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Synthesis of [guanido-^{13}C]-γ-hydroxyarginine

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This report describes an efficient method of synthesizing [guanido-^{13}C]-γ-hydroxyarginine HCl salt. Iodolactonization of *N*-Boc-protected allylglycine mainly provided the *cis* iodo compound 2. This was converted to an amine through azide 4. The amine 5 was reacted with *N*-Boc-protected [^{13}C]thiourea to afford *N*-Boc-protected [^{13}C]guanidine 6, which underwent base catalyzed ring opening. Removal of the *N*-Boc group afforded [guanido-^{13}C]-γ-hydroxyarginine HCl salt 7 giving a 30% overall yield of the final product from *N*-Boc protected allylglycine 1 in five steps.

Keywords: carbon-13 synthesis; [guanido-^{13}C]-γ-hydroxyarginine; iodolactonization

Introduction

Enduracidin is a lipodepsipeptide, composed of 17 amino acids and an unsaturated fatty acid side chain, that exerts potent *in vitro* and *in vivo* antibacterial activity against a wide spectrum of Gram-positive organisms, including methicillin resistant *Staphylococcus aureus* (MRSA).[1–5] Minimal inhibitory concentrations are as low as 0.05 μg/mL and the effect is bactericidal.[4,6] The peptide was first isolated in Japan from the soil bacteria *Streptomyces fungicidicus* B5477 in 1968.[7,8] Enduracidin has found commercial use as a poultry feed additive. The unique cyclic structure of enduracidin is constructed with 15 amide and one ester bonds.[9,10] Among these amino acids, D- and L-enduracididine (see Figure 1), L-3,5-dichloro-4-hydroxyphenylglycine, D- and L-hydroxyphenylglycine, D-ornithine, and L-citrulline are rare nonproteinogenic amino acids. Enduracididine (aminodihydrohistidine) is a unique amino acid with an imidazolidin-2-imine moiety.[11,12] It was also shown to be a component of the antibiotic minosaminomycin and was identified in the cytotoxic fraction of extracts of a marine ascidian.[13–15] The mannopeptimycins are antibiotics isolated from a strain of *S. hygroscopicus* that contain D- and L-β-hydroxyenduracididine.[16]

Little is known about the formation of enduracididine other than it originates from L-arginine.[17] Thus, in order to conduct studies on the biosynthesis of enduracididine required for the formation of enduracidin, [guanido-^{13}C]-γ-hydroxyarginine was required for labeled precursor incorporation experiments. This report describes the complete experimental details for the preparation of [guanido-^{13}C]-γ-hydroxyarginine from *N*-Boc-protected allylglycine **1**.

Results and discussion

As illustrated in the Scheme 1, iodolactonization of *N*-Boc-protected allylglycine **1** in tetrahydrofuran in the presence of sodium bicarbonate provided a 93% yield of *cis* iodolactone **2** and *trans* iodolactone **3** in a 10 to 1 ratio. The two isomers were separated by crystallization. The same reaction previously reported in the literature claimed to obtain only *cis* iodolactone **2**.[18] In the case of *N*-acetyl-protected allylglycine, a similar iodolactonization was reported to afford 7–8 to 1 ratio of *cis* to *trans* iodolactone.[19] S_N2 reaction of **2** with sodium azide in *N*,*N*-dimethylformamide at 40°C yielded azidolactone **4** as an oil with a yield of 98%. Reduction of the azidolactone **4** under hydrogen in ethanol in the presence of 5% Pd/C gave aminolactone **5** as a foamy solid. Attempts to purify the compound for characterization studies were unsuccessful, possibly due to competing δ-lactam formation. Hence, the crude product of reductive hydrogenation of azidolactone **4** was directly treated with *N*,*N*′-di-(*tert*-butoxycarbonyl)-[^{13}C]thiourea[20] and triethylamine in the presence of 2-chloro-*N*-methylpyridinium iodide,[21] giving **6** with a yield of 70%. Although it was easier to isolate product **6** when the guanylation of amine **5** was carried out in the presence of mercuric chloride rather than 2-chloro-*N*-methylpyridinium iodide, the yield was quite variable. Base (1 N NaOH) catalyzed hydrolysis of *N*-Boc-protected [^{13}C]guanidinolactone **6**, followed by removal of three *N*-Boc groups using 4 N HCl provided crude [guanido-^{13}C]-γ-hydroxyarginine as the HCl salt. This crude product was loaded onto a column (1.5 cm × 10 cm) of Dowex 50W X 8 (hydrogen form, 200 mesh) ion exchange resin. The column was washed sequentially with water and 1 N HCl then the bound product was eluted with 4 N HCl. The 4 N HCl solution was evaporated *in vacuo* to give a white solid that was crystallized from water–ethanol to afford [guanido-^{13}C]-γ-hydroxyarginine HCl salt **7.**

γ-Hydroxyarginine has previously been synthesized from histidine or epichlorohydrin.[22,23] But these processes demand tedious separation or provide very low yield of the product. Our

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synthetic procedure affords a high yield of the product and it is so versatile that the four possible stereoisomers of [guanido-^{13}C]-γ–hydroxyarginine can be prepared from readily available chiral allylglycine.

Materials and methods

All reactions were carried out in dried solvents. The reaction mixtures were normally stirred magnetically except for those stirred vigorously with a mechanical stirrer. All nonvolatile samples were pumped to constant weight at room temperature after removing the solvents under reduced pressure. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Thin layer chromatography was carried out using Merck Kiesel gel 60 F-254. Column chromatography was performed using Merck 60, 70–230 mesh silica gel. The ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were obtained on Bruker Avance DRX300 spectrometers and are reported in δ (ppm) downfield from tetramethylsilane (TMS). The following abbreviations were used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

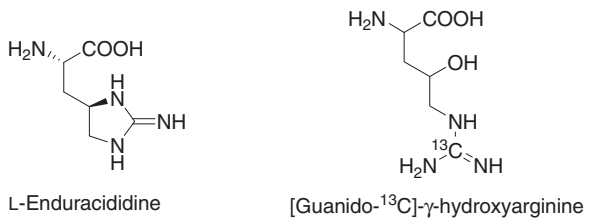


Figure 1. Structures of L-enduracididine and [guanido-^{13}C]-γ-hydroxyarginine.

Experimental

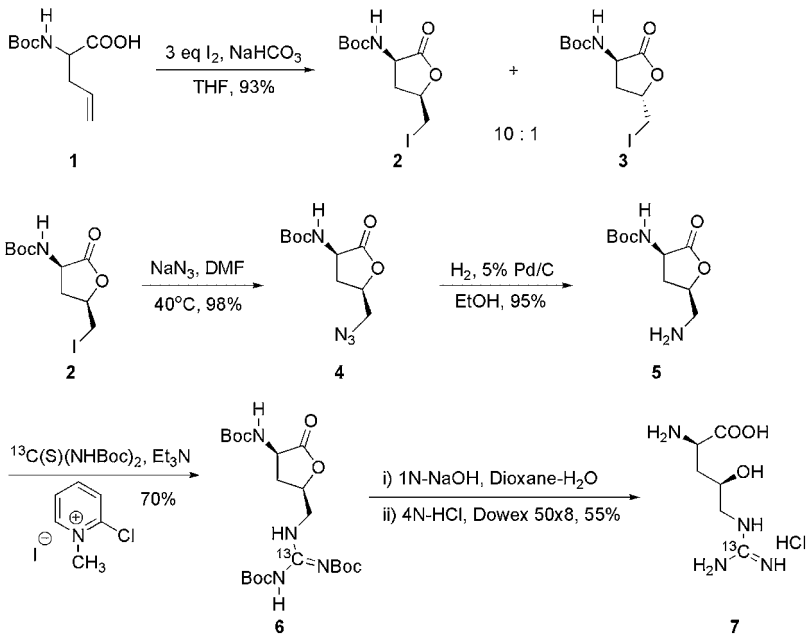
t-Butyl 5-(iodomethyl)-2-oxo-tetrahydrofuran-3-ylcarbamate (2, 3)

A solution of iodine (26.45 g, 104 mmol) in tetrahydrofuran (160 mL) was added dropwise at 0°C to a solution of **1** (7.48 g, 34 mmol) in tetrahydrofuran (120 mL) in the presence of aqueous sodium bicarbonate (29.18 g, 347 mmol, in 347 mL of water). The reaction mixture was stirred at 0–5°C for 5 h and quenched with saturated sodium sulfite (160 mL). The mixture was extracted with ethyl acetate and the combined organic extracts were washed with water as well as brine, dried over anhydrous magnesium sulfate and evaporated *in vacuo* to give 12 g of an off-white solid. Two isomers corresponding to *cis* and *trans* were separated by crystallization from ethyl acetate and hexane. *Cis* isomer (**2**, 10 g, 91%), ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.87 (dd, $J_1 = 12.3$ Hz, $J_2 = 10.5$ Hz, 1H), 3.02 (m, 1H), 3.32 (dd, $J_1 = 10.5$ Hz, $J_2 = 7.2$ Hz, 1H), 3.45 (dd, $J_1 = 10.5$ Hz, $J_2 = 4.8$ Hz, 1H), 4.45 (m, 2H), 5.07 (bs, 1H).

Trans isomer (**3**, 1 g, 9%) ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.41 (dd, $J_1 = 12.3$ Hz, $J_2 = 10.5$ Hz, 1H), 2.62 (m, 1H), 3.36 (m, 2H), 4.36 (m, 1H), 4.75 (m, 1H), 5.06 (bs, 1H).

Cis t-Butyl 5-(azidomethyl)-2-oxo-tetrahydrofuran-3-ylcarbamate (4)

To a solution of the iodide **2** (0.50 g, 1.47 mmol) in *N,N*-dimethylforamide (8 mL) sodium azide (0.47 g, 7.3 mmol) was added and the mixture was stirred at 40°C for 17 h. After cooling to room temperature, the reaction mixture was poured into ice and extracted with ethyl acetate. The combined organic extracts were washed with water as well as brine, dried and evaporated *in vacuo* to give an oil which was purified by flash chromatography (ethyl acetate: hexane = 3:7) to afford 0.37 g (98%) of **4**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.87 (q, $J = 11.4$ Hz, 1H), 2.77 (m, 1H), 3.50 (dd, $J_1 = 13.5$ Hz, $J_2 = 5.4$ Hz, 1H), 3.64 (dd,



Scheme 1. Synthesis of [guanido-^{13}C]-γ-hydroxyarginine.

$J_1 = 13.5$ Hz, $J_2 = 3.6$ Hz, 1H), 4.48 (m, 1H), 4.57 (m, 1H), 5.09 (bs, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 28.63, 33.04, 50.98, 53.29, 75.65, 80.80, 155.31, 173.83.

Cis 1-((4-*N*-Boc-amino-5-oxo-tetrahydrofuran-2-yl)methyl)-*N′*,*N″*-di-boc-[^{13}C]guanidine (6)

To a solution of the azide **4** (0.36 g, 1.4 mmol) in absolute ethanol (15 mL) 5% Pd/C (80 mg, 50% water wet) was added and the mixture was stirred under hydrogen atmosphere (a hydrogen-filled balloon was used and the reaction flask was purged three times before initiating the reaction) at room temperature for 3 h. The reaction mixture was filtered through a pad of celite and anhydrous magnesium sulfate and the filtrate was evaporated to give 310 mg (95%) of amine **5** as a white foamy solid. The amine **5** (0.31 g, 1.35 mmol) was dissolved in *N*,*N*-dimethylformamide (2 mL) and *N*,*N′*-di-(*tert*-butoxycarbonyl)-[^{13}C]thiourea (0.37 g, 1.35 mmol) was added to the solution, followed by triethylamine (0.56 mL, 4.0 mmol). The reaction mixture was then cooled to 0°C. 2-Chloro-*N*-methylpyridinium iodide (0.34 g, 1.35 mmol) was added in small portions to the reaction mixture for 5 min and the mixture was initially stirred at 0°C for 25 min and then at room temperature for 5 min. The reaction mixture was poured into ice and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate, filtered and evaporated to give 0.90 g of oil. Flash chromatography of this oily product with a 1:4 mixture of ethyl acetate:hexane gave 0.45 g (70%) of foamy solid **6**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 27H), 1.84 (q, $J = 11.4$ Hz, 1H), 2.81 (m, 1H), 3.50 (m, 1H), 3.98 (m, 1H), 4.41 (m, 1H), 4.61 (m, 1H), 5.09 (bs, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 27.97, 28.03, 28.24, 28.25, 33.42, 44.07, 51.12, 76.01, 79.58, 80.68, 83.54, 152.95, 155.27, 156.58, 163.26, 174.20.

[Guanido-^{13}C]-γ-hydroxyarginine HCl salt (7)

To a solution of **6** (0.23 g, 0.48 mmol) in dioxane (4 mL) was added 1 N NaOH (5 mL) and the mixture was stirred at room temperature overnight. After being cooled to 0°C, 4 N HCl (5 mL) was added slowly and the mixture was stirred at room temperature for 4 h. Then the solvents were evaporated to give 0.44 g of white solid. The product was chromatographed on a Dowex 50W X 8 (hydrogen form, 200 mesh) column (1.5 × 10 cm) and eluted using a gradient, with water and 4 N HCl. The compound was crystallized from water–ethanol to afford 60 mg (55%) of white crystal. Mp 187–89°C; ^{1}H-NMR (300 MHz, D_2O) δ 2.09 (q, $J = 12.0$ Hz, 1H), 2.81 (m, 1H), 3.43 (dt, $J_1 = 15.3$ Hz, $J_2 = 5.4$ Hz, 1H), 3.66 (d, $J_1 = 15.3$ Hz, 1H), 4.50 (dd, $J_1 = 12.0$ Hz, $J_2 = 9.0$ Hz, 1H), 4.78 (m, 1H); ^{13}C-NMR (75 MHz, D_2O) δ 29.23, 43.35, 49.24, 77.60, 157.26, 172.85.

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