Total Synthesis of (±)-Leuhistin

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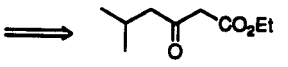
An efficient and diastereoselective total synthesis of the natural product leuhistin is described. The synthesis proceeds in nine steps and 9.4% overall yield starting with isovaleraldehyde.

The enzyme aminopeptidase M, found in membranes of a variety of eukaryotic cells, has attracted considerable interest as a potential drug target because of its function as an enkephalin-degrading protease.[1] This attention has led to the discovery of potent natural product inhibitors of aminopeptidase M, such as actinonin[2] and probestin.[3] More recently, the isolation and characterization of leuhistin (1), a potent and highly specific inhibitor of aminopeptidase M, was reported.[4] We desired a sample of leuhistin for testing in a variety of biological assays; we now report an efficient total synthesis of (±)-1.

Our retrosynthetic analysis envisioned late installment of the amino group by a formal reductive amination of ketone 2; compound 2 in turn would be derived from a double functionalization (alkylation and oxidation) of β-keto ester 3.

leuhistin (1) 2



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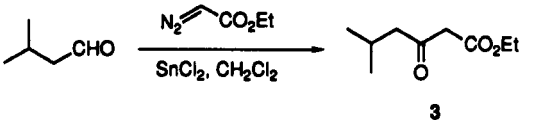
Reaction of isovaleraldehyde with ethyl diazoacetate and tin(II) chloride in dichloromethane affords β-keto ester 3 in quantitative yield.[5] Alkylative oxidation of 3 with benzoyl peroxide (NaH, benzene; 74%) affords benzoyloxy derivative 4. Further alkylation with (chloromethyl)-imidazole hydrochloride[6] 5 (NaOEt, EtOH; 65%) provides compound 6, in which the entire carbon skeleton of leuhistin is intact.

Although conversion of compound 6 to the natural product requires only a reductive amination and two ester hydrolyses, the execution of the former proved to be problematic. Standard conditions of sodium cyanoborohydride and ammonium acetate in methanol gave only slow reduction to the corresponding alcohol; use of titanium



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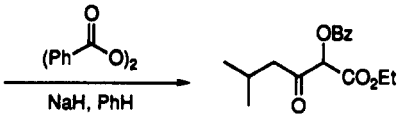

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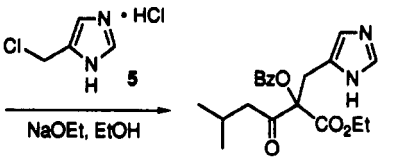

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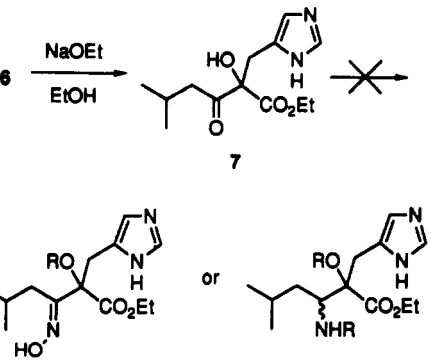
isopropoxide[7] to activate the ketone (benzylamine, sodium cyanoborohydride) proved equally unsuccessful. Treatment of either compound 6 or its debenzoyl derivative 7 with hydroxylamine hydrochloride under a variety of conditions (ethanol, sodium carbonate, reflux; acetic acid, reflux; pyridine, reflux) gave only unreacted starting material and none of the desired oxime.



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Conversion of intermediate 4 to its oxime methyl ether 8 appeared to be a convenient way to insert the desired nitrogen atom. However, alkylation of 8 with (chloromethyl)imidazole hydrochloride could not be effected.

Treatment of compound 7 with hydrazine hydrochloride in pyridine at reflux affords cyclic derivative 9. Although at first we were greatly encouraged by this result, all attempts to effect reduction of the carbon–nitrogen double bond were unsuccessful. Conditions of sodium in am-

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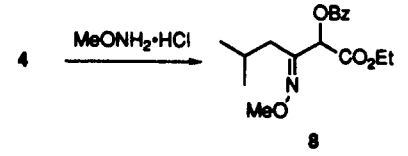

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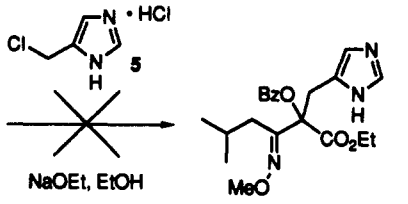
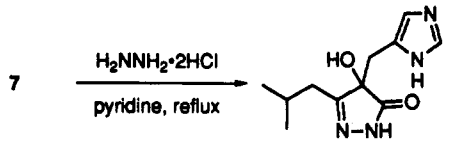


monia, with or without added *tert*-butyl alcohol, cause reduction but concomitant dehydration, giving compound **10**. A wide variety of other reducing agents were tried, without success (sodium cyanoborohydride, sodium triacetoxyborohydride, borane–dimethyl sulfide, Raney nickel, lithium aluminum hydride, lithium triethylborohydride).



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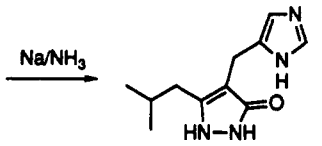
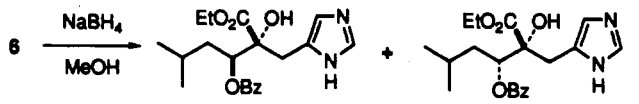


10

Having failed to achieve direct reductive amination of the ketone, reduction to an alcohol, followed by activation and displacement with a nitrogen nucleophile, appeared to be a viable alternative approach. Reduction of ketone **6** using sodium borohydride in methanol affords a ca. 3:1 mixture of diastereomeric products **11** and **12** (83%), in which the benzoate group has migrated to the newly formed secondary alcohol; these structure assignments are based on an NOE study of an epoxide derived from compound **11** (vide infra).

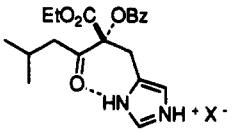


11 12

A variety of reducing agents and conditions were examined in order to improve the diastereoselectivity of the reduction of compound **6** (Table I). Lowering the reaction temperature to −78 °C affords a slight increase in selectivity (entry 2). However, decreasing the solvent polarity (entry 3), use of a chelating reducing agent (entry 4), and use of a bulky reducing agent (entry 5) provide no improvement. Ultimately, we were gratified to discover that sodium borohydride reduction of the preformed trifluoroacetate salt of **6** results in almost exclusive conversion to compound **11** (entry 6). Although our rationale for performing the reduction of a salt of compound **6** was to promote activation of the carbonyl by intramolecular hydrogen bonding, as in **13**, the actual cause of our improved selectivity has not been established.

Table I. Conditions for Reduction of Compound 6

entry	conditions	ratio (11:12)
1	$NaBH_4$, MeOH, 0 °C	3:1
2	$NaBH_4$, MeOH, −78 °C	4:1
3	$NaBH_4$, 5% MeOH/CH_2Cl_2, −78 °C	2.5:1
4	$ZnBH_4$, Et_2O, 0 °C	1.5:1
5	$LiEt_3BH$, THF, −78 °C	1:1.5
6	TFA; $NaBH_4$, MeOH, 0 °C	19:1



13

Cleavage of the benzoate ester of **11** with sodium ethoxide in ethanol proceeds cleanly to provide diol **14** in 92% yield (Scheme I). Attempts to activate the secondary alcohol of **14** by treatment with methanesulfonyl chloride taught us that, although sulfonylation of the imidazole nitrogen is rapid, reaction of the hindered secondary alcohol is quite slow. We therefore turned to the more reactive sulfonylation agent, trifluoromethanesulfonic anhydride (triflic anhydride, Tf_2O). Treatment of a solution of diol **14** (pyridine, dichloromethane) with triflic anhydride directly affords epoxide **15**. An NOE experiment on **15** showed significant enhancement between the epoxide proton and the methylene protons adjacent to the imidazole, establishing that these groups are cis to each other. Assuming that the mechanism of epoxide formation involves activation of the secondary alcohol followed by nucleophilic closure by the tertiary alcohol, this allows one to conclude that the major diastereomer from sodium borohydride reduction is the (2*RS*,3*SR*) isomer **11**. Furthermore, this is the correct diastereomer for synthesis of leuhistin if epoxide **15** can be opened at C(3) by a nitrogen nucleophile.

Upon scaleup, the conversion of diol **14** to epoxide **15** under the conditions described above proved to be somewhat capricious. Collidine was examined as an alternative base, and we were surprised to observe that this leads to a much more complex reaction mixture. Ultimately, we discovered that this reaction proceeds best when the pyridine and triflic anhydride are premixed and allowed to react together at 0 °C; cooling to −78 °C, addition of diol **14**, and warming to room temperature allows isolation of epoxide **15** in a reproducible 69% yield.

Treatment of **15** with sodium azide and ammonium chloride in refluxing methanol effects cleavage of the *N*-triflate as well as epoxide opening, affording azide **16** (81%). After trying numerous methods for azide reduction (thioacetic acid,[8] hydrogenation, tin chloride,[9] propanethiol,[10] triphenylphosphine[11]), we ultimately settled upon transfer hydrogenation[12] with formic acid in ethanol over palladium on carbon, which provides amine **17** in 50% yield. Saponification of the ethyl ester with aqueous

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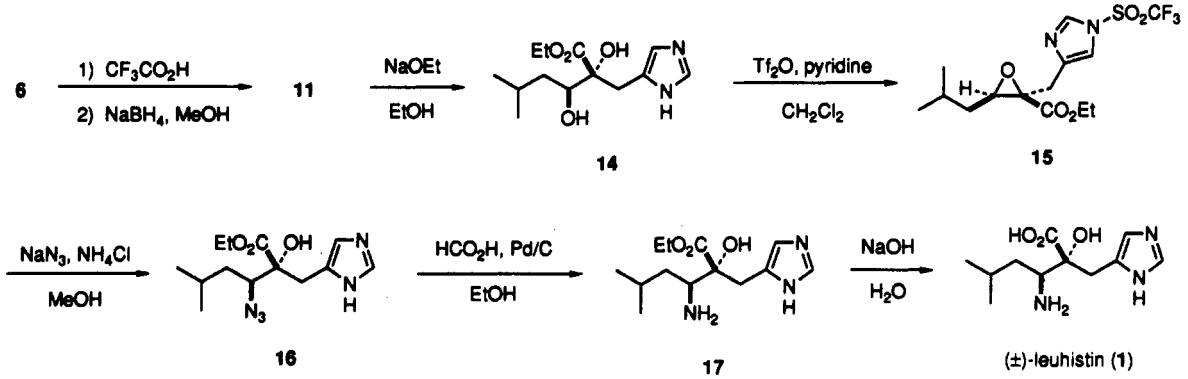
Scheme I



sodium hydroxide, followed by ion-exchange chromatography, affords (±)-leuhistin in 94% yield. The ^1H and ^{13}C NMR data for our material are in excellent agreement[13] with the reported values for the natural product.[4]

In summary, we have described an efficient and diastereoselective total synthesis of (±)-leuhistin. Our synthesis proceeds in nine steps and 9.4% overall yield from isovaleraldehyde and utilizes a minimum of protecting groups. This synthesis should prove useful in the preparation of significant quantities of material for biological testing and also could be adapted easily to the preparation of analogs.

Experimental Section

General. Unless stated otherwise, solvents and reagents were obtained from commercial suppliers and were used without further purification. ^1H NMR spectra were determined at 300 MHz and unless otherwise specified were recorded in $CDCl_3$. J values are in hertz. IR values are in inverse centimeters. In the standard reaction workup, the organic solution containing the product was dried over $MgSO_4$ and filtered, and the solvent was removed with a rotary evaporator.

Benzoic Acid 1-(Ethoxycarbonyl)-4-methyl-2-oxopentyl Ester (4). To a stirring suspension of sodium hydride (1.30 g of a 60% dispersion in oil, 32.5 mmol) in benzene (150 mL) at rt was added ethyl 5-methyl-3-oxohexanoate[5] (3; 11.2 g, 65.0 mmol). After 30 min, benzoyl peroxide (7.86 g, 32.5 mmol) was added. The mixture was stirred for 3.5 h. Chloroform was added, and the mixture was washed with 1 M aqueous H_3PO_4, saturated $NaHCO_3$, and brine. After standard workup, the crude product was purified by flash chromatography on silica gel (520 g, 4% ethyl acetate/hexane) to afford 7.00 g (74%) of 4 as a colorless oil. IR ($CHCl_3$): 2970, 1735, 1250, 1115. ^1H NMR: δ 0.96 (d, 3, J = 6), 0.99 (d, 3, J = 6), 1.33 (t, 3, J = 7), 2.27 (m, 1), 2.60 (dd, 1, J = 7, 16), 2.69 (dd, 1, J = 7, 16), 4.32 (q, 2, J = 7), 5.71 (s, 1), 7.50 (bt, 2, J = 8), 7.63 (bt, 1, J = 8), 8.15 (dd, 2, J = 1, 8). Anal. Calcd for $C_{16}H_{20}O_5$; C, 65.74; H, 6.90. Found: C, 66.04; H, 6.54. ^{13}C NMR: 199.5;, 165.1, 164.6, 133.8, 130.0, 128.6, 78.1, 62.4, 48.5, 24.0, 22.4, 22.3, 14.0.

Benzoic Acid 1-(Ethoxycarbonyl)-1-(3*H*-imidazol-4-ylmethyl)-4-methyl-2-oxopentyl Ester (6). To a stirring solution of sodium ethoxide (100 mL of a 0.378 M solution) was added compound 4 (5.50 g, 18.9 mmol). The mixture was cooled to 0 °C and 4-(chloromethyl)imidazole hydrochloride[6] (5; 2.87 g, 18.9 mmol) was added. After 1 h at 0 °C, the mixture was allowed to warm to rt and stir for 30 min. Chloroform was added, and the mixture was washed with water. After standard workup, the crude product was purified by flash chromatography on silica gel (320 g, 4% methanol/chloroform) to afford 4.60 g (65%) of 6 as a yellow oil. IR ($CHCl_3$): 3462, 2960, 1730, 1280, 1098. ^1H NMR: δ 0.89 (d, 6, J = 7), 1.22 (t, 3 J = 7), 2.18 (m, 1), 2.49 (dd, 1, J = 7, 18), 2.72 (dd, 1, J = 7, 18), 3.69 (d, 1, J = 14), 3.79 (d, 1, J 14), 3.79 (d, 1, J = 14), 4.23 (q, 2, J = 7), 6.70 (s, 1), 7.45 (s, 1), 7.45 (bt, 2, J = 8), 7.58 (bt, 1, J = 8), 7.97 (bd, 2, J = 8). HRMS: calcd for $C_{20}H_{24}N_2O_5$ 372.1685, found 372.1657. ^{13}C NMR 203.3, 166.7, 164.9, 134.9, 133.5, 129.7, 129.1, 128.5, 118.1, 88.3, 62.2, 47.6, 30.9, 23.4, 22.3, 13.8.

(1'*RS*,1*SR*)-Benzoic Acid 1-[1'-(Ethoxycarbonyl)-1'-hydroxy-2'-(3*H*-imidazol-4-yl)ethyl]-3-methylbutyl Ester (11). To a stirring solution of compound 6 (2.47 g, 6.64 mmol) in methylene chloride (20 mL) was added trifluoroacetic acid (0.563 mL, 7.31 mmol). The solvent was removed on a rotary evaporator. The resulting solid was dissolved in methanol (25 mL) and the solution was cooled to 0 °C. To the stirring solution was added sodium borohydride (326 mg, 8.62 mmol). After 0.5 h, additional sodium borohydride (125 mg, 3.32 mmol) was added, and the reaction was stirred for 20 min. Chloroform was added, the mixture was washed with saturated sodium bicarbonate solution and brine. Standard workup afforded a mixture of compound 11 and its precursor in which the benzoate had not migrated. The mixture was dissolved in chloroform (20 mL) and heated to 50 °C for 15 min. The solvent was removed on a rotary evaporator to afford 2.01 g (81%) of compound 11 and its diastereomer 12 (in a 19:1 ratio, as determined by ^1H NMR) as a yellow solid, mp 49–54 °C. IR ($CHCl_3$): 3452, 2945, 1725, 1240, 1090. ^1H NMR: δ 0.91 (d, 3, J = 6), 1.00 (d, 3, J = 6), 1.12 (t, 3, J = 7), 1.60 (t, 1, J = 10), 1.59 (m, 1), 1.85 (t, 1, J = 10), 2.99 (d, 1, J = 14), 3.18 (d, 1, J = 14), 4.03 (q, 2, J = 7), 5.57 (d, 1, J = 10), 6.79 (s, 1), 7.40 (bt, 2, J = 8), 7.49 (s, 1), 7.53 (bt, 1, J = 8), 8.00 (dd, 2, J = 1,8). HRMS: calcd for $C_{20}H_{26}N_2O_5$ 374.1841, found 374.1936. ^{13}C NMR: 173.5, 165.8, 135.0, 133.2, 131.1, 129.8, 129.7, 128.4(2), 118.9, 80.2, 75.5, 62.0, 38.3, 32.4, 24.4, 23.8, 21.6, 14.0.

(2*RS*,3*SR*)-2,3-Dihydroxy-2-(3*H*-imidazolyl-4-ylmethyl)-5-methylhexanoic Acid Ethyl Ester (14). To a solution of sodium ethoxide (4.50 mL of a 0.241 M solution) was added a solution of compound 11 (340 mg, 0.909 mmol) in ethanol (2.00 mL). The mixture was stirred for 0.5 h. Chloroform was added, and the mixture was washed with water. The aqueous layer was extracted three times with chloroform and the organic layers were combined. After standard workup, the crude product was purified by flash chromatography on silica gel (20 g, 10% methanol/chloroform) to afford 225 mg (92%) of 14 as a colorless oil. IR ($CHCl_3$): 3460, 3280, 2930, 1725, 1365, 1230, 1070. ^1H NMR: δ 0.85 (d, 3, J = 6), 0.91 (d, 3, J = 6), 1.16 (t, 3, J = 7), 1.31 (ddd, 1, J = 2, 8, 12), 1.42 (ddd, 1, J = 2, 8, 12), 1.83 (m, 1), 2.92 (d, 1, J = 14), 3.02 (d, 1, J = 14), 3.76 (dd, 1, J = 2, 10), 4.10 (q, 2, J = 7), 6.71 (s, 1), 7.42 (s, 1). HRMS: calcd for $C_{13}H_{22}N_2O_4$ 270.1579, found 270.1615. ^{13}C NMR: 174.7, 135.0, 131.2, 119.0, 81.3, 73.4, 61.7, 39.8, 31.8, 24.4, 24.0, 21.3, 14.1.

(2*RS*,3*RS*)-3-Isobutyl-2-[[1-[(trifluoromethyl)sulfonyl]-1*H*-imidazol-4-yl]methyl]oxirane-2-carboxylic Acid Ethyl Ester (15). To a stirred solution of pyridine (1.42 mL, 17.6 mmol) in methylene chloride (15 mL) at 0 °C was added trifluoromethanesulfonic anhydride (1.45 mL, 8.64 mmol). After stirring at 0 °C for 10 min, the mixture was cooled to −78 °C and a solution of compound 14 (1.06 g, 3.92 mmol) in methylene chloride (5 mL) was added dropwise by addition funnel. The mixture was allowed to warm to rt. After 1 h at rt, methylene chloride was added, and the mixture was washed with water. After standard workup, the crude product was purified by flash chromatography on silica gel

(13) Our ^{13}C NMR chemical shifts are all exactly 1.9 (± 0.1) ppm downfield of the published values, for which no internal standard is reported (ref 4).

(20 g, 10% ethyl acetate/hexane) to afford 1.05 g (69%) of **15** as a yellow oil. IR ($CHCl_3$): 3120, 2950, 1740, 1415, 1220, 1130, 1055. ^1H NMR: δ 0.90 (d, 3, J = 7), 0.93 (d, 3, J = 7), 1.20 (t, 3, J = 7), 1.48 (m, 2), 1.79 (m, 1), 2.82 (d, 1, J = 14), 3.08 (t, 1, J = 7), 3.53 (d, 1, J = 14), 4.19 (q, 2, J = 7), 7.20 (s, 1), 7.88 (s, 1). HRMS: calcd for $C_{14}H_{20}N_2O_5F_3S$ 385.1045, found 385.1040. ^{13}C NMR: 168.8, 141.3, 137.3, 115.8, 62.2, 61.6, 61.0, 36.3, 32.2, 26.3, 22.6, 22.2, 14.1.

(2*RS*,3*SR*)-3-Azido-2-hydroxy-2-(3*H*-imidazol-4-ylmethyl)-5-methylhexanoic Acid Ethyl Ester (16). To a stirred solution of epoxide **15** (960 mg, 2.49 mmol) in a mixture of methanol (8 mL) and water (1 ml) were added ammonium chloride (290 mg, 5.48 mmol) and sodium azide (810 mg, 12.5 mmol). The mixture was heated at reflux for 14 h and cooled to rt. Chloroform was added, the mixture was washed with water, the aqueous phase was extracted three times with chloroform, and the organic layers were combined. After standard workup, the crude product was subjected to flash chromatography on silica gel (40 g, 5% methanol/chloroform) to afford 700 mg (95%) of **16** as a yellow oil. IR ($CHCl_3$): 3460, 3280, 2955, 2100, 1730, 1230. ^1H NMR: δ 0.95 (d, 3, J = 6), 1.00 (d, 3, J = 6), 1.23 (t, 3, J = 7), 1.46 [(ddd, 1, J = 2, 8, 12), 1.77 (ddd, 1, J = 2, 8, 12), 1.82 (m, 1), 2.97 (d, 1, J = 14), 3.06 (d, 1, J = 14), 3.46 (dd, 1, J = 2, 12), 4.17 (q, 2, J = 7), 6.79 (s, 1, 7.48 (s, 1), 7.75 (br s, 1). Anal. Calcd for $C_{13}H_{21}N_5O_3$: C, 52.87; H, 7.17; N, 23.71. Found: C, 53.14; H, 6.99; N, 23.32. ^{13}C NMR: 173.7, 135.1, 131.6, 118.0, 81.3, 64.7, 62.1, 36.3, 32.9, 25.1, 23.6, 21.2, 14.0.

(2*RS*,3*SR*)-3-Amino-2-hydroxy-2-(3*H*-imidazol-4-ylmethyl)-5-methylhexanoic Acid Ethyl Ester (17). To a solution of azide **16** (118 mg, 0.400 mmol) in ethanol (2 mL) were added palladium on carbon (10%, 300 mg) and formic acid (151 μL, 4.00 mmol). The reaction mixture was heated at 50 °C for 45 min. The reaction was filtered through filter paper and was washed with hot methanol. The solvent was removed on a rotary evaporator. The crude product was purified by flash chromatography on silica gel (6 g, 0.2:4:96 aqueous NH_4OH/MeOH/$CHCl_3$) to afford 54 mg (50%) of **17**. IR ($CHCl_3$): 3458, 3250, 2940, 1725, 1230, 1090. ^1H NMR: δ 0.90 (d, 3, J = 6), 0.97 (d, 3, J = 6), 1.18 (t, 3, J = 7), 1.28 (ddd, 1, J = 2, 8, 12), 1.40 (ddd, 1, J = 2, 8, 12), 1.80 (m, 1), 2.92 (t, J = 8), 2.92 (d, 1, J = 14), 3.06 (d, 1, J = 14), 4.12 (q, 2, J = 7), 6.81 (s, 1), 7.52 (s, 1). HRMS: calcd for $C_{13}H_{23}N_3O_3$ 269.1739, found 269.1762. ^{13}C NMR: 176.8, 136.7, 133.2, 121.4, 83.0, 63.5, 57.4, 42.5, 34.7, 26.7, 25.6, 22.5, 15.4.

(±)-Leuhistin (1). To a stirred solution of compound **17** (62.0 mg, 0.230 mmol) in methanol (460 μL) was added 1 N aqueous sodium hydroxide solution (460 μL). The reaction mixture was heated at 50 °C for 2 h. The solvent was removed on a rotary evaporator. The crude reaction mixture was loaded onto 15 mL of Amberlite IR 120+ ion-exchange resin. The product was eluted with aqueous ammonium hydroxide/methanol/water (2:47:51), to afford 52.1 mg (94%) of **1**. A solution of acetyl chloride (14.7 μL, 0.207 mmol) in methanol (500 μL) was stirred for 0.5 h. To this solution was added a solution of **1** (50 mg, 0.207 mmol) in water (300 μL). The solvent was removed with a rotary evaporator to afford 57 mg of leuhistin hydrochloride. ^1H NMR (500 MHz, D_2O): δ 0.95 (d, 3, J = 7). 1.00 (d, 3, J = 7), 1.58 (bt, 1, J = 12), 1.70 (bt, 1, J = 12), 1.73 (m, 1), 3.02 (d, 1, J = 14), 3.23 (d, 1, J = 14) 3.54 (dd, 1, J = 2, 12), 7.26 (s, 1), 8.59 (s, 1). HRMS: calcd for $C_{11}H_{19}N_3O_3$·HCl 242.1504, found 242.1451. ^{13}C NMR (D_2O, ppm downfield from DSS (sodium 3-(trimethylsilyl)-1-propanesulfonate):[13] δ 178.7, 135.8, 130.6, 119.9, 80.1, 57.7, 39.1, 33.1, 26.7, 25.5, 22.8.

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Supplementary Material Available: NMR spectra for compounds **1**, **4**, **6**, **11**, and **14–17** (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.