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CONTROLLED REDUCTION AND OXIDATION OF ACTINOBOLIN

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Abstract

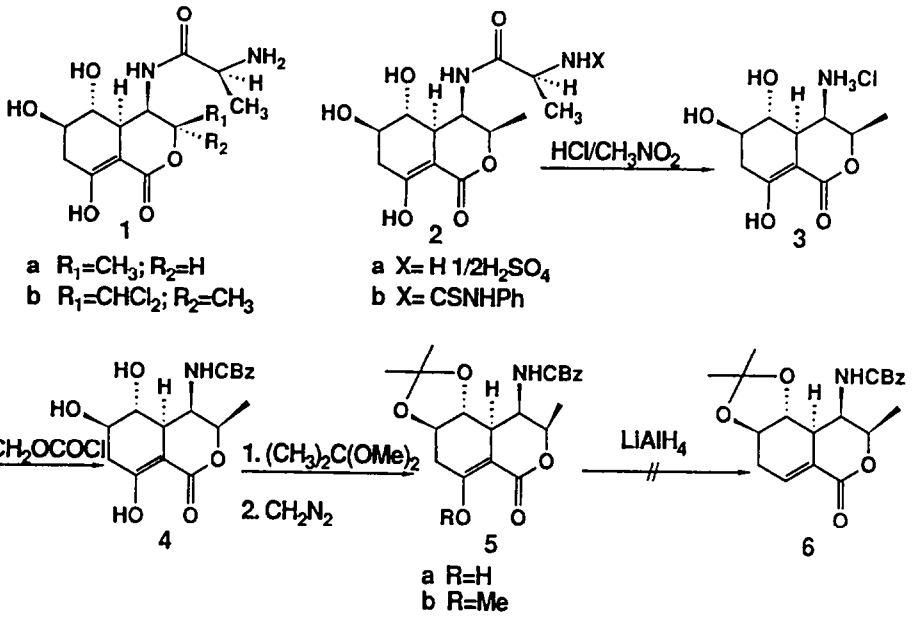
The Edman degradation has been applied to actinobolin sulfate (2a) and the resulting hydrochloride 3 was converted into the carbobenzyloxy derivative 4. Treatment of the latter with a variety of reducing agents did not lead to any useful products; however, the corresponding N-acetyl derivative, 7, reacted with sodium borohydride to give a mixture of the hemi-acetal, 8, and the lactone 9. Although both of the latter underwent acid-catalyzed dehydrations, it was possible to obtain a mixture of diastereomeric glycosides, 12, by treatment with methanolic hydrogen chloride. Hydrolysis of 12 with aqueous sulfuric acid, Swern oxidation, and saponification then regenerated the actinobolin system 5a.

Actinobolin 1a was isolated in 1959 from Strepomyces griseoviridies by Haskell and Bartz,[1] and initially the antibiotic attracted only minimal attention. However, interest has been reawakened because actinobolin has been found to interact strongly with human tooth enamel,[2] which implies that it could be a cariostatic agent. Additional interest stems from the recent isolation of a structurally related substance bactobolin, 1b, which has been found to display improved antibiotic activity and to be an anti-tumor agent.[3]

Total syntheses of actinobolin[4,5] and its N-acetyl analogue[6,7] have appeared recently, but to date, no synthesis of bactobolin has been reported.

The exhaustive studies by the groups of Haskell and Munk made it clear that the actinobolin skeleton was very labile towards a wide variety of reaction conditions.[3b,8,9] In order to prepare the ground for the final stages of our synthesis[6] and to establish useful comparison points along the synthetic route, we undertook some transformation studies on actinobolin, since the actinobolin derivatives which had been described[8,9] did not promise to be suitable relay intermediates.

In order to lessen the complexity of the highly reactive functionalities of the molecule, the alanine moiety was removed by use of the Edman procedure.[10] Thus, treatment of actinobolin sulfate, 2a, with phenylisothiocyanate in pyridine provided the thiourea derivative (2b) in 90% yield. Reaction of the latter with anhydrous hydrogen chloride in nitromethane then led to the crystalline hydrochloride, 3, isolated after column chromatography in 90% yield. This compound could be stored without decomposition, and could be used not only for the substitution of new amino acids, but also for the formation of a wide variety of modified derivatives.[11]



The carbobenzyloxy group was considered an appropriate choice for N-protection since it had been shown that it could be removed easily without destruction of the actinobolin skeleton.[12] Accordingly, the hydrochloride 3 was converted into the free base *in situ* by treatment with excess triethylamine in ethyl acetate (conditions which were found to suppress O-acetylation), and then with benzylchloroformate to afford compound 4 in 80% yield. The *trans*-diequatorial hydroxyl groups were then ketalised to give 5 in excellent yield by use of acetone, dimethoxypropane and a catalytic amount of pyridinium-p-toluenesulfonate[13] at room temperature. It may be noted that Munk and co-workers prepared the acetonide of N-acetyl actinobolin (13) by use of acetone and p-toluenesulfonic acid at reflux.[3b]

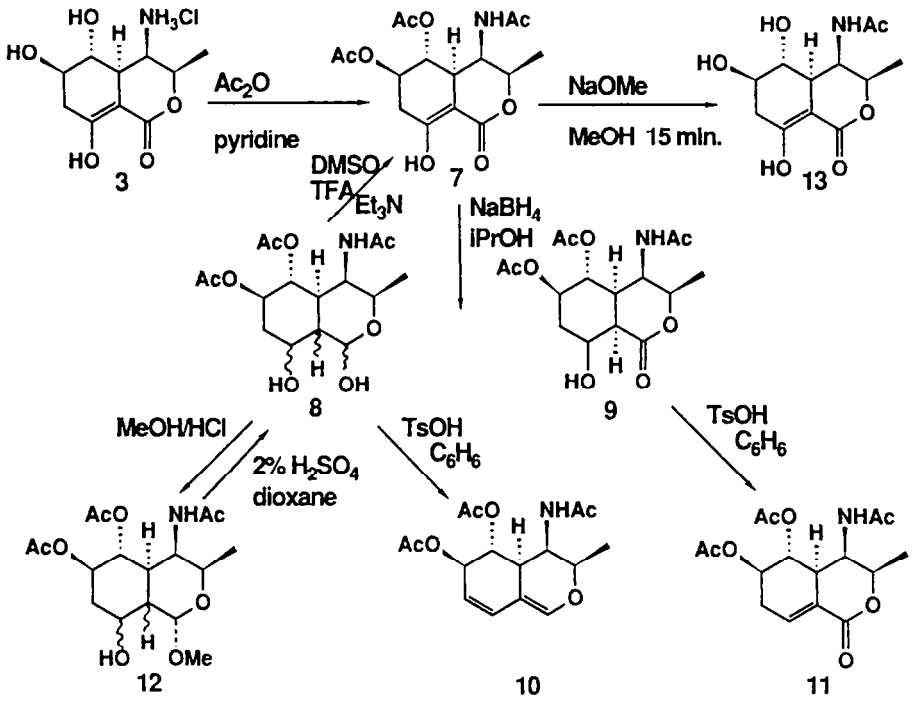
The enol 5a was methylated with diazomethane using methanol as a solvent to afford the fully protected intermediate 5b. The last reaction showed a strange solvent dependency, in that with ethanol, the reaction took three days for completion and gave less than 50% yield.

There are literature precedents[14] which show that compounds such as 5b afford α, β-unsaturated lactones, for example 6, upon reduction with diisobutyl aluminum hydride. However, when compound 5b was subjected to comparable reaction conditions, there were no isolable products.

In view of these failures with compound 5b, we wished to see whether other protecting groups would cause a difference in the outcome of the reaction. Accordingly, the hydrochloride 3 was treated with acetic anhydride in pyridine, and the triacetyl derivative, 7, was isolated in 80% yield. Reduction with sodium borohydride in isopropanol now gave a mixture of compounds 8 and 9 in good yield, the structures of which were confirmed by dehydration. Thus, treatment of lactol 8 with p-toluenesulfonic acid in benzene at room temperature provided diene 10, whose high resolution mass spectrum and ^{1}H NMR data were in agreement with the presence of a conjugated diene. Similarly, dehydration of 9 with p-toluenesulfonic acid in benzene led to the α, β-unsaturated lactone 11.

All attempts to oxidize **9** led to the elimination product **11**. However, in spite of the ready acid catalyzed dehydration of **8**, it was found that upon treatment of the compound with methanolic hydrogen chloride for 12 hours, the methyl glycoside **12** could be formed, albeit in only 36% yield.

Although compounds **8** and **12** appeared as single substances on thin layer chromatograms, they were undoubtedly mixtures. Thus, in the case of **12**, high pressure liquid chromatography revealed the presence of three components, and the most abundant (~60%) was shown to be the 1α, 7β diastereomer, which has been prepared by synthesis[6].



The glycosidic mixture **12** was hydrolyzed to the lactol **8** by use of aqueous sulfuric acid (2%) in dioxane-water (4:1) at 50°C. Attempts to oxidize the lactol **8** with pyridinium chlorochromate,[15] pyridinium dichromate,[16] or silver carbonate/Celite,[17] failed to regenerate the actinobolin skeleton. However, the Swern oxidation medium containing trifluoracetic anhydride, dimethyl sulfoxide and triethylamine[18] proved suitable, affording **7** in 70% yield. It is noteworthy that under the same conditions, but using oxalyl chloride instead of trifluoroacetic anhydride, a dehydration product was obtained.

Hydrolysis of the O-acetyl groups of **7** was achieved using sodium methoxide in methanol for 15 minutes, which afforded N-acetyl desalanylactinobolin **13** in 80% yield.

It was therefore, shown confidently that the glycosidic mixture **12** could be converted into N-acetyl desalanylactinobolin **13** in three steps and was therefore a logical relay target for our synthetic plan.

Experimental

General Elemental analyses were performed either by Dr. F. Kasler (Department of Chemistry, University of Maryland, College Park, Maryland) or by M-H-W Laboratories, Phoenix, Arizona. IR spectra were recorded using chloroform as solvent, or sodium chloride plates for thin films. Optical rotations were determined at the sodium D line. ^{1}H NMR spectra were determined on a Brucker 250 MHz spectrometer using $CDCl_2$ (Me_4Si). The coupling constants were verified by homonuclear decoupling experiments. For the purpose of ^{1}H NMR interpretation, compound structures have been numbered in the Schemes. High resolution mass spectra (HRMS) were performed at the Research Triangle Park, North Carolina, with a VG7070F instrument. The progress of all reactions was monitored by thin-layer chromatography (TLC) which was performed on aluminum plates precoated with silica gel HF-254 (0.2 mm layers) containing a fluorescent indicator (Merck, 5539), with detection by UV (254 nm), charring with sulfuric acid spray, or with a solution of ammonium molybdate (VI) tetrahydrate (12.5 g) and cerium (IV) sulfate tetrahydrate (5.0 g) in 10% aqueous sulfuric acid (500 mL). Flash chromatography was performed using Kieselgel 60 (230-400 mesh, E. Merck). High pressure liquid chromatography was performed on a Varian model 5000 (HPLC) with Micro Pak CN-5, 15 cm x 4 mm column.

Thiourea derivative of actinobolin (2b). Actinobolin sulfate (2a; 367 mg, 1 mmol) was dissolved in dry pyridine (4 mL) and stirred at room temperature for 10 min. Excess phenylisothiocyanate (0.35 ml, 2.9 mmol) was then added, and after further stirring for 3h, the reaction mixture was extracted (CH_2Cl_2, 3 x 20 mL), and the combined extracts washed with water and dried (Na_2SO_4). The solvent was evaporated, and the crude product was chromatographed on a silica gel column, using EtOAc:Me_2CO (4:1) as eluent. Evaporation gave crystalline **2b**, (391.5 mg, 90% yield): m.p. 148 - 150°. R_f0.40 (EtOAc: Me_2CO, 4:1). ν_{max}^{CHCl} 3500, 3300 (OH,NH), 1720 (enol lactone >C=O), 1650 (amide >C=O), 1600 (enol C=C), 1500 cm^{-1} (amide II). ^{1}H NMR, δ 1.40 (d, $J_{5,6}$ = 6.50 Hz, $J_{12,13}$ = 6.50 Hz, $6CH_3$, $13CH_3$), 2.40 (m, 1, H-8ax), 2.60 (m, 1, H-3), 2.90 (dd, 1, $J_{8ax-8eq}$ = 18.0 Hz, H-8eq), 3.10 (m, 2, H-10, OH), 3.85 (q, 1, H-9), 4.30 (m, 1, H-4) 4.50 (br, 1, OH), 4.60 (q, 1, H-5), 5.10 (m, 1, H-12), 6.40 (d, 1, NH), 7.30 (m, 5, C_6H_5), 8.0 (br, 1, enolic OH), NH and OH exchanged with D_2O.

Anal. Calcd. for $C_{20}H_{25}O_6N_3S$; C, 55.17, H, 5.75, N, 9.65, S, 7.35: Found: C, 54.58, H, 6.03, N, 9.28, S, 6.79.

Desalanylactinobolin hydrochloride (3). To a solution of the thiourea derivative **2b** (500 mg, 1.149 mmol) in dry CH_3NO_2 (10 mL) was added a saturated solution of anhydrous hydrogen chloride in dry CH_3NO_2 (20 mL) under argon. A solid precipitated after 5 min., and the reaction mixture was stirred for another 0.5 h at room temperature after which the solvent was removed on a rotary evaporator. The residue was then chromatographed on silica gel, eluting first with EtOAc: petroleum ether (3:7) to remove the less polar impurities, and then with EtOAc:EtOH (3:2). Evaporation of the latter solvent mixture gave **3** as a solid material (274:5 mg, 90% yield). m.p. 174-171° (decomp.). $[\alpha]_D^{23}$ + 38.7°. (c, 1.0, EtOH) ^{1}H NMR (D_2O), δ 1.35 (d, 3, $J_{5,6}$ = 6.50 Hz, $6CH_3$), 2.40 (d of q, 1, H-8ax), 2.70 (m, 1, H-3), 2.80 (m, 1, H-8eq), 3.35-3.40 (m, 2, H-9, H-10), 3.65 (m, 1, H-4), 3.80 (q, 1, H-5).

N-Carbobenzyloxydesalanylactinobolin (4). To a solution of **3** (118 mg, 0.466 mmol) in EtOAc (5 mL) was added Et_3N (0.193 mL, 1.39 mmol) and benzyl chloroformate (0.513 mL, 0.278 mmol) at 0°. The reaction mixture was stirred overnight at room temperature after which the solvents were evaporated. The residue was chromatographed on silica gel eluting first with EtOAc: petroleum ether (3:7) to remove the less polar impurities, and then with EtOAc. Evaporation of the latter solvent gave **4** as a solid compound (134.4 mg, 80% yield): m.p. 86°. $[\alpha]_D^{20}$ + 10.26° (c, 0.1, $CHCl_3$), R_f 0.40 (EtOAc). ν_{max}^{CHCl} 3600, 3400

(OH, NH), 1720 (lactone >C=O), 1650 (amide>C=O), 1600 (enolic C=C), 1520 cm^{-1} (amide II). ^1H NMR, δ 1.40 (d, 3, $J_{5,6}$ = 6.50 Hz, CH_3), 2.40 (dddd, 1, H-8ax), 2.60 (m, 1, H-3), 2.90 (dd, 1, $J_{8ax-8eq}$ = 18.0 Hz, H-8eq), 3.0 (br, 1, OH), 3.20 (t, 1, H-10) 3.90 (q, 1, H-9), 4.10 (m, 1, H-4), 4.50 (br, 1, OH), 4.60 (q, 1, H-5), 5.0 (d, 1, NH), 5.15 (s, 2, H-12), 7.40 (m, s, C_6H_5-), 10.0 (br, 1, enolic OH).

HRMS Calcd. for $C_{18}H_{21}O_7N$; 363.1317. Found: 363.1317.

Isopropylidene derivative of N-Carbobenzyloxydesalanylactinobolin (5a). Compound 4 (100 mg, 0.275 mmol) was dissolved in dry Me_2CO (5 mL) and excess of 2,2-dimethoxypropane (15 mL) was added to the solution. A catalytic amount of pyridinium-p-toluenesulfonate[13] (10 mg) was added, and after 18h at room temperature, the solvents were evaporated, and the residue was chromatographed on silica gel eluting with EtOAc. The product was a syrup (99.74 mg, 90% yield): $[\alpha]_D^{20}$ + 41.66° [c, 0.1, $CHCl_3$]. R_f 0.82 (EtOAc: petroleum ether, 3:7). ν_{max}^{CHCl} 3500 (NH, OH), 1720 (lactone >C=O), 1650 (amide >C=O), 1500 cm^{-1} (amide II). ^1H NMR, δ 1.35 (d, 3, $J_{5,6}$ = 6.50 Hz, $6CH_3$), 1.40 (s, 6, $C(CH_3)_2$), 2.60 (m, 1, H-8ax), 2.85 (m, 1, H-3), 2.90 (m, 1, H-8eq), 3.30 (t, 3, H-10), 3.70 (m, 1, H-9), 4.30 (m, 1, H-4), 4.55 (q, 1, H-5), 4.70 (d, 1, NH), 5.10 (s, 1, H-12), 5.15 (s, 1, H-12′), 7.30 (s, 5, C_6H_5), 9.65 (br, 1, enolic OH).

Methyl enol ether derivative (5b) of compoupd (5a). To a stirred solution of compound 5a (100 mg, 0.248 mmol) in MeOH (10 mL) was added excess of diazomethane solution (5 mL) at 0° and after 3 h, the excess diazomethane was destroyed by the dropwise addition of acetic acid. The solvents were evaporated and the residue was chromatographed on silica gel using EtOAc as eluent. Removal of the solvent gave 5b as a syrupy liquid (82.5 mg, 80% yield): $[\alpha]_D^{20}$ + 38.63°. R_f 0.54 (EtOAc: petroleum ether: EtOH 5:5:1). ν_{max}^{neat} 3300 (NH), 1710 (lactone >C=O), 1650 (amide >C=O), 1530 cm^{-1} (amide II). ^1H NMR δ 1.30 (d, 3, $J_{5,6}$ = 6.0 Hz, $6CH_3$), 1.40 (2s, 6, $C(CH_3)_2$), 2.50 (m, 1, H-8ax) 2.90 (m, 1, H-3), 3.0 (dd, 1, $J_{8ax-8eq}$ = 18.0 Hz, H-8eq), 3.30 (t, 1, H-10), 3.65 (m, 1, H-9), 3.85 (s, 3, OCH_3), 4.25 (m, 1, H-4), 4.50 (q, 1, H-5), 4.78 (d, 1, NH), 5.10 (s, 1, H-12), 5.15 (s, 1, H-12′), 7.35 (m, 5, C_6H_5).

HRMS Calcd. for $C_{22}H_{27}O_7N$, 417.1788. Found: 417.1787.

N-acetyl-9,10-di-O-acetyldesalanylactinobolin (7). Desalanylactinobolin hydrochloride (3; 50 mg, 0.188 mmol) was dissolved in dry pyridine (2 mL), and excess acetic anhydride (0.2 mL) was added to the solution at 0°. After being stirred at room temperature overnight, the mixture was extracted (CH_2Cl_2, 3 x 10 mL), and the extract was washed with dilute HCl and saturated $NaHCO_3$ solutions, and water. The residue obtained from the extract was purified by column chromatography on silica gel using first EtOAc: petroleum ether (3:7) to remove the less polar uv active impurities, and then with EtOAc. Removal of the latter solvent gave 7 as a semi-solid material (55 mg, 82% yield): $[\alpha]_D^{20}$ + 53.09° [c, 0.55, $CHCl_3$]. R_f 0.37 (EtOAc) $\nu_{max}^{CHCl_3}$, 3650, 3500, 3300 (OH, NH), 1740 (ester >C=O), 1710 (lactone >C=O), 1650 (amide >C=O), 1600 (enolic C=C), 1510 cm^{-1} (amide II). ^1H NMR, δ 1.25 (d, 3, $J_{5,6}$ = 6.5 Hz, $6CH_3$), 1.93 (s, 3, $NHCOCH_3$), 1.98 (s, 3, $OCOCH_3$), 2.0 (s, 3, $OCOCH_3$), 2.50 (dddd, 1, H-8ax), 2.95 (dd, 1, H-3), 3.0 (dd, 1, $J_{8ax-8eq}$ = 18.0 Hz, H-8eq), 4.50 (m, 2, H-4, H-5), 4.90-5.10 (m, 2, H-9, H-10), 5.80 (d, $J_{4,11}$ = 10.0 Hz, NH).

Anal. Calcd. for $C_{16}H_{21}O_8N$; C, 54.08, H, 5.91, N, 3.94. Found: C, 53.92, H, 6.17, N, 3.79.

Sodium borohydride reduction of 7. To a stirred solution of compound 7 (50 mg, 0.14 mmol) in isopropanol (5 mL), was added excess of sodium borohydride (30 mg, 0.81 mmol) at 0°. The reaction mixture was stirred at room temperature for 24 h after which the sodium borohydride was destroyed by dropwise addition of acetic acid until the solution was neutral. The solvent was evaporated and the residue was dissolved in a small volume of water (1 mL) and then extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extracts

were washed with saturated $NaHCO_3$ solution (2 x 5 mL), dried (Na_2SO_4) and evaporated. The crude product was column chromatographed on silica gel using EtOAc as eluent. The major components were **8** (16.1 mg, 32% yield) and **9** (10 mg, 20% yield). For **8**: $[\alpha]_D^{20}$ + 32.70° [c, 0.085, $CHCl_3$]. R_f 0.33 (EtOAc). $\nu_{max}^{CHCl_3}$ 3400 (OH, NH), 1740 (ester >C=O), 1660 (amide >C=O), 1500 cm^{-1} (amide II). ^1H NMR, δ 1.20 (d, 3, $J_{5,6}$ = 6.0 Hz $6CH_3$), 1.65 (m, 1, H-8ax), 1.90 (s, 3, $NHCOC\underline{H}_3$), 2.0 (2s, 6, $OCOCH_3$), 2.30 (m, 1, H-8eq), 2.65 (m, 1, H-2), 3.0 (m, 1, H-3), 3.90 (q, 1, H-5), 4.30 (m, 1, H-7), 4.45 (dd, $J_{4,5}$ = 5.0 Hz, $J_{3,4}$ = 5.0 Hz, H-4) 4.80-5.0 (m, 2, H-9, H-10), 5.35 (d, 1, NH), 6.60 (s, 1, H-1), 10.0 (br, 1, OH).

For **9**: $[\alpha]_D^{20}$ + 44.50 [c, 0.4, $CHCl_3$]. R_f 0.36 (EtOAc). $\nu_{max}^{CHCl_3}$ 3300 (OH, NH), 1750 (ester >C=O, lactone>C=O), 1660 (amide >C=O), 1515 cm^{-1} (amide II). ^1H NMR ($CDCl_3$, 250 MHz), δ 1.30 (d, 3, $J_{5,6}$ = 6.0 Hz, $6CH_3$), 2.0 (s, 3, $NHCOC\underline{H}_3$), 2.01 (2s, 6, $OCOCH_3$), 2.55 (m, 1, H-8ax), 3.0 (m, 2, H-2, H-3), 3.01 (dd, 1, $J_{8ax-8eq}$ = 18.0 Hz, H-8eq), 4.10 (q, 1, H-7), 4.50 (m, 2, H-4, H-5), 5.0 (m, 2, H-9, H-10), 5.50 (d, 1, NH) (br, 1, OH).

HRMS Calcd. for $C_{16}H_{23}O_8N$; 357.1418. Found: 357.1418.

Dehydration of **9** to the α,β-unsaturated lactone **11**. To a stirred solution of compound **9** (10 mg, 0.028 mmol) in dry benzene (2 mL) was added a catalytic amount of p-toluene sulfonic acid. After standing at room temperature for 3 h, the solvent was evaporatored and the residue was chromatographed on silica gel using EtOAc as eluent. Compound **11** was recovered as a syrup (7 mg, 74% yield). $[\alpha]_D^{20}$ + 44.66° [c, 0.15, $CHCl_3$]. R_f 0.56 (EtOAc). $\nu_{max}^{CHCl_3}$ 3500 (NH), 1750 (ester >C=O), 1710 (α,β-unsaturated lactone), 1600 (amide >C=O), 1530 cm^{-1} (amide II). ^1H NMR, δ 1.30 (d, 3, $J_{5,6}$ = 6.50 Hz $6CH_3$), 2.0 (s, 3, $NHCOC\underline{H}_3$), 2.01 (2s, 6, $OCOCH_3$), 2.55 (dddd, 1, H-8ax), 3.0 (m, 1, H-3), 3.10 (dd, 1, $J_{8ax-8eq}$ = 18.0 Hz, H-8eq), 4.10 (t, 1, H-7), 4.55 (m, 2, H-4, H-5), 4.98-5.15 (m, 2, H-9, H-10), 5.80 (d, 1, NH). m/e 340 (M + 1), 295 (M^+ - $COCH_3$).

Dehydration of **8** to the diene **10**. To a stirred solution of compound **8** (10 mg, 0.278 mmol) in dry benzene (2 mL) was added a catalytic amount of p-toluene sulfonic acid. After 3 h at room temperature, the solvent was removed and the residue was chromatographed on silica gel using EtOAc as eluent to afford compound **10** (8.08 mg, 90% yield) as an oil: R_f 0.40 (EtOAc). $\nu_{max}^{CHCl_3}$ 3400 (NH), 1740 (ester >C=O), 1650 (amide >C=O), 1600 (conjugated C=C) 1500 cm^{-1} (amide II). $\nu_{max}^{CH_3OH}$ 247 nm (ε max 1.52 x 10^4). ^1H NMR, δ 1.20 (d, 3, $J_{5,6}$ = 6.0 Hz, $6CH_3$), 2.0 (s, 3, $NHCOC\underline{H}_3$), 2.01 (2s, 6, $OCOCH_3$), 2.90 (m, 1, H-3), 4.01 (q, 1, H-5), 4.50 (dd, 1, H-4), 5.0 (dd, 1, $J_{3,10}$ = 8.0 Hz, $J_{9,10}$ = 8.0 Hz, H-10), 5.30 (dd, 2, H-8, H-9), 5.60 (d, 1, NH), 6.08 (dd, 1, $J_{7,8}$ = 6.50 Hz, H-7), 6.55 (s, 1, H-1).

HRMS Calcd. for $C_{16}H_{21}O_6N$; 323.1361. Found: 323.1361.

Preparation of the glycoside **12**. To a solution of compound **8** (20 mg, 0.0577 mmol) in dry CH_3OH (2 mL) was added anhydrous methanolic hydrogen chloride (0.25 mL, 1% solution), and the reaction mixture was stirred at room temperature for 12 h under argon. The solution was neutralized by addition of Et_3N at 0°, the solvents were removed, and the residue was chromatographed on a silica gel column using EtOAc: petroleum ether: EtOH (5:5:1) as eluent. Removal of the solvent gave **12** (7.2 mg, 36% yield), as an oil: R_f 0.33 (EtOAc). ν_{max}^{neat} 3400 (OH, NH), 1730 (ester >C=O), 1650 (amide >C=O), 1510 cm^{-1} (amide II). ^1H NMR, δ 1.10 (d, 3, $J_{5,6}$ = 6.50 Hz, $6CH_3$), 1.90 (m, 1, H-8ax), 2.0 (s, 3, $NHCOC\underline{H}_3$), 2.01 (2s, $OCOCH_3$), 2.10 (m, 1, H-8eq), 2.65 (m, 1, H-2), 3.10 (q, 1, H-3), 3.30 (s, 3, OCH_3), 3.90 (m, 1, H-5), 4.0 (m, 1, H-4), 4.50 (m, 1, H-7), 4.80 (s, 1, H-1), 5.0 (m, 1, H-9), 5.45 (m, 1, H-10). HPLC showed that the major component had a retention time of 10.81 minutes in 2% isopropanol in hexane. The synthetic 1α, 7β isomer[6] had a retention time of 10.83 minutes in the same solvent system.

HRMS Calcd. for $C_{17}H_{27}O_8N$: 373.1733. Found: 373.1733.

Hydrolysis of the glycoside 12 to give compound 8. To a solution of compound 12 (10 mg, 0.0268 mmol) in a 4:1 mixture of dioxane and water (2 mL), was added aqueous sulfuric acid (25 μL, 2% solution), and the reaction mixture was heated in an oil bath at 50° for 3 h. The reaction mixture was then extracted with Et_2O (3 x 10 mL), and the combined extracts were washed with brine (2 x 5 mL), and dried over (Na_2SO_4). The recovered product was chromatographed on silica gel using EtOAc as eluent. Removal of the solvent gave a compound (4 mg, 42% yield) which was identical to that obtained from sodium borohydride reduction of 7.

Oxidation of compound 8 to 7. A complex of dimethyl sulfoxide (10 μL, 0.0141 mmol) and trifluoroacetic anhydride (10.92 μL, 0.028 mmol) was prepared at -78°, and compound 8 (10 mg, 0.0282 mmol) in dry CH_2Cl_2 (2 mL), was added. The reaction mixture was stirred at -78° for 1 h, and then Et_3N (10.66 μL, 0.028 mmol) was added. After 15 min, the cooling bath was removed and the reaction mixture was allowed to warm up at room temperature. The mixture was extracted with CH_2Cl_2 (3 x 10 mL), washed with $NaHCO_3$ solution and dried (Na_2SO_4). Filtration and evaporation of the solvent gave a syrupy product which was purified by column chromatography on silica gel using EtOAc as eluent. The recovered material 7, (7 mg, 70% yield) which was identical to that obtained by direct acetylation of desalanylactionobolin hydrochloride 3.

Synthesis of N-acetyldesalanylactinobolin (13). To a solution of compound 7 (10 mg, 0.0281 mmol) in MeOH was added sodium methoxide (3 mg, 0.055 mmol), and the reaction mixture was stirred at room temperature for 15 min. The solvent was evaporated and the residue was chromatographed on silica gel using EtOAc: petroleum ether: EtOH (5:5:1) as eluent, to give 13 as a waxy material for (7 mg, 80% yield): $[\alpha]_D^{20}$ + 15.02 (c, 0.1; $CHCl_3$). R_f 0.28 (EtOAc). ν_{max}^{neat} 3400 (OH, NH), 1730 (lactone 3 >C=O), 1650 (amide >C=O), 1510 cm^{-1} (amide II). ^1H NMR, δ 1.35 (d, 3, $J_{5,6}$ = 6.50 Hz, 6-CH_3), 2.10 (s, 3, NHCOCH_3), 2.40 (m, 1, H-8ax), 2.60 (m, 1, H-3), 2.90 (dd, 1, $J_{8ax-8eq}$ = 18.0 Hz, H-8eq), 3.10 (t, 1, H-10), 3.90 (m, 1, H-9), 4.30 (m, 1, H-4), 4.60 (q, 1, H-5), 4.80 (br, 1, OH), 5.8 (d, 1, NH), 10.0 (br, 1, enolic OH).

HRMS Calcd. for $C_{12}H_{17}O_6N$; 271.1043. Found: 271.1043.

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