nitrogen. The residual solid was then dissolved in 1 mL of 0.2 N TFA in CH_3CN–H_2O (1:1) and heated for 10 min at 80 °C. The solution was concentrated to approximately half volume. The (phenylthio)hydantoin (PTH) of the N-terminal amino acid was separated by Et_2O extraction (3 × 0.5 mL) and concentrated for TLC and MS analyses. The aqueous layer containing the degraded peptide was evaporated to dryness under a stream of nitrogen and then subjected to another cycle of Edman degradation.

Scaled-Up Edman Degradation of Lysobactin To Form Desleucyllysobactin (17). PhNCS (5 mL) was added to lysobactin·2TFA (326.7 mg) dissolved in C_5H_5N–H_2O, 1:1 (30 mL). The mixture was allowed to stand at 37 °C under nitrogen for 1 h with occasional swirling. Toluene extraction (3 × 10 mL) removed the excess reagent (C_5H_5N–H_2O, 1:1, was added as necessary to maintain a biphasic extraction). The aqueous residue was concentrated and lyophilized to yield the (phenylthio)-carbamyl derivative as a white solid. TFA (3 mL) was added to the solid, and the solution was heated at 40 °C for 15 min under a nitrogen atmosphere. After being chilled to 0 °C, the reaction mixture was diluted with Et_2O (0 °C) to precipitate desleucyllysobactin. The cleaved amino acid derivative was removed by Et_2O trituration (2 × 10 mL), and the residual peptide was vacuum dried to yield **17** as a white solid: FABMS (+ ion) m/z 1163; FABMS (− ion) m/z 1161. Anal. Calcd for $C_{52}H_{86}N_{14}O_{16}$·2TFA·H_2O: C, 45.38; H, 6.67; N, 13.24; F, 7.7. Found: C, 45.73; H, 6.34; N, 13.43; F, 6.4.

Acylation of Desleucyllysobactin with *N*-Carboxy Anhydrides. General Method (Scheme VI). All *N*-carboxy amino acid anhydrides (NCA's) were prepared according to ref 30. The details given below for synthesis of **19** also apply to **18**. Desleucyllysobactin (800 mg) was dissolved in 50 mL of DMF containing triethylamine (250 μL), diluted with 150 mL of solvent mix A (THF–EtOAc–DMF, 12:4:1), and chilled to −65 °C. The NCA of D-alanine (115.7 mg) was dissolved in 50 mL of solvent mix A via sonication and was slowly added to the peptide solution under a nitrogen atmosphere at −65 °C with rapid stirring. After the solution was mixed for 10 min, 25 mL of 1 M HOAc (0 °C) was added with stirring. The reaction mixture was concentrated to an oily residue in vacuo, from which the Et_2O-insoluble material was chromatographed on a column of MCI Gel CHP20P resin (5 × 42 cm), eluting with a 4-L linear gradient from 0.1% TFA–H_2O to 0.1% TFA in CH_3CN–H_2O (4:1) at a flow rate of 4 mL/min. The bioactive fractions were pooled on the basis of TLC appearance and lyophilized to give **18** as a white solid: mp 206–212 °C; FABMS (+ ion) m/z 1235; FABMS (− ion) m/z 1223.

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A Synthesis of (−)-Talaromycin A

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The total synthesis of (−)-talaromycin A utilizing the addition of the lithium acetylide of 1-methoxy-1-buten-3-yne to 4(*R*)-ethylvalerolactone is described. The ethylvalerolactone is prepared in seven steps from allyl bromide. The required 1,3-diol of talaromycin A is introduced in a regio- and stereospecific fashion via a tin-mediated radical cyclization.

The talaromycins A and B (**1** and **2**) are toxic metabolites that were isolated by Lynn and co-workers in 1982 from the fungus *Talaromyces stipitatus*.[2] Their structures were assigned primarily through the use of two-dimensional ^1H NMR studies of a mixture of the two toxins. More recently Lynn[3] has isolated and identified the minor components that accompany talaromycins A and B, and these have been assigned the names talaromycins C, D, E,[4] and F (**3**–**6**). It is important to note that the less stable talaromycin A (**1**), which possesses an axially disposed hydroxymethyl, can be quantitatively converted to talaromycin B (**2**) (equatorial hydroxymethyl) by acid catalysis. Several reports of syntheses of talaromycin B have appeared.[5-8,13,14] Most of these approaches take advantage of a key thermodynamically controlled spiroketalization to establish all stereogenic centers on the spiroketal and thus preclude (with the exception of Schreiber's[9] approach) the preparation of talaromycin A. In contrast, the thermodynamically less stable talaromycin A has received less attention.[10-12] We describe here a highly enantioselective synthesis of talaromycin A (**1**), uncontaminated by talaromycin B, which takes advantage of the dioxaspiro-[5.5]undecene system as a template to control the stereochemistry of the 1,3 diol function.

Results and Discussion

Previously we had reported a method for the preparation of spiroketals such as **7** by the addition of the lithium acetylide of 1-methoxy-1-buten-3-yne to lactones. The acetylenic ketones that resulted were exposed to a two-step

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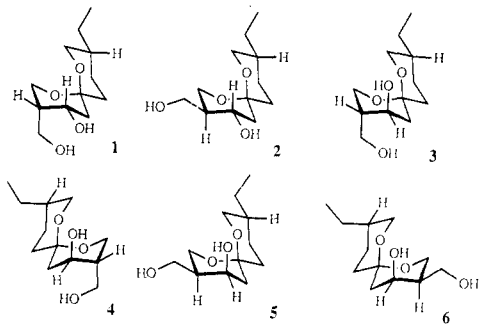
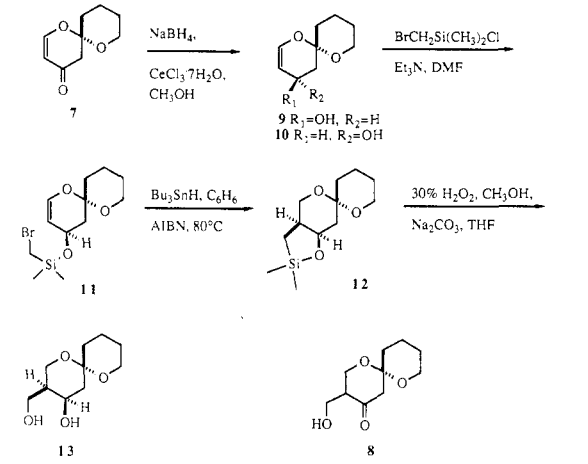


hydrolysis–cyclization sequence.[13] It seemed reasonable that if a method could be found to convert the unsaturated carbonyl of **7** to the required 1,3 diol, a system of this type would be an excellent precursor for the talaromycins. It was initially felt that the unsaturated carbonyl functionality might be reductively alkylated with lithium–ammonia and formaldehyde to produce the α-hydroxymethyl ketone **8**, an intermediate that had been utilized by Smith in his synthesis of the talaromycins. However, this approach failed as did efforts to alkylate the enolate derived from the silyl enol ether (formed from enone **7** by treatment with triethylsilane in the presence of $Rh(PPh_3)_3Cl$ according to the procedure outlined by Magnus).[16]

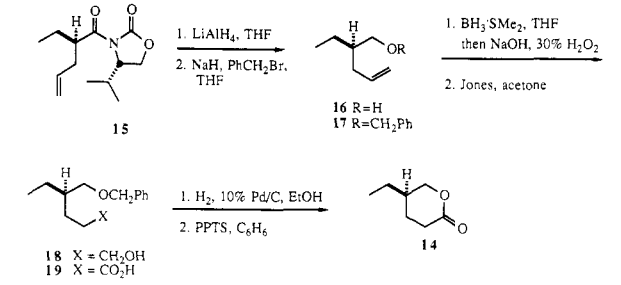
Synthesis of Desethyltalaromycin A. The lack of success of this route as well as the realization that this approach might give good regioselectivity but at best modest stereoselectivity led us to search for alternate solutions. If the carbonyl of **7** could be reduced with reasonable stereocontrol, the resultant allylic hydroxyl might be utilized to direct the delivery of the hydroxymethyl to the adjacent carbon and thus stereoselectively introduce the 1,3-diol. A radical cyclization of the (bromomethyl)dimethylsilyl ether of the allylic alcohol seemed a logical choice (Scheme I). With this strategy in mind, spiroketal **7** was reduced with sodium borohydride–cerium chloride[17] in methanol at 25 °C to give a 2:1 mixture of the moderately unstable equatorial/axial alcohols **9**:**10**, respectively, in 95% overall yield. The equatorial alcohol **9** was converted to its (bromomethyl)dimethylsilyl ether **11** in 87% yield. Subsequent reductive cyclization of **11** by the slow addition of a solution of tributyltin hydride in benzene[18] at 80 °C produced the siloxane **12** in near quantitative yield. Oxidation of **12** with hydrogen peroxide (CH_3OH, Na_2CO_3, THF) provided desethyltalaromycin A (**13**) in 77% yield from **11**. The radical cyclization–reduction sequence could be performed equally efficiently without isolation of the siloxane, thus producing **13** in five efficient operations from valerolactone. That the hydroxymethyl group in **13** was indeed axial was evident from the coupling in the 200-MHz ^1H NMR spectrum ($J_{2,3}$ = 2.8 and 5.5 Hz).

Synthesis of Talaromycin A. To implement this strategy in a synthesis of (−)-talaromycin A, an efficient route to 4(*R*)-4-ethylvalerolactone (**14**)[20] was required. The valine-derived oxazolidinone was alkylated with allyl bromide to give **15** (Scheme II) and reductive removal of the chiral auxiliary provided alcohol **16**[10] in 73% yield.[21]

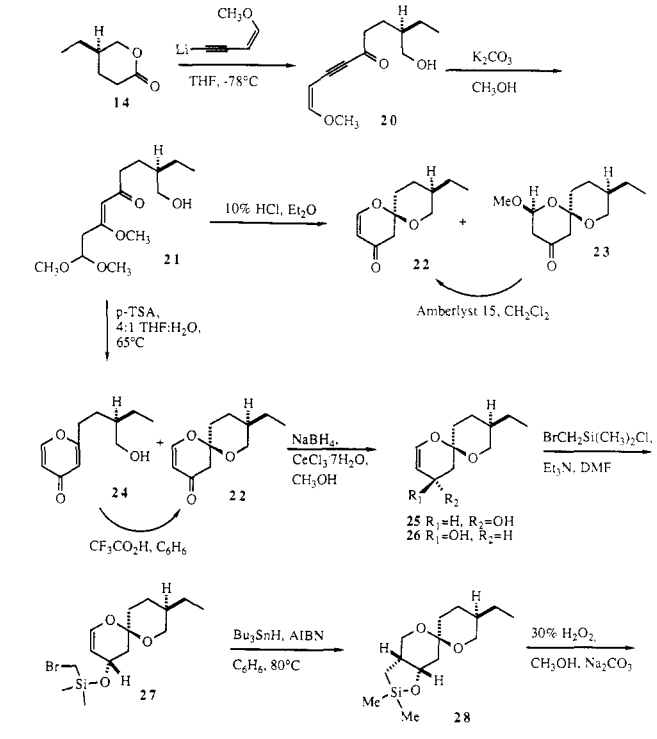
Scheme I



Scheme II



Scheme III



Chiral shift studies showed that this alcohol was produced in >97% ee. Protection of the alcohol as its benzyl ether gave 97% of the ether **17**. Hydroboration of the terminal olefin with borane–methyl sulfide produced the primary alcohol **18**, which was subjected to Jones oxidation to give the acid **19** in 76% overall yield. Hydrogenolysis of the

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benzyl ether (H_2, Pd/C, EtOH, cat. $HClO_4$) followed by exposure to pyridinium *p*-toluenesulfonate in benzene to complete the lactonization provided lactone **14** in near quantitative yield.

Completion of talaromycin A closely paralleled the model study with valerolactone (Scheme III). Addition of the lithium acetylide of 1-methoxy-1-buten-3-yne to **14** in THF at −78 °C produced the keto alcohol **20** in 95% yield. Treatment of **20** with potassium carbonate in methanol produced acetal **21** in 92% yield. While our previously described procedure[15] utilizing 30% aqueous perchloric acid and dichloromethane in a rapidly stirred biphase produced only trace amounts of the spiroketal **22** from acetal **21**, irradiation of the mixture with ultrasound yielded 22% of **22** and 20% of the methanol adduct **23**. The effect of the ultrasonic waves is most likely due to the increase in the efficiency of mixing of the two phases. Despite this improved result, further improvement was realized when an ether solution of the acetal **21** was stirred in contact with 10% HCl. This procedure produced 37% of the spiro enone **22** accompanied by 11% of the β-methoxy spiroketal **23**. Additionally, the byproduct methoxy spiroketal **23** could be converted to the desired **22** by exposure to Amberlyst 15 in dichloromethane at reflux.

While this procedure allowed the preparation of the enone **22** in >40% overall yield from the lactone **14**, still further improvement was sought. After considerable experimentation it was determined that treatment of the acetal **21** with *p*-toluenesulfonic acid in 4:1 tetrahydrofuran/water produced a quantitative yield of a 1:3 mixture of enone **22** and pyrone **24**. Also, further experimentation revealed that the pyrone **24** could be quantitatively converted to a 3.5:1 mixture of the enone **22**/starting pyrone **24** by the action of trifluoroacetic acid in benzene. In this manner the acetal **21** could be transformed into the enone **22** in 80% overall yield after a single recycle of the byproduct pyrone **24**.

Reduction of the carbonyl ($NaBH_4$, $CeCl_3{\cdot}7H_2O$, CH_3OH, 70%)[17] gave a 2.4:1 mixture of allylic alcohols **25** and **26** of which the equatorial **25** was the major isomer. These alcohols were readily separated by flash chromatography and the minor isomer could be recycled via oxidation–reduction. Protection of the alcohol **25** as its (bromomethyl)dimethylsilyl ether gave **27** in excellent yield. Exposure of **27** to tributyltin hydride in benzene produced the siloxane **28**, which was immediately oxidized[19] to provide a 78% overall yield of (−)-talaromycin A (**1**) from alcohol **25**. The synthetic talaromycin A was spectrally identical with that described by Lynn.[2] Since talaromycin A can be quantitatively converted to talaromycin B by acid catalysis,[2,5,9] this also constitutes a total synthesis of talaromycin B.

Experimental Section

General Experimental Procedures. Preparative column chromatography was performed with "silica gel for flash chromatography" manufactured by J. T. Baker Chemical Co. "Dry" solvents were distilled immediately prior to use from an appropriate drying agent. Diethyl ether, benzene, and tetrahydrofuran (THF) were distilled from sodium-benzophenone. Triethylamine, pyridine, dimethylformamide, and dichloromethane were distilled from calcium hydride. Melting points and boiling points are uncorrected. The purity of all title compounds was shown to be ≥95% by elemental analyses or homogeneous by TLC and ^1H NMR.

Alcohols 9 and 10. To a solution of 30 mL of methanol, 0.9480 g (5.58 mmol) of racemic spiro enone **7**,[15] and 2.2855 g (6.13 mmol) of $CeCl_3{\cdot}7H_2O$ was slowly added 0.2331 g (6.13 mmol) of $NaBH_4$. After 5 min the reaction was quenched with saturated NH_4Cl and washed with ether. The organic layer was dried over $MgSO_4$, filtered, concentrated, and chromatographed (silica gel, 25% EtOAc/hexanes) to yield 0.3028 g (32%) of axial alcohol **10** and 0.5958 g (63%) of equatorial alcohol **9** as unstable oils that were homogeneous (≥95%) by ^1H NMR. Axial alcohol **10**: ^1H NMR (200 MHz, $CDCl_3$) δ 3.92 (1 H, m, C*H*OH), 5.13 (1 H, ddd, *J* = 6 Hz, 1.5 Hz, 6 Hz, OCH=C*H*), 6.31 (1 H, d, *J* = 6 Hz, OC*H*=CH). Equatorial alcohol **10**: ^1H NMR (200 MHz, $CDCl_3$) δ 1.45–2.07 (7 H, m, CH_2CH_2 and HCHOHCHH_{ax}), 2.20 (1 H, ddd, *J* = 1.5 Hz, 6 Hz, 13.5 Hz, -HCOHCHH_{eq}), 3.60–3.87 (2 H, m, OCH_2), 4.51 (1 H, br s, *H*COH), 4.91 (1 H, ddd, *J* = 7 Hz, 1.5 Hz, 1.5 Hz, OCH=C*H*), 6.26 (1 H, dd, *J* = 2 Hz, 7 Hz, OC*H*=CH).

Silyl Ether 11. To a solution of 10 mL of DMF, 0.555 g (2.77 mmol) of alcohol **9**, 0.284 g (4.17 mmol) of imidazole, and 0.58 mL (4.17 mmol) of triethylamine was added 0.45 mL (3.33 mmol) of (bromomethyl)dimethylsilyl chloride. After 5 h the reaction was quenched with 10% $NaHCO_3$ and diluted with ether. The organic phase was dried over $MgSO_4$ and concentrated. Chromatography (silica gel, 10% EtOAc/hexanes) gave 0.817 g (87%) of the labile silyl ether **11**, which was homogeneous (≥95%) by ^1H NMR: ^1H NMR (200 MHz, $CDCl_3$) δ 0.25, 0.30 (6 H, 2s, $Si(CH_3)_2$), 1.45–1.92 (7 H, m, $CH_2CH_2CH_2$ and HCHOHCHH_{ax}), 2.08 (1 H, ddd, *J* = 1.5 Hz, 6 Hz, 13.5 Hz, -HCOHCHH_{eq}), 2.45 (2 H, s, CH_2Si) 3.60–3.85 (2 H, m, OCH_2), 4.57 (1 H, m, *H*COSiR_3), 4.80 (1 H, dt, *J* = 7 Hz, 2 Hz, OCH=C*H*), 6.23 (1 H, dd, *J* = 2 Hz, 7 Hz, OC*H*=CH).

Desethyltalaromycin (13). A solution of 13 mL of degassed benzene and 0.248 g (0.734 mmol) of silyl ether **11** was stirred and heated at reflux as 0.24 mL (0.881 mmol) of Bu_3SnH and 0.04 g (0.220 mmol) of AIBN in 1.5 mL of degassed benzene were added by syringe pump over 2 h. After heating at reflux for 3 additional h, the reaction was cooled and concentrated to give a quantitative crude yield of the silacycle **12**. This material was unstable to silica gel chromatography and was used without further purification or characterization. A solution of 0.22 mL of 30% H_2O_2, 1.5 mL of THF, 1.5 mL of methanol, 0.040 g (0.367 mmol) Na_2CO_3, and 0.0889 g (0.367 mmol) of silacycle **12** was stirred and heated at reflux for 20 h. After cooling and diluting the reaction mixture with water, it was extracted 5 times with ether. The aqueous layer was then saturated with NaCl and extracted continuously with ether for 7 h. Concentration afforded 54.5 mg (77%) of (±)-desethyltalaromycin (**13**) as a thick oil, which subsequently crystallized; mp 87–90 °C: IR (film) 3380 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.33–2.12 (6 H, band), 3.45–3.81 (6 H, band), 3.96 (1 H, d, *J* = 5 Hz), 4.10 (1 H, t, *J* = 9 Hz), 4.32 (1 H, m). Anal. Calcd for $C_{10}H_{18}O_4$: C, 59.39; H, 8.97. Found: C, 59.12; H, 8.98.

(*R*)-2-Ethyl-4-penten-1-ol (16).[10] A solution of 3.0532 g (80.35 mmol) of lithium aluminum hydride and 100 mL of dry ether was cooled in an ice–water bath, and an ether solution of 6.401 g (26.8 mmol) of the oxazolidinone **15**[10] was added dropwise. After being stirred for 15 min, the reaction was quenched with 10% aqueous sodium hydroxide and the salts were removed by filtration. The ether solution was washed with 10% HCl and brine, then dried over magnesium sulfate, and concentrated to yield 2.659 g of 4(*R*)-(hydroxymethyl)-1-hexene and recovered oxazolidone chiral auxiliary. Chromatography (ethyl acetate/hexanes, 25:75) gave 2.230 g (73%) of alcohol **16**[10] as a colorless oil. Distillation (bp 73 °C, 25 mm) provided analytically pure material: IR (film) 3200, 1640 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.92 (3 H, t, *J* = 7.5 Hz, CH_2CH_3), 1.38 (2 H, m, CH_2CH_3), 1.53 (1 H, m, CH_3CH_2CH), 2.12 (2 H, m, CH_2), 1.85 (1 H, br s, OH), 3.55 (2 H, d, *J* = 6.0 Hz, CH_2OH), 5.05 (2 H, m, CH=CH_2), 5.82 (1 H, m, CH=CH_2); $[\alpha]^{23}_D$ = −1.20° ($CHCl_3$, *c* = 2.505); lit.[10] $[\alpha]^{20}_D$ = −0.06° ($CHCl_3$, *c* = 6.55).

(*R*)-4-[(Benzyloxy)methyl]-1-hexene (17). A 250-mL three-neck flask was charged with 2.685 g (61.14 mmol) of sodium hydride (60% suspension in mineral oil). The sodium hydride was washed twice with hexanes and suspended in 100 mL of THF. A solution of 2.552 g (22.38 mmol) of 4(R)-(hydroxymethyl)-1-hexene (**16**) in THF was then added dropwise. After hydrogen evolution had ceased, 3.2 mL (26.86 mmol, 1.2 equiv) of benzyl bromide was added. After 24 h the reaction was quenched with 10% HCl, diluted with ether, washed twice with brine, and dried over magnesium sulfate. Concentration gave a quantitative crude yield of 4(*R*)-[(benzyloxy)methyl]-1-hexene, which was chromatographed on silica gel (ethyl acetate/hexanes, 10:90) to yield 4.415 g (97%) of **17**. Purification could also be accomplished by dis-

tillation; bp 81 °C (0.1 mm): IR (film) 1645 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.89 (3 H, t, J = 7.5 Hz, CH_2CH_3), 1.40 (2 H, m, CH_2CH_3), 1.65 (1 H, m, CH_3CH_2CH), 2.12 (2 H, m CH_2), 3.35 (2 H, d, J = 6.0 Hz, CH_2OBz), 4.49 (2 H, s, OCH_2Ph), 5.00 (2 H, m, $CH=CH_2$), 5.77 (1 H, m, $CH=CH_2$), 7.32 (5 H, s, Ph); $[\alpha]^{23}_D$ = +3.63° ($CHCl_3$, c = 3.525). Anal. Calcd for $C_{14}H_{20}O$: C, 82.30; H, 9.87. Found: C, 82.10; H, 9.84.

(*R*)-4-[(Benzyloxy)methyl]hexan-1-ol (18). A solution of 6.666 g (32.63 mmol) of olefin **17** in 50 mL of THF was cooled in an ice–water bath whereupon a solution of borane–dimethyl sulfide (2 M in THF, 16.3 mL; 32.6 mmol) was added dropwise. After stirring for 5 h, 12 mL of 10% NaOH followed by 12 mL of 30% hydrogen peroxide were added carefully. After stirring for an additional 16 h, the reaction mixture was diluted with ether and the aqueous phase was saturated with sodium chloride and extracted 3 times with ether. The organic layer was dried over $MgSO_4$ and concentrated to give a quantitative yield of the crude alcohol. Flash chromatography on silica gel (ethyl acetate/hexanes, 1:9) provided 6.611 g (91%) of alcohol **18** as a colorless oil. Purification could also be accomplished by distillation; bp 115 °C (0.1 mm): IR (film) 3370 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.89 (3 H, t, J = 7.5 Hz, CH_2CH_3), 1.47 (7 H, m, $CH_3CH_2CHCH_2CH_2CH_2OH$), 2.04 (1 H, br s, OH), 3.36 (2 H, ddd, AB of ABX, J_{obs} = 3 Hz, 6 Hz, 9 Hz, CH_2OBz), 3.61 (2 H, t, J = 7.5 Hz, $-CH_2OH$), 4.50 (2 H, s, OCH_2Ph), 7.34 (5 H, s, Ph); $[\alpha]^{23}_D$ = +2.036° ($CHCl_3$, c = 3.045). Anal. Calcd for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97. Found: C, 75.43; H, 9.98.

4(*R*)-[(Benzyloxy)methyl]hexanoic Acid (19). A solution of chromium trioxide in sulfuric acid (Jones reagent) was added dropwise to a solution of 300 mg (1.351 mmol) of 4(R)-[(benzyloxy)methyl]hexan-1-ol (**18**) and 20 mL of acetone until the reaction mixture retained a brownish orange tint. The mixture was stirred for 30 min and then quenched by adding 2-propanol until the orange tint dissipated and the mixture was green. Celite was added and the salts were filtered and washed with acetone. The solvent was removed to give the crude acid. This could be purified by taking the oil up in ether and washing with 10% NaOH. The base extract was then acidified and extracted 3 times with ether. The combined ether washes were dried over $MgSO_4$ and concentrated to give 267 mg (84%) of 4(*R*)-[(benzyloxy)-methyl]hexanoic acid (**19**) as a colorless oil: IR (film) 3500, 1710 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.88 (3 H, t, J = 8.0 Hz, CH_2CH_3), 1.23–1.78 (5 H, band, $CH_3CH_2CHCH_2CH_2COOH$), 2.38 (2 H, t, J = 10 Hz, CH_2COOH), 3.38 (2 H, ddd, AB of ABX, J_{obs} = 6 Hz, 6 Hz, 10 Hz, CH_2OBz), 4.49 (2 H, s, OCH_2Ph), 7.34 (5 H, s, Ph), 11.72 (1 H, br s, COOH); $[\alpha]^{23}_D$ = −2.544° ($CHCl_3$, c = 1.14). Anal. Calcd for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53. Found: C, 71.10; H, 8.51.

(*R*)-5-Ethyltetrahydro-2(3*H*)-pyranone (14). A mixture of 3.00 g (12.71 mmol) of acid **19**, 350 mg of 10% Pd on charcoal, and 5 drops of concentrated $HClO_4$ in 150 mL of ethyl acetate was magnetically stirred under an atmosphere of hydrogen for 8 h, whereupon the uptake of hydrogen had stopped. Solid sodium bicarbonate was added and, the reaction mixture was filtered through Celite and concentrated to give a colorless oil. This material was treated directly with pyridinium *p*-toluenesulfonate in benzene at reflux with continuous removal of water for 12 h. The mixture was then cooled, washed with half-saturated brine, and dried over magnesium sulfate. Filtration and concentration of the solution provided 1.73 g (100%) of lactone **14** (unstable to silica gel chromatography) as a colorless liquid that was essentially pure and used without further purification. Kugelrohr distillation (60 °C, 0.1 mm) provided analytically pure material: IR (film) 1745 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.96 (3 H, t, J = 7.5 Hz, CH_2CH_3), 1.20–1.62 (3 H, band), 1.72–2.12 (2 H, m), 2.40–2.73 (2 H, m, $-CH_2COOR$), 3.97 (1 H, dd, J = 12 Hz, 9 Hz, OCH*H* axial), 4.36 (1 H, ddd, J = 12 Hz, 6 Hz, 3 Hz, OCH*H* equatorial); $[\alpha]^{22}_D$ = +3.658° ($CHCl_3$, c = 3.39). Anal. Calcd for $C_7H_{12}O_2$: C, 65.60; H, 9.44. Found: C, 65.40; H, 9.30.

(*R*)-2-Ethyl-1-hydroxy-9-methoxynon-8-en-6-yn-5-one (20). After cooling a solution of 50 mL of dry THF and 1.2 mL (12.7 mmol) of freshly distilled 1-methoxy-1-buten-3-yne to −78 °C, 5.1 mL (12.7 mmol) of a 2.5 M solution of *n*-butyllithium was added dropwise and the reaction then stirred. After 45 min, 1.627 g (12.7 mmol) of lactone **14** in 5 mL of THF was added and the reaction mixture was stirred for an additional hour. The reaction was quenched with saturated ammonium chloride and diluted with ether. The organic phase was then washed with brine and dried over $MgSO_4$. Filtration and concentration gave 2.523 g (95%) of the crude ketone **20** as a pale yellow, unstable oil, which was homogeneous (≥95%) by ^1H NMR and was used without further purification: IR (film) 3430, 21270, 1665, 1620 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.90 (3 H, t, J = 7.5 Hz, CH_2CH_3), 1.14–1.79 (5 H, band), 2.63 (2 H, dd, J = 7.5 Hz, 7.0 Hz, CH_2CO), 2.74 (1 H, br s, O*H*), 3.52 (2 H, dd, J = 5 Hz, 3 Hz, CH_2OH), 3.86 (3 H, s, OCH_3), 4.67 (1 H, d, J = 6.0 Hz, $CH=CHOMe$), 6.61 (1 H, d, J = 6.0 Hz, $CH=CHOMe$).

(*R*)-2-Ethyl-1-hydroxy-7,9,9-trimethoxynon-6-en-5-one (21). After 527 mg (3.87 mmol) of finely ground K_2CO_3 had dissolved in 50 mL of methanol, 3.475 g (19.1 mmol) of 2(*R*)-ethyl-1-hydroxy-9-methoxynon-8-en-6-yn-5-one (**20**) was added to the solution. After stirring for 24 h most of the methanol was removed in vacuo. The resulting oil was taken up in ether, dried over $MgSO_4$, and concentrated to yield 4.556 g (92%) of (*R*)-2-ethyl-1-hydroxy-7,9,9-trimethoxynon-6-en-5-one (**21**) as a pale yellow oil, which was homogeneous (≥95%) by ^1H NMR: IR (film) 3450, 1685, 1590 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.90 (3 H, t, J = 7.5 Hz, CH_2CH_3), 1.48–1.76 (7 H, band), 2.50 (2 H, t, J = 7.5 Hz, CH_2CO), 2.93 (1 H, br s, O*H*), 3.10 (2 H, d, J = 6 Hz, $CH_2CH(OMe)_2$), 3.33 (6 H, s, $CH(OCH_3)_2$), 3.48 (2 H, t, J = 7 Hz, CH_2OH), 3.69 (3 H, s, OCH_3), 4.70 (1 H, t, J = 6.0 Hz, $CH(OMe)_2$), 5.54 (1 H, s, J = 6.0 Hz, $CH=COMe$).

(*R*)-8-Ethyl-1,7-dioxaspiro[5.5]undec-2-en-4-one (22). A mixture of 52 mL of ether, 527 mg (1.92 mmol) of trimethoxy ketone **21**, and 13 mL of 10% HCl was stirred for 1 h. The reaction was then quenched by the cautious addition of solid $NaHCO_3$ until the reaction mixture was at pH 7. The aqueous layer was saturated with sodium chloride and extracted with ether. The combined ether extracts were dried over $MgSO_4$, filtered, and concentrated to provide 386 mg of crude spiroketal, which was flash chromatographed (ethyl acetate/hexanes, 1:9) to give 47 mg (11%) of methoxy spiroketal **23** and 142 mg (37%) of spiro enone **22** as a colorless oil. Spiroketal **22**: IR (film) 1690, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.90 (3 H, t, J = 7.5 Hz, CH_2CH_3), 1.20 (2 H, m, CH_2CH_3), 1.65 (4 H, m, CH_2's), 2.05 (1 H, m, $CHCH_2CH_3$), 2.63 (2 H, AB, J = 16.5 Hz, $\Delta\nu$ = 37.6 Hz, J_w = 1 Hz, CH_2CO), 3.35 (1 H, dd, J = 10.5 Hz, 10.5 Hz, $OCH_{ax}H_{eq}$), 3.66 (1 H, ddd, J = 10.5 Hz, 2 Hz, 4.5 Hz, $OCH_{ax}H_{eq}$), 5.45 (1 H, dd, J = 6 Hz, J_w = 1 Hz, $OCH=CHCO$), 7.21 (1 H, d, J = 6 Hz, $OCH=CHCO$); $[\alpha]^{22}_D$ = −223.3° ($CHCl_3$, c = 4.235). Anal. Calcd for $C_{11}H_{16}O_3$: C, 67.35; H, 8.16. Found: C, 67.36; H, 8.26. Methoxy spiroketal **23**: ^1H NMR ($CDCl_3$, 200 MHz) δ 0.92 (3 H, t, J = 7.5 Hz, CH_2CH_3), 1.11–1.77 (5 H, m), 2.45 (1 H, dd, J = 15, 7.5 Hz, $MeOCHCH_2CO$), 2.48 (2 H, AB, J = 15 Hz, $\Delta\nu$ = 37.6 Hz, J_w = 1.5 Hz, CH_2CO), 2.76 (1 H, ddd, J = 1.5, 3, 15 Hz, $MeOCHCH_2CO$), 3.36 (1 H, dd, J = 10.5 Hz, 7.5 Hz, OCH_2), 3.55 (3 H, s, OCH_3), 3.63 (1 H, m, OCH_2), 4.87 (1 H, dd, J = 7.5 Hz, 3 Hz, CH_3OCH-).

Conversion of 23 to 22. A suspension of 2 g of Amberlyst 15 in a solution of 966 mg (4.24 mmol) of spiroketal **23** and 50 mL of dichloromethane was heated at reflux for 16 h, then cooled, filtered, and concentrated. The residue was flash chromatographed (25% ethyl acetate in hexanes) to provide 484 mg (58%) of spiroketal **22**, identical with that prepared above.

Pyrone 24. A solution of 4.162 g (14.55 mmol) of (*R*)-2-ethyl-1-hydroxy-7,9,9-trimethoxy 6-en-5-one **21** in 100 mL THF and 300 mg of *p*-toluenesulfonic acid in 25 mL of H_2O was heated at reflux for 10 h. The reaction was quenched with solid $NaHCO_3$, diluted with ether, and saturated with NaCl. The organic phase was dried over $MgSO_4$ and concentrated to give 2.809 g (98%) of a mixture of pyrone **24** and enone **22** as a pale yellow oil. Chromatography (9:1 EtOAc/methanol, silica gel) yielded 2.111 g (74%) of pyrone **24**: IR (film) 3400, 1663, 1608 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.92 (3 H, t, J = 7.5 Hz, $-CH_2CH_3$), 1.28–1.87 (5 H, band), 2.58 (2 H, t, J = 8.0, $CH=CHCH_2$-), 3.39 (1 H, bs, O*H*), 3.58 (2 H, ddd, AB of ABX, J = 5.5, 10.9, 20.8 Hz), 6.19 (1 H, d, J = 2.5 Hz, $OC(CH_2)=CH$), 6.28 (1 H, dd, J = 2.5, 5.8 Hz, $OCH=CH$-), 7.75 (1 H, d, J = 5.8 Hz, $OCH=CHCO$-); $[\alpha]^{20}_D$ = −0.567° ($CHCl_3$, c = 4.06) and 698 mg (24%) of enone **22**.

Enone 22 from Pyrone 24. Ten drops of trifluoroacetic acid was added to a solution of 150 mL of benzene and 2.111 g (10.76 mmol) of pyrone **24**. After stirring for 3 days, the reaction was

concentrated and chromatographed (silica gel, 10% ethyl acetate in hexanes followed by 10% methanol in ethyl acetate) to provide 1.224 g (58%) of the spiroketal **22** and 0.760 g (36%) of recovered pyrone **24**. The pyrone **24** was resubjected to trifluoroacetic acid in benzene as before to produce an additional 440 mg of spiroketal **22**, giving a total of 2.373 g (81%) of spiroketal **22** from the acetal **21**.

8(*R*)-Ethyl-4(*S*)-hydroxy-1,7-dioxaspiro[5.5]undec-2-ene (25) and 8(*R*)-Ethyl-4(*R*)-hydroxy-1,7-dioxaspiro[5.5]undec-2-ene (26). A solution of 85 mg (0.432 mmol) of spiroketal **22** and 177 mg (0.475 mmol) of $CeCl_3 \cdot 7H_2O$ in 15 mL of methanol was cooled to −78 °C and 18 mg (0.475 mmol) of sodium borohydride was added. The bath was removed and the reaction mixture was allowed to warm to room temperature. The reaction was quenched with aqueous NH_4Cl and diluted with ether and the biphase was stirred for 16 h. The ether layer was then separated and dried over $MgSO_4$. Concentration gave a quantitative yield of a 2.4:1 mixture of two labile allylic alcohols **25**:**26**. Flash chromatography (ethyl acetate/hexanes, 1:9) gave 17.4 mg (20%) of the α-alcohol **26** and 40.6 mg (48%) of alcohol **25**. Allylic alcohol **25**: IR (film) 3380, 1655 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.88 (3 H, t, J = 7.5 Hz, CH_2CH_3), 1.07–1.92 (8 H, band), 2.16 (1 H, ddd, J = 1 Hz, 6 Hz, 12 Hz, $HCOHCH_{eq}H$), 3.36 (1 H, t, J = 10.5 Hz, OCH_2), 3.61 (1 H, -OCH*H*, m), 4.49 (1 H, m, C*H*OH), 4.89 (1 H, ddd, J = 1 Hz, 1 Hz, 6 Hz, CH=C*H*CHOH), 6.25 (1 H, dd, J = 1 Hz, 6 Hz, -OC*H*=CH); $[\alpha]^{22}_D$ = −192.88° ($CHCl_3$, c = 1.53). Anal. Calcd for $C_{11}H_{18}O_3$: C, 66.64; H, 9.15. Found: C, 66.20; H, 9.15.

Allylic alcohol **26**: IR (film) 3380, 1655 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.88 (3 H, t, J = 7.5 Hz, CH_2CH_3), 1.16–1.83 (7 H, band), 1.90 (1 H, dd, J = 10 Hz, 6 Hz, $HCOHCH_{eq}H$), 2.13 (1 H, ddd, J = 2 Hz, 15 Hz, 2 Hz, $CHOHCH_2$), 3.37 (1 H, dd, J = 12 Hz, 4 Hz, OCH_2), 3.61 (1 H, -OCH*H*, m), 3.95 (1 H, m, C*H*OH), 5.15 (1 H, ddd, J = 1.5 Hz, 2 Hz, 6.5 Hz, CH=C*H*CHOH), 6.31 (1 H, d, J = 6.5 Hz, -OC*H*=CH).

Silyl Ether 27. To a solution of 38.3 mg (0.192 mmol) of allylic alcohol **25** in 2 mL of dry DMF was added 19.6 mg (0.287 mmol) of imidazole and 40 μL (0.287 mmol) of triethylamine. After stirring for 5 min, 40 μL (0.287 mmol) of (bromomethyldimethyl)silyl chloride was added, and the reaction mixture was stirred for 3 h. The reaction was then quenched with saturated $NaHCO_3$ and diluted with ether. The ether layer was washed with brine, dried over $MgSO_4$, concentrated, and chromatographed (ethyl acetate/hexanes, 1:1) to yield 62.2 mg (93%) of silyl ether **27** as a labile colorless oil, which was homogeneous (≥95%) by ^1H NMR: ^1H NMR ($CDCl_3$, 200 MHz) δ 0.26 (2 H, AB, J_{AB} = 10.5 Hz, $BrCH_2Si$), 0.29 (6 H, $Si(CH_3)_2$), 0.88 (3 H, t, 7.5 Hz, CH_2CH_3), 1.39–1.90 (7 H, band), 1.75 (1 H, dd, J = 9 Hz, 12 Hz, $CH(OSiR_3)CH_2$), 2.07 (1 H, ddd, J = 1 Hz, 6 Hz, 12 Hz, $CHOSiR_3CH_2$), 3.50 (2 H, m, OCH_2), 4.59 (1 H, m, C*H*OSiR_3), 4.80 (1 H, ddd, J = 1 Hz, 1 Hz, 6 Hz, OCH=C*H*), 6.22 (1 H, dd, J = 1 Hz, 6 Hz, OC*H*=CH).

Talaromycin A (1). A solution of 62.2 mg (0.178 mmol) of silyl ether **27** and 4 mL of degassed benzene was heated to reflux, whereupon a solution of 60 μL (0.213 mmol) of Bu_3SnH and 8.6 mg (0.053 mmol) of AIBN in 1 mL of benzene was added by syringe pump over 2 h. The reaction was heated at reflux for an additional 5 h and then concentrated in vacuo to provide the crude silacycle **28**, which was not characterized and was used without further purification. The crude silacycle from above was added to a mixture of 0.2 mL of 30% H_2O_2, 30 mg of Na_2CO_3, 2 mL of methanol, and 2 mL of THF. The mixture was heated at reflux for 12 h, cooled to room temperature, diluted with water, 10% $NaHSO_3$, and 10% $NaHCO_3$. This mixture was then placed in a continuous extractor and extracted with ether for 4 h. Concentration of the ether layer and flash chromatography (ethyl acetate/hexanes 1:9; then 100% ethyl acetate) gave 34.1 mg (84%) of (−)-talaromycin A (**1**) which gave spectral data identical with that reported by Lynn:[2] IR (film) 3381 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.92 (3 H, t, J = 7.7 Hz, CH_2CH_3), 1.11–1.25 (2 H, m), 1.37–1.75 (5 H, band), 1.75 (1 H, B of ABX, J = 13, 16.8 Hz, $-CH(OH)CH_{eq}CH_{ax}$), 1.93 (1 H, A of ABX, J = 5, 13 Hz, $-CH(OH)CH_{eq}CH_{ax}$), 2.18 (1 H, m), 3.22 (1 H, B of ABX, J = 10, 10 Hz, $-OCH_{eq}CH_{ax}$-CHEt), 3.55 (1 H, A of ABX, J = 10, 5, 2 Hz, $-OCH_{eq}CH_{ax}CH$Et), 3.62 (1 H, B of ABX, J = 11.8, 1.7, -OC*H*H-CH(CH_2OH)-), 3.78 (1 H, A of ABX, J = 11.8, 3.4, -OC*H*H-CH(CH_2OH)-), 3.83 (1 H, B of ABX, J = 5, 10 Hz, -C*H*HOH), 4.24 (1 H, A of ABX, J = 8.4, 10 Hz, -C*H*HOH), 4.44 (1 H, ddd, J = 11.8, 5, 5 Hz, C*H*OH); $[\alpha]^{22}_D$ = −105.7° (c = 0.505, $CHCl_3$); lit.[10] $[\alpha]^{20}_D$ = −110.2° (c = 0.83, $CHCl_3$).

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Biosynthesis of Antibiotics of the Virginiamycin Family. 7. Stereo- and Regiochemical Studies on the Formation of the 3-Hydroxypicolinic Acid and Pipecolic Acid Units[1]

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Details of the biosynthesis of two components of virginiamycin S_1 (**1**) derived from (*S*)-lysine have been studied in *Streptomyces virginiae* by incorporation of lysines labeled with stable isotopes. Both the (*S*)-4-oxopipecolic acid (**2**) and 3-hydroxypicolinic acid (**3**) portions incorporate (*RS*)-[6-^{13}C,6-^{15}N]lysine (**11**) with retention of the labeled nitrogen. Thus, the cyclization of lysine in both cases occurs with the loss of the α-nitrogen and retention of the ε-nitrogen. In addition, the 3-hydroxypicolinic acid unit incorporates deuterium from (2*RS*,5*R*)-[5-^{2}H]lysine (**20b**) but not from (2*RS*,5*S*)-[5-^{2}H]lysine (**20a**). The 5-*pro*-*R* hydrogen of lysine is thus retained in the biogenesis of 3-hydroxypicolinic acid.

In the previous paper in this series,[1] we described our studies on the basic biosynthetic pathways leading to the cyclic peptidolactone antibiotic virginiamycin S_1 (**1**). A key finding to emerge from this work was that the amino

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