcohol (**2**) in eight steps provided 56% overall yield.

4. EXPERIMENTAL

4.1. General. Methylene chloride and tetrahydrofuran were distilled prior to use. All other reagents and solvents were obtained from commercial sources and used without further purification. Reactions were routinely carried out under an atmosphere of dry nitrogen with magnetic stirring. Products in organic solvents were dried with anhydrous magnesium sulfate before concentration *in vacuo*. All reported melting temperatures were uncorrected.

4.2. (2*S*,4*R*)-1-*t*-Butoxycarbonyl-4-hydroxy-2-methyoxycarbonylpyrrolidine (4)[19]

Thionyl chloride (2.50 g, 21.0 mmol) was added to a stirred solution of **3** (1.31 g, 10.0 mmol) in methanol

(20 mL) at –78 °C for 10 min. The mixture was stirred in an ice bath for 30 min then at rt for 30 min, followed by reflux for 3 h. Concentration *in vacuo* followed by azotropic removal of water using benzene gave methyl 4-hydroxyproline-HCl (1.81 g, 100%). Sodium bicarbonate (1.68 g, 20.0 mmol) and di-*t*-butyl dicarbonate (2.4 g, 11.0 mmol) were added to a solution of the resulting product (1.81 g) in THF (20 mL) and water (20 mL) in an ice bath. After stirring at the same temperature for 2 h, the mixture was concentrated *in vacuo*. Ethyl acetate (40 mL) was added to the residue, and the solution was washed with 0.1N HCl, saturated sodium bicarbonate solution and brine, dried, filtered, then concentrated *in vacuo*. The residue was purified by column chromatography (hexane/ethyl acetate=2/1) to yield **4** (2.25 g, 92%): viscous oil; $[\alpha]_D$ –57.4 ° (*c* 1.0, CH_3OH), [lit.,[19] $[\alpha]_D$ –60.2 ° (*c* 1.0, CH_3OH)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.46-4.35 (m, 2H), 3.70 (s, 3H), 3.63-3.44 (m, 2H), 2.30-2.25 (m, 1H), 2.06-1.97 (m, 2H), 1.43 (br s, 9H); Anal. Calcd for $C_{11}H_{19}NO_5$ C, 53.87; H, 7.81. Found C, 53.68; H, 7.70.

4.3. (2*S*,4*R*)-1-*t*-Butoxycarbonyl-2-hydroxymethyl-4-[*t*-butyldimethylsilyloxy]pyrrolidine (5)[20]

tert-Butyldimethylsilyl chloride (1.51 g, 10.0 mmol) and imidazole (1.0 g, 14.7 mmol) were added to a solution of **4** (2.2 g, 9.0 mmol) in DMF (10 mL) at rt. After stirring at the same temperature for 2 h, the mixture was concentrated *in vacuo*. Ethyl acetate (40 mL) was added to the residue, and the solution was washed with 0.1N HCl, saturated sodium bicarbonate solution and brine, dried, filtered, then concentrated *in vacuo* to give the crude product (3.0 g, 93%). Without further purification, lithium chloride (1.22 g, 28.8 mmol), and sodium borohydride (1.09 g, 28.8 mmol) were added to a solution of the crude product (2.9 g) in THF (20 mL) and methanol (20 mL) at rt. After stirring at the same temperature for 12 h, the mixture was concentrated *in vacuo*. Ethyl acetate (40 mL) was added to the residue, and the solution was washed with 0.1N HCl, saturated sodium bicarbonate solution and brine, dried, filtered, then concentrated *in vacuo*. The residue was purified by column chromatography (hexane/ethyl acetate=2/1) to yield **5** (2.6 g, 97%): oil; $[\alpha]_D$ –51.2 ° (*c* 1.0, CH_3OH); EI-MS: $C_{16}H_{33}NO_4Si$ m/z (%) = 331; HRMS (EI, M^+) calcd for $C_{16}H_{33}NO_4Si$ 331.2179, found 331.2180; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28-4.24 (m, 1H), 4.12-4.05 (m, 1H), 3.67 (dd, *J* = 2.5, 11.3 Hz, 1H), 3.52 (dd, *J* = 7.3, 11.3 Hz, 1H), 3.42 (dt, *J* = 2.0, 11.6 Hz, 1H), 3.31 (dd, *J* = 4.1, 11.6 Hz, 1H), 3.25 (br s, 1H), 1.96-1.90 (m, 1H), 1.65-1.58 (m, 1H), 1.45 (s, 9H), 0.84 (s, 9H), 0.36 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 80.38, 69.80, 66.96, 58.92, 55.99, 38.00, 28.42 (6x),

25.67, 17.92, -4.87 (2x); Anal. Calcd for $C_{16}H_{33}NO_4Si$ C, 57.97; H, 10.03. Found C, 57.66; H, 9.79.

4.4. (2*S*,4*R*)-1-*t*-Butoxycarbonyl-2-{1-hydroxy-1-[4-methyoxyphenylmethyl]}-4-[*t*-butyldimethyl-silyloxy]pyrrolidine (6)

A solution of oxalyl chloride (200 mg, 1.56 mmol) in CH_2Cl_2 (10 mL) was mixed with dimethyl sulfoxide (200 mg, 2.56 mmol) together carefully at –78 ^oC. The solution was warmed to –40 ^oC for 5 min and recooled to –78 ^oC, and then a solution of **5** (400 mg 1.21 mmol) in CH_2Cl_2 (5 mL) was added dropwise for 20 min followed by excess triethylamine (4 mL, 28.5 mmol) for 30 min. The reaction mixture was warmed to rt and poured into saturated aqueous ammonium chloride solution (2 mL), and concentrated. The residue was diluted with water (15 mL) and extracted with ethyl acetate (3 x 30 mL). The organic layer was washed with brine and water, dried, filtered and concentrated to produce crude product (362 mg). Without further purification, 4-methoxyphenylmagnesium bromide (1M in THF, 1.4 mL, 1.4 mmol) was added to a solution of the resulting crude aldehyde (362 mg, 1.1 mmol) in THF (10 mL) –78 ^oC. After stirring at the same temperature for 3 h, the mixture was concentrated *in vacuo*. Ethyl acetate (20 mL) was added to the residue, and the solution was washed with 0.1N HCl, saturated sodium bicarbonate solution and brine, dried, filtered, then concentrated *in vacuo*. The residue was purified by column chromatography (hexane/ethyl acetate=2/1) to yield **6** (445 mg, 93%): viscous oil; EI-MS: $C_{23}H_{39}NO_5Si$ m/z (%) = 437; HRMS (EI, M^+) calcd for $C_{23}H_{39}NO_5Si$ 437.2598, found 437.2592; ^1H NMR (400 MHz, $CDCl_3$) δ 7.25-7.20 (m, 2H), 6.88-6.75 (m, 2H), 4.81-4.72 (m, 1H), 4.50-4.20 (m, 3H), 3.76 (s, 3H), 3.52-3.34 (m, 1H), 3.30-3.15 (m, 1H), 1.60-1.94 (m, 2H), 1.37 (s, 9H), 0.92 (s, 9H), 0.05 (s, 6H).

4.5. (2*S*,4*R*)-1-Benzyloxycarbonyl-2-[4-methyoxyphenylmethyl]-4-hydroxypyrrolidine (2)[16]

A mixture of **6** (250 mg, 0.57 mmol), trifluoroacetic acid (1 mL), and triethylsilane (1.01 mL, 0.63 mmol) was stirred at rt for 15 h, and then the mixture was concentrated *in vacuo*. Ethyl acetate (20 mL) was added to the residue, and the solution was washed with 0.1N HCl, saturated sodium bicarbonate solution and brine, dried, filtered, then concentrated *in vacuo* to give crude **7** (107 mg). Without further purification, a mixture of triethylamine (102 mg, 1.0 mmol) and benzyloxycarbonyl chloride (0.12 mL, 0.86 mmol) was added to a solution of the resulting **7** (107 mg) in THF (10 mL) in an ice bath. After stirring at the same temperature for 3 h, the mixture was concentrated *in vacuo*. Ethyl acetate (20 mL)

was added to the residue, and the solution was washed with 0.1N HCl, saturated sodium bicarbonate solution and brine, dried, filtered, then concentrated *in vacuo*. The residue was purified by column chromatography (hexane/ethyl acetate=1/1) to yield **2** (153 mg, 88%): oil; $[\alpha]_D$ –78.2 ^{o} (*c* 1.12, $CHCl_3$); EI-MS: $C_{20}H_{23}NO_4$ m/z (%) = 91 (100), 121 (23), 176 (46), 220 (43), 341 (4); HRMS (EI, M^+) calcd for $C_{20}H_{23}NO_4$ 341.1627, found 341.1616; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41-7.31 (m, 5H), 7.00 (br s, 2H), 6.79 (br d, *J* = 7.5 Hz, 2H), 5.25-5.16 (m, 2H), 4.23-4.20 (m, 2H), 3.78 (s, 3H), 3.58-3.48 (m, 1H), 3.30 (dd, *J* = 4.5, 12.0 Hz, 1H), 3.12-2.97 (m, 1H), 2.76-2.69 (m, 1H), 1.89-1.86 (m, 2H), 1.83 (br s, 1H); Anal. Calcd for $C_{20}H_{23}NO_4$ C, 70.36; H, 6.79. Found C, 70.61; H, 6.88.

The retention time of alcohol (**2**) was 19.67 min by an RP-HPLC analysis. Water and acetonitrile (H_2O/MeCN=5~95%, 0~30 min both containing 0.1% TFA) were used as the solvent system at a flow rate of 1.0 $mlmin^{-1}$ on a Nucleosil C18 column (4.6 x 250 mm) at rt.

5. ACKNOWLEDGEMENT

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