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Studies Towards the Synthesis of Radiolabeled R106-1(LY295337)

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SUMMARY

A unique semisynthetic pathway has been used as a route to acquire radiolabeled material of a complex natural product, R106. The retro-aldol reaction of R106-1 gave a key intermediate R106-sarcosine that was used in a subsequent aldol reaction to incorporate acetone-[2-^{14}C] .

Keywords: Aureobasidins, retro-aldol, C-14, R106, sarcosine

INTRODUCTION

R106-1 (LY295337), **1** is part of the R106 family also known as the aureobasidins. These macrocyclic depsipeptides are a new class of promising fungicidal agents produced by fermentation of the microorganism *Aureobasidium pullulans,* R106.[1,2] R106-1 is one of the most potent factors that exhibits excellent *in vitro* activity against several clinically important fungal pathogens such as *Candida albicans* and *Cryptococcus neoformans.*[3] During the course of our development of R106-1, labeled material was needed for biological probes. We wish to describe our approach to synthesize labeled material by the retro-aldol, aldol alkylation of R106-1. An alternative approach, namely the tritiation of a related fermentation factor R106-4 is also discussed.

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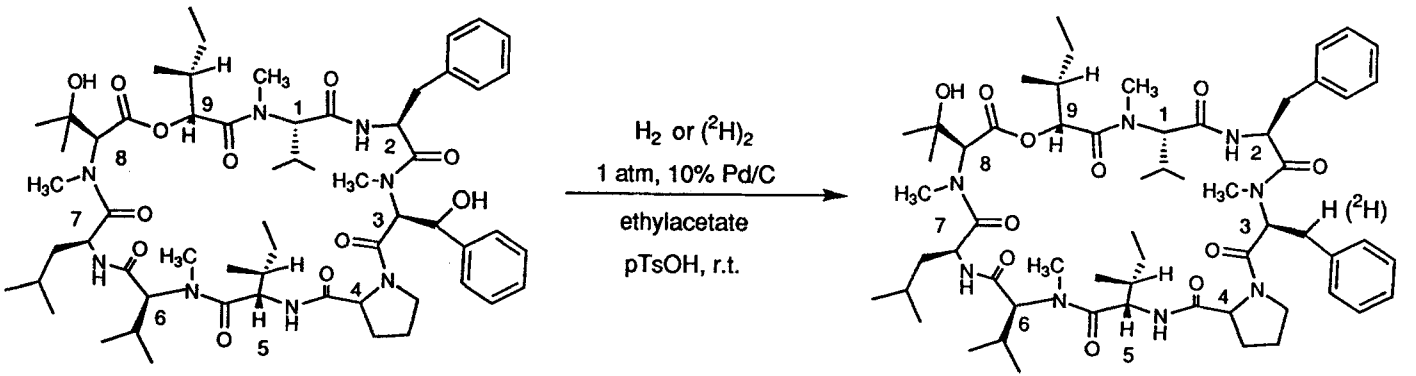
RESULTS AND DISCUSSION

The natural product, **1**, is highly lipophilic and has proven to be a challenge to modify chemically. R106-1 is a cyclic depsipeptide consisting of an octapeptide linked by a 2*R*, 3*R*-2-hydroxy-3-methylpentanoic acid (Hmp) group at residue 9. All amino acids have the *L* configuration. There are few chemical handles available for direct modification with the exception of a hydroxylated *N*-methyl valine residue (HO-MeVal-8). Another closely related factor R106-4 is identical to R106-1 except that residue 4 has an HO-Phe-3.[4] We recognized that selective hydrogenolysis of the hydroxy group might be possible by acid catalyzed tritiation. If successful, this would provide a single synthetic step to tritiated R106-1. R106-4 was subjected to standard hydrogenolysis conditions commonly used on benzylic alcohols. As shown in Scheme 1, the treatment of R106-4 with 10% Pd/C, 10 mole% pTsOH in ethyl acetate under 1 atm of hydrogen was successful in converting R106-4 to R106-1. We were encouraged to find similar results when hydrogen was replaced with deuterium. Unfortunately, all attempts using tritium failed.

The tritiation result prompted us to find an alternative solution by using a synthetic intermediate that was first reported by Takara Shuzo known as R106-sarcosine, **2**.[5] R106-sarcosine was obtained from R106-1 in high yields via a retro-aldol reaction.[6] We successfully alkylated the sarcosine residue on this class of cyclic peptides with a variety of electrophiles including acetone without epimerization of the stereogenic centers.[7] We recognized that this methodology, i.e., aldol condensation of R106-sarcosine with acetone-[2-^{14}C] as shown in Scheme 2, would provide direct access to [^{14}C]-R106 derivatives.

Typically, the aldol reaction consisted of dissolving R106-sarcosine in dry THF treated with LiCl. Excess LDA was used to generate a polylithiated species. Subsequent addition of an equal amount of *n*-butyllithium produced an emerald green colored solution, at which point excess acetone-[2-^{14}C] was added and allowed to stir for several hours before quenching with pH 7 buffer.[8]

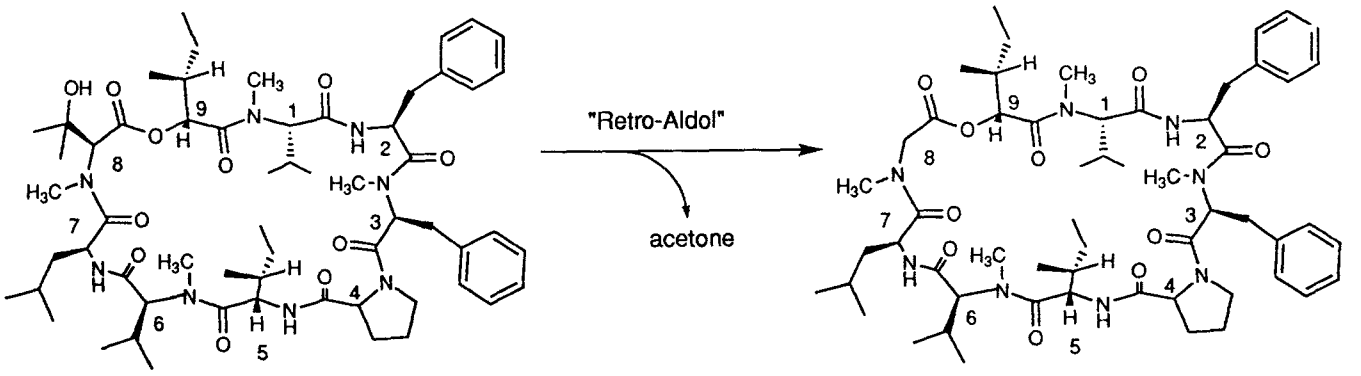
Scheme 1



R106-4

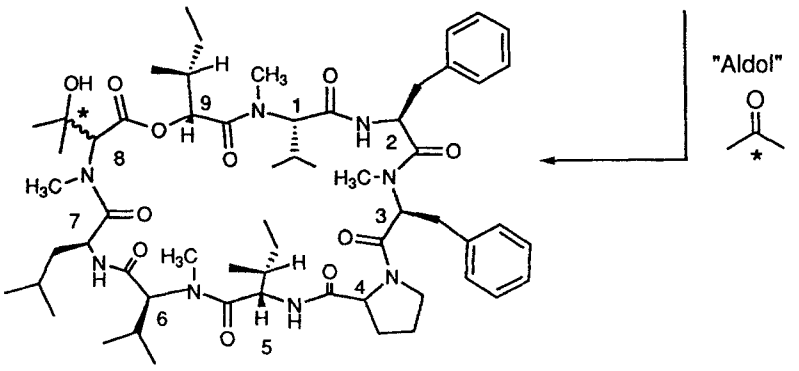
R106-1 or [^{2}H]R106-1

Scheme 2



R106-1(LY295337), **1**

R106-sarcosine, **2**



D/L R106-1, **3**

Determination of Product Ratios. Unlabeled acetone played a unique role in optimizing the reaction conditions since the aldol products were easily compared and analyzed against the natural product standard, R106-1. By C18 reverse phase HPLC, a single peak having an identical retention time as the standard was detected. The diastereomeric ratios were determined by examining proton NMR signals at 5.75 and 6.49 ppm corresponding to 2-C-H Hmp and C_δ(Ar)-H MePhe (residues 9 and 3, respectively). By ^{1}H NMR, the single peak detected by HPLC was a 2:1 mixture of *D* and *L* isomers of R106-1.[9] This mixture had identical spectrum and activity to that of the natural product.[10]

CONCLUSION

We have prepared [^{14}C]-labeled R106 with the desired specific activity and reasonable radiochemical yield. A 2:1 mixture of *D*:*L* isomers of [^{14}C]-R106-1 was obtained from the aldol reaction. For future studies requiring tritium labeled material, this route can be easily modified by utilizing tritiated 2-propanone.

EXPERIMENTAL

General. ^{1}H NMR spectra were determined at 500 MHz. Chemical shifts are reported in parts per million (δ) relative to trimethylsilane as internal standard. Spin multiplicities are reported using the following abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Infared spectra were recorded on a Nicolet 510P FT-IR spectrometer. FAB mass spectra were obtained using a V6 ZAB-2SE mass spectrometer. High-resolution mass spectra were generated for all final products and were consistent with the theoretical empirical formulas. Analytical reverse phase HPLC work was done using the Waters 600E system with Waters Novapak® or μ-bondapak® (C18, 3.9 X 300 mm) columns. Preparative HPLC work was performed with a Waters Prep 2000 system using either a Dynamax 60-A 21.4 x 250 mm C18 column or a Waters C18 Nova-pak® 3x (40mm X 100mm) radial cartridge column. All final products were >90% pure as determined by analytical HPLC and by ^{1}H NMR.

Radiochemical purity (RCP) was assessed by autoradiography employing silica gel (Merck 5715) thin-layer chormatography plates (*n*-butanol:water:acetic acid, 12:5:3) and by HPLC (Apex ODS SYMM 5u 150mm X 4.6mm ID, (40:60 -> 10:90 water/acetonitrile over 30 min gradient; 250 nm UV detection). Specific activity was determined by gravimetric analysis.

Hydrogenolysis of R106-4. To a stirred solution of R106-4 (9 mg) in 7.5 ml reagent grade ethyl acetate was added 28 mg p-toluenesulfonic acid. After 5 minutes of stirring, 4.5 mg 10% Pd/C was added. The solution was degassed under vacuum and loaded with 1 atm of hydrogen (repeated degassing/loading procedure 7 times). The reaction was stirred vigorously at room temperature (25-30°C) and monitored every 24 hours by analytical HPLC [Waters C18 μ-bondapak® (2 ml/min, 10/90 water/acetonitrile, 250 nm)] for four days (70% conversion). The reaction was worked-up on the fourth day by removing the catalyst and reducing the solvent volume to approximately 4 ml under vacuum at room temperature. The concentrated reaction mixture was purified by preparative HPLC (Rainin C18 Dynamax® 60-A, 21.4 x 250 mm, 20 ml/min, 20/80 water/acetonitrile, 250 nm) to give 2 mg of R106-1. HRMS calcd for $C_{60}H_{93}N_8O_{11}$ 1101.6964, found 1101.6980.

D/L R106-1, 3 To a -78°C solution of R106-sarcosine (100 mg, 0.096 mmol) in dry THF (2.0 mL), treated with LiCl (0.032 mg, 0.77 mmol) was added 0.35 M LDA (1.37 mL) dropwise over a 5 minute period. The reaction was allowed to stir for an additional 2 h before the addition of 1.6 M *n*-butyllithium (0.30 mL). After 10 minutes of stirring, dry acetone (0.11 ml, 1.5 mmol) was added dropwise and the reaction was allowed to stir for additional 3 h before quenching with pH 7 buffer. After warming to room temperature, cold 10% HCl was added to the reaction mixture, and the solution was extracted with EtOAc. The organic phase was washed with water, $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated. The residue was purified by C18 reversed phase chromatography (25:75 water/acetonitrile, Waters 3x [40mm X 100mm] Nova-Pak® radial cartridges, 75 ml/min, 250 nm). The first eluate was unreacted

starting material, R106-sarcosine (24 mg). The second eluate gave the desired product **3**, a 2:1 D/L mixture of R106-1(20 mg, 19 %) as a white solid. Analytical HPLC [Waters C18 Novapak® column (30/70 water/acetonitrile, 1.5 mL/min, UV detection 250 nm] retention time, 11.59 min; IR (KBr) ν 3308, 2967, 2935, 2878, 1733, 1648, 1632 cm^{-1}; ^1H NMR (500 MHz) δ 1.18 (d, 7.35 Hz), 1.20 (d, *J* = 7.35 Hz), 1.25 (s), 1.30 (s), 1.32 (s), 1.37 (s), 1.39 (s), 1.42-1.52 (br m), 1.53 (s), 1.62 (s), 1.64-1.85 (m), 1.9 (m), 2.0-2.15 (m), 2.30 (m), 2.46 (s), 2.48 (s), 2.50 (s), 2.60 (s), 2.62 (s), 2.65 (m), 2.90 (s), 2.95 (m), 3.03 (s), 3.05 (m), 3.07 (s), 3.10 (m), 3.14 (s), 3.16 (s), 3.18 (s), 3.27 (m), 3.29 (s), 3.31 (s), 3.3 (s), 3.5 (s), 3.41 (s), 3.55 (m), 3.65 (m), 3.75 (d, *J* = 8.08 Hz), 3.80 (d, *J* = 8.08 Hz), 4.23 (m), 4.5 (m), 4.57 (m), 4.62 (d, *J* = 11.02 Hz), 4.77 (d, *J* = 7.35 Hz), 4.8 (d, *J* = 7.35 Hz), 4.08-5.0 (m), 5.03 (d, *J* = 11.02 Hz), 5.15-5.35 (m), 5.4 (s), 5.44 (m), 5.63 (d, *J* = 0.73 Hz), 5.69 (d, *J* = 0.73 Hz), 5.8 (d, *J* = 0.73 Hz), 6.5 (d, *J* = 7.35 Hz), 6.58 (d, *J* = 7.35 Hz), 6.92 (m), 7.05-7.35 (m), 7.4 (d, *J* = 7.35 Hz), 7.85 (d, *J* = 8.08 Hz), 7.93 (d, *J* = 7.35 Hz), 7.95 (d, *J* = 7.35 Hz), 8.0 (d, *J* = 7.35 Hz), 8.75 (d, *J* = 7.35 Hz), 8.83 (d, *J* = 7.35 Hz), 8.85 (d, *J* = 7.35 Hz); exact mass calcd for $C_{60}H_{93}N_8O_{11}$ 1101.6964, found 1101.6970.

D/L R106-1-[^{14}C]^{11}. To a -78°C solution of R106-sarcosine (447 mg, 0.428 mmol) in dry THF (4.0 mL), treated with LiCl (143 mg) was added 0.35 *M* LDA (1.8 mmol) dropwise over a 5 minute period. The reaction was allowed to stir for an additional 2 h before the addition of 1.6 *M* *n*-butyllithium (0.30 mL). After 10 minutes of stirring, acetone-[2-^{14}C] (80 mCi @ 58 mCi/mmol, 1.38 mmol) was added *via* syringe and the reaction was allowed to stir for additional 2 h before quenching with pH 7 buffer. After warming to room temperature, the solution was extracted with EtOAc and the combined organics were washed with 10% HCl and brine. The solvent was removed by rotary evaporation. The residue was redissoved in CH_2Cl_2, dried over Na_2SO_4, and filtered. The solvent was removed and the residue was purified by preparative C18 reverse phase chromatography with a Dynamax® ODS II column (30:70 -> 80:20 water/acetonitrile over 30 min gradient; 250 nm UV detection) to give a 2:1 *D/L* mixture of **3** as a white solid with a radiochemical yield of 10.4% (8.4 mCi). The

specific activity was diluted to ~ 25 mCi/mmol by the addition of 209.2 mg of carrier LY295337. The material was pumped to a constant weight in a dessicator to obtain 371.4 mg with a specific activity of 22.3 μCi/mg (8.28 mCi). The radiochemical purity was ≥ 98% in all systems examined.

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