solution of cinnamaldehyde was added dropwise over 10 min. After nitrogen evolution had stopped[12] (~1 h) the reaction was transferred to a separatory funnel with saturated brine (NaCl, 40 mL)[13] and extracted with diethyl ether (2×, 80 mL).[14] The organic layers were combined and dried ($MgSO_4$) and the volatiles removed under vacuo. The remaining oil was either chromatographed on silica gel 60 (hexane/ethyl acetate) or the smaller keto esters could be distilled (the higher molecular weight ones tend to undergo a fair amount of decomposition).

(12) Aromatic and tertiary aldehydes reacted much slower with only a very moderate rate of nitrogen evolution. When initial nitrogen evolution had stopped with these substrates the reactions were somewhat cloudy and still had a greenish color, characteristic of the diazoacetate. We added an additional amount of tin(II) chloride to these reactions and observed additional nitrogen evolution. This procedure was repeated until there was no longer any nitrogen evolution observed.

(13) To this was added 1% KOH, which helped in reducing or eliminating the emulsion formed from the tin chloride. This was not used in every case to avoid any base-catalyzed reactions.

(14) Alternatively the methylene chloride can be removed in vacuo and the crude product chromatographed directly.

Articles

Total Synthesis of 3(*S*)-Carboxy-4(*S*)-hydroxy-2,3,4,5-tetrahydropyridazine, an Unusual Amino Acid Constituent of Luzopeptin A†

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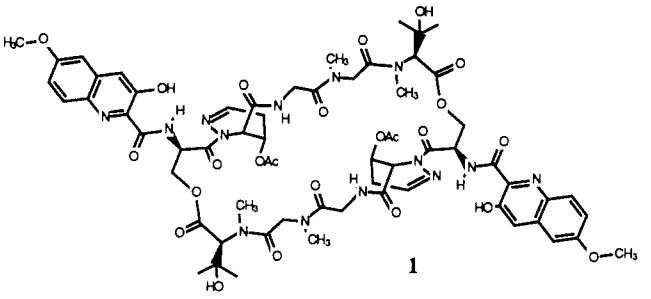
The enantiospecific synthesis of 3(*S*)-carboxy-4(*S*)-hydroxy-2,3,4,5-tetrahydropyridazine (2), a novel constituent of the antibiotic antitumor agent luzopeptin A is described. Structural corroboration of the synthetic compound is based on three points. The ^{13}C NMR spectrum of synthetic **2** was compared with that of a similar degradation product of luzopeptin. The ^{1}H NMR spectrum of **2** was compared with that of the synthetic cis isomer. Finally, the synthetic methodology developed for the synthesis of **2** was applied to the synthesis of the known L-*trans*-3-hydroxyproline.

Introduction

An increasingly popular approach to the development of therapeutic agents involves the design of compounds that closely mimic natural peptide hormones or substrates. Generally, this entails reducing the natural peptide to a minimally required size and replacing normal amino acids and peptide regions with synthetic analogues that confer desired properties such as enhanced binding, hydrolytic stability, or antagonism verses agonism. New nonstandard amino acids, which might serve to replace normal amino acids, are often discovered in natural products. Such compounds also offer, besides their possible therapeutic utility, challenging synthetic targets.

A soil screening program at the Bristol-Banyu Research Institute in Tokyo, aimed at finding new compounds with promising antitumor activity, recently led to the isolation of a series of antitumor antibiotics from *Actinomadura luzonesis*. Chemical degradation studies by Konishi and co-workers[1] and single-crystal X-ray diffraction studies by Arnold and Clardy[2] defined the structure of the major and most active compound as **1**, originally named BBM-928A but since renamed luzopeptin A.

Luzopeptin, a dimeric cyclic depsipeptide, is a bis-intercalator of DNA and this is thought to play a role in its activity.[3] The six unique constituents of luzopeptin are four known amino acids, a new quinoline, and an interesting new "amino acid": 2(*S*)-carboxy-3(*S*)-hydroxy-2,3,4,5-tetrahydropyridazine (**2**). This ring system was previously unknown in nature though it has since been found in the cirratiomycin antibiotics.[4] The hexahydropyridazine carboxylic acids are known, occurring in the monamycin antibiotics.[5] The synthesis of luzopeptin analogues where the pyridazine has been replaced with a simpler amino acid has also been reported.[6] We report here an enantioselective synthesis of this new amino acid.



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† Taken from the thesis of P. Hughes, Cornell University, 1983.

‡ Present address: Wyeth-Ayerst Research, Box CN 8000, Princeton, NJ 08543-8000.

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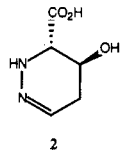


2

Results

Because hydrazines and their derivatives are sensitive to oxidation and reduction, and the state of the art for the formation of N–N bonds is poor,[7] it was felt that the best approach to tetrahydropyridazine **2** would involve addition of hydrazine to a five-carbon unit at the oxidation state of the final product. This could be accomplished by a two-step addition of hydrazine to glycidic acid **3** via epoxide opening and subsequent hydrazone formation (Scheme I). The chirality of glycidic acid **3** could be derived from allylic alcohol **4** via the chiral Sharpless epoxidation.

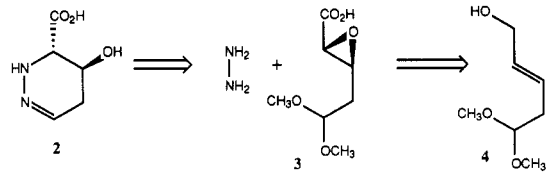
Synthesis of glycidic acid **3** was straightforward (Scheme II). Treatment of malonaldehyde dimethyl acetal[8] with the Emmons–Horner–Wadsworth reagent, trimethyl phosphonoacetate, in methanolic potassium methoxide gave ester **5** in quantitative yield. Only trans product was seen by ^{1}H NMR. Attempted reduction of ester **5** with LAH led to numerous products. However, DIBAL cleanly reduced ester **5** to the desired allylic alcohol **4** (87% yield).

Using the procedure of Sharpless,[9] titanium-mediated epoxidation of allylic alcohol **4** using L-(+)-diethyl tartrate gave the desired epoxy alcohol **6** (81% yield). The sodium fluoride workup[10] was used due to the hydrophilicity of the product.

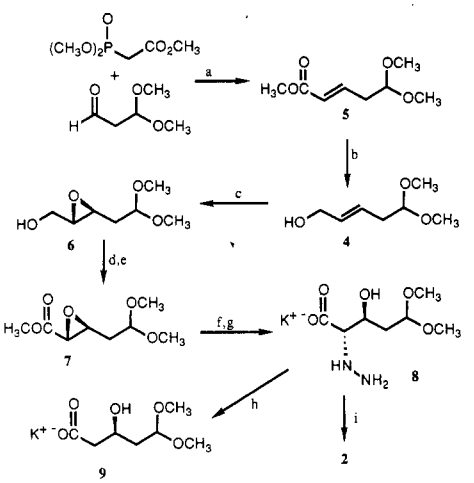
Epoxy alcohol **6** was oxidized to glycidic acid with use of ruthenium tetraoxide[11] and then esterified with diazomethane to give the more stable glycidic ester **7** (70% yield from **6**). Analysis of ester **7** by 300-MHz ^{1}H NMR with a chiral shift reagent (Eu(tfc)$_3$) showed an enantiomeric excess of >97%. The ^{1}H NMR spectrum of the chiral ester showed only two small extraneous singlets (≈1%), which arise from the methyls of the enantiomer. Other signals from the enantiomer were undetectable. The ^{1}H NMR spectrum of racemic glycidic ester **7**, prepared from MCPBA oxidation of **5**, showed well-resolved peaks at similar concentrations of ester and shift reagent.

The glycidic ester **7** was then converted in one pot to the final product, tetrahydropyridazine **2**. Saponification of glycidic ester **7** with potassium carbonate in aqueous methanol gave the potassium salt of glycidic acid **3**. The saponification mixture was concentrated and treated with hydrazine hydrate (30 equiv) and stirred under nitrogen at room temperature for 24 h. ^{1}H NMR analysis of the reaction mixture (after hydrazine removal under vacuum) showed clean epoxide opening by hydrazine at the α-position to give hydrazino acid **8**. None of the isomer arising from opening at the β-position was detected. The hydrazino acid **8** proved to be somewhat unstable. On standing, with occasional exposure to air, **8** was converted over a few days to β-hydroxy acid **9**, probably by oxidation to the azo derivative followed by β-elimination of nitrogen.[12] However, the hydrazino acid **8**, if used promptly, posed no stability problems. The hydrazino acid **8** was redissolved in water, titrated to pH = 1.3 with trifluoroacetic acid and stirred at room temperature until starting material was not detectable by TLC (usually about 30 min). It is important that the reaction not run longer than

Scheme I

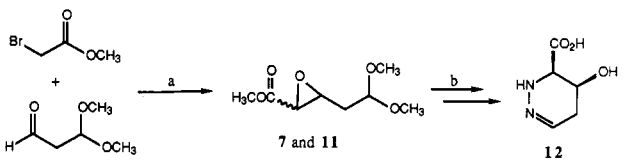


Scheme II[a]



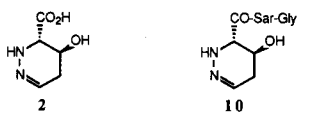
[a] (a) $KOCH_3$, CH_3OH; (b) DIBAL; (c) *t*BuOOH, Ti(O*i*Pr)$_4$, L-(+)-diethyl tartrate; (d) RuO_4; (e) CH_2N_2; (f) K_2CO_3, CH_3OH, H_2O; (g) H_2NNH_2, H_2O; (h) air; (i) CF_3CO_2H, H_2O.

Scheme III[a]



[a] (a) LiN(Si(CH$_3$)$_3$)$_2$, THF, (b) (i) H_2NNH_2, H_2O; (ii) CF_3CO_2H, H_2O.

Table I. ^{13}C NMR Chemical Shifts (ppm) and Their Multiplicities



carbon	synthetic tetrahydropyridazine **2**	tripeptide **10**
1	178.32 (s)	173.4 (s)
2	62.09 (d)	61.2 (d)
3	63.11 (d)	61.5 (d)
4	30.59 (t)	30.2 (t)
5	141.85 (d)	140.7 (d)

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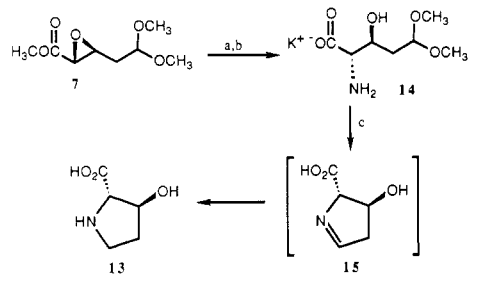
Scheme IV[a]



[a] (a) K_2CO_3, CH_3OH, H_2O; (b) NH_3, H_2O; (c) EtOH, H_2O, CF_3CO_2H, PtO_2, H_2.

necessary for conversion because the product is not infinitely stable under the reaction conditions. The reaction mixture was then neutralized to pH = 6.5 with 1 N KOH and concentrated under vacuum. The residue was chromatographed to remove salts and a minor impurity[13] and then concentrated to give the product **2** as a glassy solid (65% yield from ester **7**).

Although the synthetic approach used should give the target compound **2**, we were unable to obtain a crystalline sample for X-ray analysis and the tetrahydropyridazine itself was never isolated from nature. We therefore felt that some structural corroboration was in order.

One derivative, tetrahydropyridazine-Src-Gly **10**, was isolated from degradation studies and its ^{13}C NMR spectrum was reported.[1] The carbon chemical shifts (Table I) differed very little (<1.5 ppm) except for the carboxyl (≈5 ppm), which is an amide in the peptide and an acid in the synthetic material.

To test the remote possibility that the synthesis would give the *cis*-tetrahydropyridazine **12** (possibly via selective epimerization), it was synthesized in racemic form for comparison. A Darzen condensation of methyl bromoacetate with malonaldehyde dimethyl acetal gave a 3:2 mixture of cis **11** and trans **7** glycidic esters (Scheme III). The mixture was converted to the tetrahydropyridazines by using the procedure described above and the cis tetrahydropyridazine **12** was partially purified by chromatography. The coupling constants for the protons on carbons 2 and 3 (5.5 Hz for trans and 3.9 Hz for cis) offer consistent, though not compelling evidence for the stereochemical assignments.

As a final structural corroboration and an extension of the methodology described above, L-*trans*-3-hydroxyproline (**13**) was synthesized. A known though rare amino acid, L-*trans*-3-hydroxyproline was originally isolated from bovine tendon collagen[14] and has since been found in the teleomycin antibiotics.[15] Although a number of syntheses have been described, these generally are demanding, give poor yields, and, in most cases, produce mixtures of the cis and trans racemates.[16]

The synthesis of L-*trans*-3-hydroxyproline (**13**) (Scheme IV) proceeds in a manner analogous to the synthesis of tetrahydropyridazine **2**. The glycidic ester **7** was saponified as before and the epoxide was opened with aqueous ammonia to give the β-hydroxy-α-amino acid **14**. Again, no opening at the β-position was observed. The final product, L-*trans*-3-hydroxyproline (**13**) was formed by acid hydrolysis of the dimethyl acetal and in situ hydrogenation (PtO_2, H_2) of the subsequently formed cyclic imine **15**. Neutralizing the reaction mixture followed by desalting with ion exchange resins gave the salt-free L-*trans*-3-hydroxyproline (**13**) (87% yield). The ^1H NMR spectrum of the synthetic material was identical with that reported for the natural product.[17]

Discussion

This paper describes an efficient synthesis (overall yield = 32% from malonaldehyde dimethyl acetal) of 3(*S*)-carboxy-4(*S*)-hydroxy-2,3,4,5-tetrahydropyridazine (**2**). Comparison of the ^{13}C NMR spectra of the synthetic product with that of a degradation product **10** and the synthetic cis isomer **12**, plus validation of the methodology in the synthesis of the known L-*trans*-3-hydroxyproline (**13**), provide strong support for the structure of the synthetic tetrahydropyridazine **2**.

There is one other point that warrants discussion. In our hands, nucleophilic opening of the glycidic acid proceeds with exceptionally high regioselectivity. We were unable to detect even a trace (<1%) of opening at the β-position, in contrast to what one might expect from the literature.[18] In a variety of examples reported by Sharpless, the selectivity ranged from 11:1 to 1:1.6 for α vs β attack. One difference, however, is that these studies were conducted by heating the glycidic acid in an amine or an aqueous amine solution. In our examples the potassium salt of the acid was used instead. This should greatly reduce the amount of ammonium ion present and might consequently eliminate acid-catalyzed opening at the β-position. It is well known that Lewis acid catalyzed opening of glycidic acids occurs at the β-position with high regioselectivity.[19] These results suggest that it might be possible to control the regioselectivity of glycidic acid epoxide opening by merely changing the reaction pH. Further investigation of this reaction is warranted and in progress.

Experimental Section

Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 299-B spectrophotometer. ^1H NMR spectra were recorded on a Varian EM-390 or a Bruker WM-300 instrument at 90 and 300 MHz, respectively. ^{13}C NMR spectra were recorded on a JEOL FX-90Q instrument at 22.49 MHz. Mass spectra were recorded on an AEI MS-902 spectrometer with a VG Micromass 2040 data reduction system. Microanalyses were done by Galbraith Laboratories Inc.

Methyl 5,5-Dimethoxypent-2(*E*)-enoate (5). Malonaldehyde dimethyl acetal[8] (14.39 g, 121.9 mmol) in methanol (60 mL) was added to methanol (500 mL) at 0 °C containing potassium *tert*-butoxide (16.42 g, 146.3 mmol) and trimethyl phosphonoacetate (25.66 g, 140.9 mmol), allowed to warm to room temperature, and stirred for 1 h. The reaction mixture was concentrated and then partitioned between ether (400 mL) and saturated sodium bicarbonate solution (200 mL). The ether layer

(13) Based on the ^1H NMR spectra of a somewhat impure sample, the structure of the minor impurity was proposed to be the cyclic imine arising from the closure to a five-membered ring. We were unable to obtain enough material for further structural studies.

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was washed with brine (100 mL), dried ($MgSO_4$), and concentrated to give 22.76 g (107%[20]) of the product ester as a clear oil: IR (neat) 1726, 1663 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.38 (2 H, dd, J = 5.6, 7.3 Hz), 3.20 (6 H, s), 3.58 (3 H, s), 4.33 (1 H, t, J = 5.6 Hz), 5.77 (1 H, d, J = 15.7 Hz), 6.76 (1 H, dt, J = 7.3, 15.7 Hz); ^{13}C NMR (22.49 MHz, $CDCl_3$) δ 35.58 (t), 51.08 (q), 52.75 (q), 102.68 (d), 123.18 (d), 143.15 (d), 166.27 (s); MS (EI) m/z (rel intensity) 143 (12, M - OCH_3), 86 (21), 84 (34), 75 (100, $CH_3OCHOCH_3$). Anal. Calcd for $C_8H_{14}O_4$: C, 55.16; H, 8.10. Found: C, 54.93; H, 8.04.

5,5-Dimethoxypent-2(*E*)-en-1-ol (4). Methyl 5,5-dimethoxypent-2(*E*)-enoate (**5**) (22.76 g, 131 mmol) was dissolved in ether (500 mL) and cooled in an ice bath. Diisobutylaluminum hydride (220 mL, 20% in hexanes, 44 g, 300 mmol) was added over one-half hour and the reaction mixture was stirred for two more hours. The reaction mixture was then treated dropwise with 15% sodium hydroxide (100 mL) followed by water (500 mL) and stirred for 3 h. The two layers were then allowed to separate. The organic layer was removed and the aqueous layer was extracted with ethyl acetate (2 × 400 mL). The combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), and concentrated to give 16.7 (87%) of the product as a slightly yellow oil. The product was then purified by Kugelrohr distillation (77–80 °C, 0.5 mmHg) to give 13.6 g (72%) of the product as a clear oil: IR (neat) 3410 v br, 2835, 2940, 1130, 1057 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.26 (2 H, dd, J = 6.1, 5.6 Hz), 2.93 (1 H, br s, OH), 3.22 (6 H, s), 3.95 (2 H, d, J = 5.3 Hz), 4.29 (1 H, t, J = 5.6 Hz), 5.52 (1 H, dt, J = 6.1, 15.6 Hz), 5.61 (1 H, dt, J = 5.3, 15.6 Hz); ^{13}C NMR (22.49 MHz, $CDCl_3$) δ 35.52 (t), 52.69 (q), 62.94 (t), 103.76 (d), 126.10 (d), 132.12 (d); MS (EI) m/z (rel intensity) 115 (5.3, M - OCH_3), 86 (16), 84 (26), 75 (100, $CH_3OCHOCH_3$).

5,5-Dimethoxypent-2(*E*)-en-1-ol 2(*S*),3(*S*)-Oxide (6). A 2-L, one-neck flask equipped with a magnetic stir bar was fitted with a serum cap and flushed with nitrogen. Freshly distilled methylene chloride (900 mL, distilled from CaH_2) was added and cooled to −23 °C (dry ice/CCl_4). Titanium isopropoxide (27.79 mL, 26.5 g, 93.6 mmol) and L-(+)-diethyl tartrate (16.05 mL, 19.27 g, 93.6 mmol) were then added. After being stirred for 5 min, the allylic alcohol, 5,5-dimethoxypent-2(*E*)-en-1-ol (**4**) (13.66 g, 93.6 mmol), was added followed by *tert*-butyl hydroperoxide (39.56 mL of 4.6 M dichloroethane solution, 182.2 mmol). The mixture was stored in the freezer (−20 °C) for 20 h. The reaction was deemed complete by TLC (silica gel, CH_2CL_2–CH_3OH, 19:1, R_f = 0.35) and the reaction mixture was removed from the freezer and cooled again to −20 °C. Dimethyl sulfide (27.48 mL, 23.25 g, 374 mmol) was added, the mixture stirred for 1 h, then poured into a 5% solution of sodium fluoride (2 L), and stirred for 25 h. The solution was next saturated with sodium chloride and filtered through a Celite pad. The phases were separated and the aqueous layer was washed repeatedly with methylene chloride (4 × 500 mL). The combined organic layers were dried ($MgSO_4$), concentrated, and chromatographed (silica gel, CH_2CL_2–CH_3OH, 19:1) to give 12.3 g (81%) of the desired epoxide as a clear oil: $[\alpha]^{25}_D$ −37.5° (*c* 9.63, CH_3OH); IR (neat) 3440, 2940, 1125, 1058 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.78 (1 H, ddd, J = 14.3, 4.6, 6.9 Hz), 1.93 (1 H, ddd, J = 14.3, 4.8, 6.5 Hz), 2.00 (1 H, v br s), 2.94 (1 H, ddd, J = 2.3, 2.6, 4.3 Hz), 3.04 (1 H, ddd, J = 4.6, 2.3, 6.9 Hz), 3.32 (3 H, s), 3.35 (3 H, s), 3.61 (1 H, dd, J = 12.6, 4.3 Hz), 3.88 (1 H, dd, J = 12.6, 2.6 Hz), 4.53 (1 H, dd, J = 4.8, 6.5 Hz); ^{13}C NMR (22.49 MHz, $CDCl_3$) δ 35.17 (t), 52.21 (d), 52.81 (q), 53.34 (q), 58.23 (d), 61.63 (t), 102.27 (d); MS (EI) m/z (rel intensity) 131 (4.1, M - OCH_3), 75 (100, $CH_3OCHOCH_3$).

Methyl 5,5-Dimethoxypent-2(*E*)-enoate 2(*R*),3(*S*)-Oxide (7). A three-dram vial was charged with a magnetic stirbar, carbon tetrachloride (1 mL), acetonitrile (1 mL), and sodium periodate (440 mg, 2.06 mmol, 4.1 equiv) dissolved in water (2 mL). 5,5-Dimethoxypent-2(*E*)-en-1-ol 2(*S*),3(*S*)-oxide (**6**) (81 mg, 0.5 mmol) and ruthenium dioxide dihydrate (2 mg, 2.4 mol %) were added and the reaction mixture was capped and stirred vigorously for 2 h. The reaction mixture was then partitioned between methylene chloride (10 mL) and water (4 mL) and the phases were separated. The aqueous phase was washed with methylene chloride (3 × 8 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated. The residue was diluted with ether (20 mL) and filtered through a Celite pad. The ethereal solution was treated with diazomethane and, after 15 min, concentrated with a nitrogen stream. The residue was chromatographed (silica gel, 40% ethyl acetate in hexanes) to give 67 mg (70%) of the glycidic ester as an oil: $[\alpha]^{25}_D$ −45.5° (*c* 8.71, CH_3OH); IR (neat) 1775, 1210, 1122 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.78 (1 H, ddd, J = 5, 7, 14 Hz), 1.97 (1 H, ddd, J = 5, 6, 14 Hz), 3.24 (2 H, m), 3.30 (3 H, s), 3.34 (3 H, s), 3.74 (3 H, s), 4.53 (1 H, J = 5, 6 Hz); ^{13}C NMR (22.49 MHz, $CDCl_3$) δ 35.17 (t), 52.27 (q), 52.57 (d), 52.87 (q), 53.58 (q), 54.59 (d), 101.85 (d), 169.19 (s); MS (EI) m/z (rel intensity) 159 (6.7, M - OCH_3), 75 (100, $CH_3OCHOCH_3$). Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.43; H, 7.53.

3(*S*)-Carboxy-4(*S*)-hydroxy-2,3,4,5-tetrahydropyridazine (2). Methyl 5,5-dimethoxypent-2(*E*)-enoate 2(*R*),3(*S*)-oxide (**7**) (107 mg, 0.56 mmol) was dissolved in 20% aqueous methanol (4 mL), treated with potassium carbonate (86 mg, 0.62 mmol), and stirred for 2 h. The reaction mixture was then concentrated to give the potassium salt of the glycidic acid: ^1H NMR (300 MHz, D_2O) δ 1.71 (1 H, ddd, J = 5.6, 6.7, 14.5 Hz), 1.90 (1 H, ddd, J = 4.5, 5.6, 14.5), 2.96 (1 H, ddd, J = 2.1, 4.5, 6.7 Hz), 3.09 (1 H, d, J = 2.1 Hz), 3.26 (3 H, s), 3.27 (3 H, s), 4.55 (1 H, dd, J = 5.6, 5.6 Hz). The residue was dissolved in hydrazine hydrate (4 mL) and stirred under nitrogen for 24 h. The aqueous hydrazine was then removed under vacuum to give the α-hydrazino acid as its potassium salt: ^1H NMR (300 MHz, D_2O) δ 1.63 (2 H, m, J = 4.5, 7.2, 9.2 Hz), 3.08 (1 H, d, J = 3.8 Hz), 3.21 (6 H, br s), 3.77 (1 H, pseudo dt, J = 3.8, 9.2 Hz), 4.49 (1 H, dd, J = 4.5, 7.2 Hz); ^{13}C NMR (22.49 MHz, D_2O) δ 37.01 (t), 54.11 (q), 54.23 (t), 68.23 (d), 72.76 (d), 103.69 (d), 177.94 (s). The hydrazino acid was dissolved in water (10 mL) and titrated to pH = 1.3 and stirred until starting material was not detectable by TLC. A neutralized aliquot was used for TLC (silica gel, EtOH–1 N NH_4OAc, 5:1, visualized by anisaldehyde in ethanol–sulfuric acid after evaporation of the eluting solvent, R_f(hydrazino acid) = 0.73, R_f(product) = 0.56). When no more starting material was seen (after about 30 min), the reaction mixture was neutralized (to pH = 6.5) with 1 N KOH and concentrated under vacuum. The residue was then chromatographed through sequential weak anion (DEAE-Sephadex, OH^- form) and weak cation (IONAC, CGC-270, H^+ form) exchange columns. The eluant containing the tetrahydropyridazine was concentrated under vacuum to give 53 mg (65%) of the product as a glass: $[\alpha]^{25}_D$ −57.5° (*c* 5.3, CH_3OH); IR (KBr) 1130, 1183, 1200, 1400, 1595, 1635 shoulder, 1680, 2500–3600 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.96 (1 H, m, J = 1.7, 2.0, 4.2, 19.4 Hz, D_2O exchangeable), 2.24 (1 H, ddd, J = 2.2, 5.5, 19.4 Hz, D_2O exchangeable), 3.31 (1 H, dd, J = 1.7, 5.5 Hz), 4.14 (1 H, ddt, J = 1.2, 4.2, 5.5, 5.5 Hz), 6.57 (1 H, br s, J = 1.2, 2.0, 2.2 Hz); ^{13}C NMR (22.49 MHz, D_2O) δ 30.59 (t), 62.09 (d), 63.11 (d), 141.85 (d), 178.32 (s); MS (EI) m/z (rel intensity) 144 (2.3, M), 99 (15.4, M - CO_2H), 82 (15.1, M - CO_2 - H_2O), 69 (54, $HCCCO_2$), 51 (27, CH_2CHCC), 44 (100, CO_2); MS (CI, methane) m/z 145 (5.6, M + 1), 115 (M - N_2H); HRMS exact mass calcd for $C_5H_8N_2O_3$ 144.0535, found 144.0514.

If the hydrazino acid **8**, as prepared above, is allowed to sit for a few weeks and is occasionally exposed to air, oxidation followed by reductive elimination leads cleanly to the β-hydroxy acid **9**: ^1H NMR (300 MHz, D_2O–K_2CO_3) δ 1.61 (2 H, m, J = 4.5, 6.7, 7.2, 8.7, 14.5 Hz), 2.18 (2 H, d, J = 4.4 Hz), 3.20 (3 H, s), 3.21 (3 H, s), 3.88 (1 H, m, J = 4.4, 6.7, 8.7 Hz), 4.9 (1 H, dd, J = 4.5, 7.2 Hz); ^{13}C NMR (22.49 MHz, D_2O–K_2CO_3) δ 40.11, 45.83, 54.29, 66.63, 103.69, 180.74.

***cis*-3-Carboxy-4-hydroxy-2,3,4,5-tetrahydropyridazine (12).** Hexamethyldisilazane (765 mg, 4.74 mmol) was dissolved in THF (50 mL) at −78 °C and treated with *n*-BuLi (2.3 mL, 1.9 M, 4.31 mmol). After being stirred for 20 min, ethyl bromoacetate (720 mg, 4.31 mmol) was quickly added followed, after 15 min, by malonaldehyde dimethyl acetal (509 mg, 4.31 mmol). The reaction mixture was stirred an additional 10 min at −78 °C and then quenched with potassium phosphate buffer (5%, pH = 4.4, 50 mL). The reaction mixture was extracted with ether (100 mL),

(20) The starting material was contaminated with 3-methoxypropenal, which is formed by loss of methanol from the starting material during preparation. Because it is converted back into the starting material during the reaction and has a lower molecular weight to start with, the calculated yield, based on the erroneous assumption of pure starting material, is greater than 100%.

washed with brine (25 mL), dried ($MgSO_4$), and concentrated to an oil. The oil was chromatographed (silica gel, 25% ethyl acetate in hexanes) to give 570 mg (65%) of a 60:40 mixture of cis and trans glycidic esters, **11** and **7**, respectively: ^1H NMR (300 MHz, $CDCl_3$) δ 1.17–1.23 (3 H, 2 t), 1.69–1.96 (2 H, m), 3.14–3.42 (2 H, m), 3.23, 3.24, 3.26, 3.28 (6 H, 4 s), 4.08–4.18 (2 H, 2 q), 4.4–4.5 (1 H, 2 dd).

The mixture of esters (50 mg) was converted to the cis and trans tetrahydropyridazines **12** and **2** by using the method used for the chiral ester. The mixture of products was chromatographed (Sigmacell microcrystalline cellulose Type 50, ethanol–water, 8:1) to give the partially purified *cis*-tetrahydropyridazine **12**: ^1H NMR (300 MHz, D_2O) δ 2.03 (1 H, m, J = 2.9, 5.0, 21.5 Hz), 2.42 (1 H, m, J = 1.95, 5.3, 7.3, 21.5 Hz), 3.36 (1 H, dd, J = 1.95, 3.90 Hz), 4.22 (1 H, m, J = 1.2, 3.9, 5.0, 7.3 Hz), 6.6 (1 H, m, J = 1.2, 2.9, 5.3 Hz); R_f = 0.4 (silica gel, ethanol–1 N ammonium acetate buffer pH = 6.0, 5:1).

L-*trans*-3-Hydroxyproline (13). Methyl 5,5-dimethoxypent-2(*E*)-enoate 2(*R*),3(*S*)-oxide (**7**) (100 mg, 0.53 mmol) was dissolved in 20% aqueous methanol (4 mL), treated with potassium carbonate (81 mg, 0.59 mmol), and stirred for 2 h. The reaction mixture was then concentrated to give the potassium salt of the glycidic acid: ^1H NMR (300 MHz, D_2O) δ 1.71 (1 H, ddd, J = 5.6, 6.7, 14.5 Hz), 1.90 (1 H, ddd, J = 4.5, 5.6, 14.5), 2.96 (1 H, ddd, J = 2.1, 4.5, 6.7 Hz), 3.09 (1 H, d, J = 2.1 Hz), 3.26 (3 H, s), 3.27 (3 H, s), 4.55 (1 H, dd, J = 5.6, 5.6 Hz). The residue was dissolved in concentrated ammonia (37%, 3 mL), transferred to a Fisher-Porter tube, sealed, and heated at 40 °C for 15 h. The mixture was concentrated under vacuum to give a single product, the β-hydroxyamino acid **14**, by NMR: ^1H NMR (300 MHz, D_2O) δ 1.59 (2 H, pseudo t, J = approx. 6 Hz), 3.21 (3 H, s), 3.21 (3 H, s), 3.82 (1 H, dd, J = 4.6, 5.8 Hz), 3.90 (1 H, pseudo d, J = 4.6 Hz), 4.50 (1 H, dd, J = approx. 6 Hz); ^{13}C NMR (22.49 MHz, D_2O) δ 36.29 (t), 54.05 (q), 54.59 (q), 62.45 (d), 70.32 (d), 103.81 (d), 178.36 (s). The residue was dissolved in 50% aqueous ethanol (2 mL) and transferred to a gas burning tube containing a stirbar. A slow steady stream of hydrogen was bubbled through the mixture, which was then titrated to pH = 1.5 with trifluoroacetic acid and treated with Adam's catalyst (PtO_2, 10 mg). The reaction was stirred rapidly for 15 h at room temperature. TLC (silica gel, *n*-BuOH–AcOH–H_2O, 4:1:1, detected by chlorine:starch–iodide) showed formation of a single new product. The mixture was then titrated to pH = 7.0 (1 N KOH), filtered through a Celite pad, concentrated under vacuum, and chromatographed through weak anion (DEAE-Sephadex, OH^- form) and weak cation (IONAC, CGC-270, H^+ form) exchange resins to give 60 mg (87%) of the salt-free L-*trans*-3-hydroxyproline (**13**): ^1H NMR (300 MHz, D_2O–TFA) δ 1.7 (2 H, m), 3.15 (2 H, m), 3.92 (1 H, d, J = 2.2 Hz) 4.36 (1 H, m, J = 2.2 Hz); ^{13}C NMR (22.49 MHz, D_2O–TFA) δ 32.30, 44.99, 69.66, 74.61.

Registry No. 1, 75580-37-9; (3*S*,4*S*)-**2**, 77421-35-3; (±)-**2**, 120851-22-1; **3**·K, 120828-87-7; **4**, 40156-61-4; **5**, 61752-18-9; **6**, 120924-97-2; (2*R*,3*S*)-**7**, 120789-95-9; (±)-**7**, 120851-23-2; **8**, 120789-96-0; **9**, 120789-97-1; **10**, 120789-98-2; **11**, 120851-20-9; **12**, 120851-21-0; **13**, 4298-08-2; **14**, 120789-99-3; $OHCCH_2CH(OMe)_2$, 19060-10-7; $(MeO)_2P(O)CH_2COOMe$, 5927-18-4; $BrCH_2COOEt$, 105-36-2.

Metalated Heterocycles in the Synthesis of Ellipticine Analogues. A New Route to the 10*H*-Pyrido[2,3-*b*]carbazole Ring System

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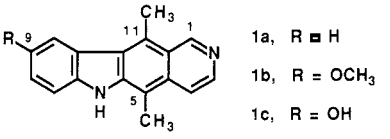
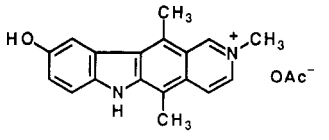
A synthesis of the 10*H*-pyrido[2,3-*b*]carbazole ring system is described, in which the key steps are regiospecific acylation of a 2-lithio-1-(phenylsulfonyl)indole (**7**) with 2,3-pyridinedicarboxylic anhydride (**8**), cyclization of the deprotected keto acid **10** to keto lactam **11** with acetic anhydride, and the addition of methyllithium to give, after reduction of the diol **12** with sodium borohydride, the target ring system. In this fashion, 5,11-dimethyl-10*H*-pyrido[2,3-*b*]carbazole (**3**) and the corresponding 7-methoxy (**4**) and 8-methoxy (**5**) derivatives were synthesized.

The 6*H*-pyrido[4,3-*b*]carbazole alkaloids ellipticine (**1a**), 9-methoxyellipticine (**1b**), and related synthetic derivatives display pronounced anticancer activity in several animal and human tumor systems.[2] A derivative of 9-hydroxyellipticine (**1c**), namely, 2-methyl-9-hydroxyellipticinium acetate (**2**) ("elliptinium"), is currently undergoing extensive clinical trials, particularly in Europe, for the treatment of metastatic breast cancer, myeloblastic leukemia, and some solid tumors.[3] Moreover, these compounds exhibit multimodal action on DNA: (a) intercalation, (b) metabolism and subsequent covalent binding, (c) generation of oxygen radicals, and (d) the inhibition of topoisomerase II.[2a]



1a, R = H
1b, R = OCH_3
1c, R = OH



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Consequently, since the initial discovery of the antitumor properties of the ellipticine alkaloids,[4] synthetic activity involving this ring system has been vigorous and unabated.[5,6]

(1) Recipient of the Dartmouth College Chandler T. White Prize for undergraduate research.

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