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Synthesis of 1*H*-2,3-dihydropyrrolizine derivatives as precursors of bifunctional alkylating agents

Shanthi Rajaraman and Leslie S. Jimenez*

Department of Chemistry and Chemical Biology, Rutgers University, 610 Taylor Road, Piscataway, NJ 08854-8087, USA

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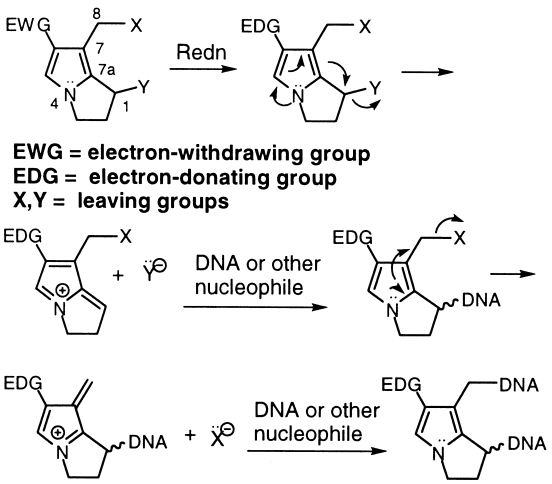
Abstract—Two 1*H*-2,3-dihydropyrrolizine derivatives bearing a nitro group at the 6 position have been synthesized and an improved method for nitrating pyrroles using potassium nitrate in trifluoroacetic acid was developed. An efficient, two-step synthesis of the butterfly pheromone, Danaidone, was also developed with an overall 33% yield.

1. Introduction

Bifunctional, pyrrole-based alkylating agents have the ability to form interstrand cross-links within the DNA double helix and are often useful as antitumor agents. The anticancer drug, mitomycin C, and the structurally related natural product, FR900482, are reductively activated inside cells to the mitosene intermediates, **1** and **2**, respectively. These highly reactive intermediates bind to the minor groove and selectively form interstrand cross-links between adjacent deoxyguanosine residues at 5′-CpG sites.[1] Another class of bifunctional alkylating agents which preferentially alkylate this site are the oxidatively activated pyrrolizine alkaloids (e.g. **3**, the dehydropyrrolizidine intermediate derived from retrorsine), which share the same reactive, pyrrole substructure as the mitomycin and FR900482 active intermediates.[1f,i,2] Interestingly, the simple pyrrole derivative **4** also exhibited a marked preference for forming deoxyguanosine-deoxyguanosine interstrand cross-links at the dinucleotide sequence 5′-CpG over 5′-GpC.[1f,3] Molecular modeling revealed that the distance between the adjacent deoxyguanosine amino groups (N2) at the 5′-CpG sites was nearly the same as the distance in the cross-linked adduct, while the amino group separation at the 5′-GpC sites was substantially larger in the starting DNA than in the adduct.[4] Proximity at CpG sites appears to greatly accelerate the second step (alkylation at C-10) of the cross-linking reaction.

Diacetate **4** lacks any kind of triggering mechanism which could modify its alkylating ability unlike the mitomycins and pyrrolizidine alkaloids. Introducing an electron-withdrawing group (e.g. the nitro group) at the 6-position of the 1*H*-2,3-dihydropyrrolizine ring system would provide a way to attenuate the leaving ability of appropriate groups at positions 1 and 8 until the electron-withdrawing group was reduced thereby unmasking the reactive bisalkylating agent (Scheme 1).

Nitro compounds that are activated by a bioreductive mechanism to form the cytotoxic species have been the subject of investigation for nearly a decade. It is known that the hypoxia-dependent activation of nitroheterocyclic drugs by cellular nitroreductases leads to the formation of intracellular adducts between the drugs and cellular macromolecules.[5] There has been considerable interest in designing potent and selective nitro compounds by using the nitro group as an electronic switch to activate a latent reactive moiety elsewhere in the molecule.[6] Some simple



Scheme 1.

Keywords: dihydropyrrolizine; bifunctional alkylating agents; antitumor agents.

* Corresponding author. Tel.: +1-732-445-0641; fax: +1-732-445-5312; e-mail: jimenez@rutchem.rutgers.edu

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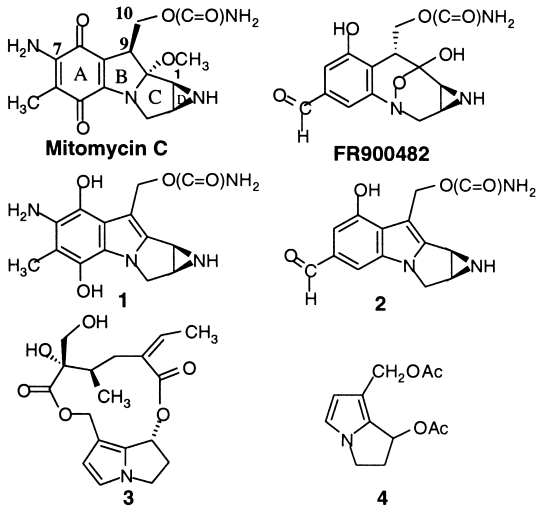
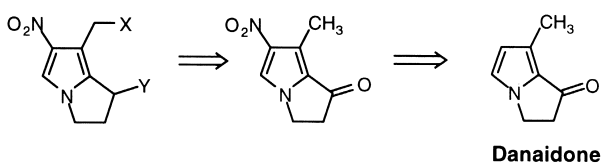


pyrrole-derived bifunctional electrophiles have also been shown to form cross-links in synthetic DNA duplexes.[1f] Studies on pyrrolic alkylating agents have shown the following: (1) pyrrolic esters are more reactive than the corresponding alcohols, (2) the position of the leaving group determines the leaving group ability; thus, when two alkylating functional groups are present, they may differ considerably in alkylating reactivity, (3) reactivity of esters depends on the structure of the acid moiety; thus, acetate esters are more reactive than the corresponding pivaloyl esters, and (4) reactivity is enhanced by electron donating substituents on the pyrrole ring.[7]

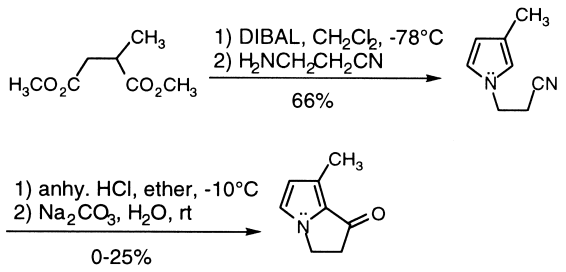
We report here the synthesis of two 1*H*-2,3-dihydropyrrolizine derivatives bearing a nitro group at the 6 position (EWG=NO_2). It is anticipated that such a compound would only become a good bisalkylating agent after reduction of the nitro group. Because of the similarity between the geometry of the C1 and C8 carbons of this reductively activated compound and the C1 and C10 carbons of the active intermediates of mitomycin C and FR900482, it is expected that these derivatives might also display a preference for forming cross-links at the 5′-CpG site.

2. Results and discussion

Looking at the structure of the target molecule, the known insect pheromone, Danaidone, seemed to be a good starting point for us. Pyrroles undergo electrophilic substitution at the 2-position preferentially. Since our target has an electron-withdrawing nitro group at the 6-position of the 1*H*-2,3-dihydropyrrolizine skeleton, we realized that we could rely on the directing effect of the keto group in Danaidone to effect that electrophilic aromatic substitution (Scheme 2).



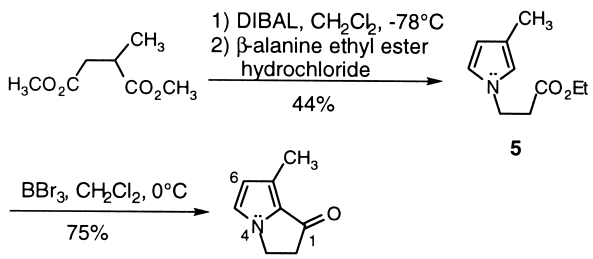
Scheme 2.



Scheme 3.

Two six-step syntheses have been previously reported for Danaidone, both with low overall yield.[8] In an attempt to improve the overall yield, *N*-cyanoethyl-3-methylpyrrole was synthesized by reducing dimethyl methyl succinate in situ with DIBAL to form the corresponding dialdehyde and reacting it with 3-aminopropionitrile.[9] This procedure resulted in moderate yields (41%) on a small scale to a reasonably high yield (66%) on a multigram scale. However, the cyclization of *N*-cyanoethyl-3-methyl pyrrole using gaseous HCl[8a] seemed to be capricious in our hands. The yield varied anywhere between 0 and 25% (Scheme 3). The failure of this reaction was probably because there was no accurate way to control the concentration of HCl in the reaction mixture and it is known that pyrroles are prone to polymerization under acidic conditions.

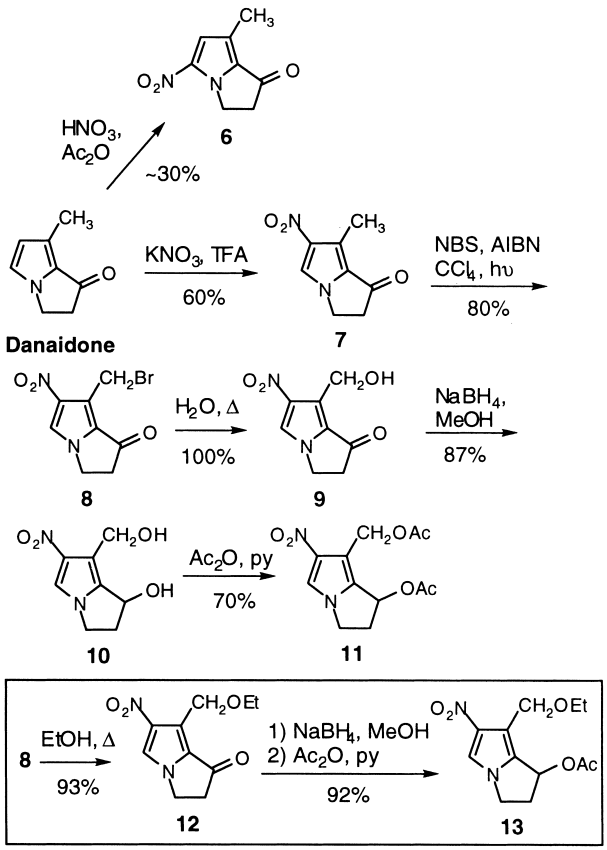
After ruling out the possibility of using hydrogen chloride to effect the cyclization, the use of boron tribromide as a Lewis acid for the cyclization of the pyrrole ester **5** seemed most promising.[10] As a modification of the first step, β-alanine ethyl ester hydrochloride was used in place of 3-aminopropionitrile to form the appropriate pyrrole ester **5** in a 44% yield (Scheme 4). Like the *N*-cyanoethyl-3-methylpyrrole, **5** was also found to be light-sensitive. Using BBr_3, the cyclization yield of **5** to form Danaidone was 75%. Having solved the cyclization problem, nitration of Danaidone was examined next.



Scheme 4.

It is well known that the high reactivity of pyrroles allows substitution by electrophilic reagents to proceed readily even under mild conditions. In addition to substituted pyrroles, the products usually include resinous substances formed by side reactions. It has been reported that the reactivities of the 2- and 3-positions of the pyrrole ring are estimated at 130000 and 30000 (with respect to benzene=1), respectively.[11] Bromination of 2-pyrrolecarboxaldehyde occurs primarily at the 4-position,[12] so it seemed reasonable to assume Danaidone's keto group might direct nitration to the 6-position.

Pyrrole is usually nitrated in acetic anhydride at low



Scheme 5.

temperatures.[11] Nitration with nitric acid in acetic anhydride resulted in the 5-nitro isomer **6** in ~30% yield (Scheme 5) while the 6-nitro isomer **7** was a minor product (<5% yield). The strongly acidic reaction conditions probably resulted in the protonation of the keto carbonyl group which promoted formation of a hemiketal (or ketal) with acetic acid; this species then proceeded to nitrate the 5-position in the absence of the directing effect of the keto functionality. Nitration under neutral conditions was attempted next. The use of nitronium tetrafluoroborate[13] in acetonitrile proceeded as expected to yield primarily the desired 6-nitro isomer **7**. The yield was 30–35%, which is common for pyrrolic systems. With the nitro group in place, it was decided to proceed forward with the functionalization of the methyl side chain.

In an attempt to effect oxidation of the methyl group to the corresponding aldehyde, ceric ammonium nitrate (CAN) in acetic acid or trifluoroacetic acid was used since this reagent has been shown to oxidize α-methyl groups of pyrroles to aldehydes and ethers[14] as well as β-alkyl groups.[15] However, no reaction of the deactivated nitropyrrole **7** occurred even under refluxing reaction conditions. Therefore, the order of reactions was changed and oxidation was attempted using CAN on Danaidone, prior to nitration. To our surprise, CAN in trifluoroacetic acid gave the 6-nitro isomer **7** as the major product. However, when the reaction was scaled up, CAN seemed to give a mixture of products and it was difficult to control the equivalents of the active nitrating species. This led to the replacement of CAN with potassium nitrate. As expected, KNO_3 gave a better yield (45% on a 0.500 g scale), which is, to our best knowledge, the highest yield known for nitrating pyrroles. The best yield obtained for this step was 60% on a 100 mg scale. Nitration using nitric acid in acetic anhydride yields the 5-nitro isomer **6** as the major product while potassium nitrate in trifluoroacetic acid exhibits better selectivity for the 6-nitro isomer **7** (Scheme 5).

With the deactivating nitro group in place, functionalization of the methyl group by bromination was attempted. Bromination on the unsubstituted pyrrole derivative, Danaidone, even under free radical conditions resulted in bromination of the pyrrole nucleus. Nitration attenuates the reactivity of the pyrrole ring so bromination of **7** using NBS and 2,2′-azobisisobutyronitrile (AIBN) proceeded smoothly to give the desired bromo derivative **8**.[16] Hydrolysis of **8** yielded alcohol **9** in a quantitative yield. Reduction of alcohol **9** with sodium borohydride yielded the diol **10**, in an 87% yield. Acetylation of the diol **10** gave the diacetate **11** in a 70% yield. Ethanolysis of the bromo derivative **8** resulted in formation of **12** in a 93% yield. Reduction of the ether **12** with sodium borohydride, followed by acetylation, gave the acetate **13** in a 92% overall yield for the two steps (Scheme 5).

Acetates **11** and **13** were unchanged by stirring in methanol at rt overnight. In an attempt to see whether reduction of the nitro group resulted in solvolysis of the acetoxy groups at C-1 and C-8, catalytic hydrogenation on Pd/C in methanol[17] was carried out on both **11** and **13**. However, this resulted in a mixture of very polar products which were very difficult to isolate and purify on a milligram scale.

3. Conclusion

Two 1*H*-2,3-dihydropyrrolizine derivatives bearing a nitro group at the 6 position have been synthesized and an improved method for nitrating pyrroles using potassium nitrate in trifluoroacetic acid was developed. Results from reducing the nitro acetates **11** and **13** by Pd-catalyzed hydrogenation were inconclusive. An efficient, two-step synthesis of the butterfly pheromone, Danaidone, was also developed with an overall 33% yield.

4. Experimental

4.1. General

Melting points were uncorrected and were recorded on a Thomas Hoover melting point apparatus. All chemicals were obtained from commercial suppliers and used without further purification, unless otherwise mentioned. All reactions were carried out under nitrogen. Thin layer chromatography (TLC) was carried out on pre-coated plates and visualized under UV light. Flash column chromatography was performed with silica (Merck 230–400 mesh), unless mentioned otherwise. Elemental analyses were processed by Quantitative Technologies Inc., Whitehouse, NJ. Mass spectra were processed by the University of California Mass Spectroscopy Facility, Riverside, CA, and the Center for Advanced Food Technology, Rutgers University, New Brunswick, NJ. Infrared spectra were recorded by using a

Matteson Genesis FT-IR spectrometer. ^{1}H and ^{13}C NMR were recorded on Varian-Gemini 200 MHz, Varian Unity 300 MHz and Varian Unity 400 MHz spectrometers using commercially available $CDCl_3$ as the solvent, unless otherwise noted. Proton and carbon chemical shifts are expressed in parts per million (ppm) downfield relative to internal tetramethylsilane. ^{1}H NMR spectra are reported on the δ scale with splitting patterns, coupling constants, and relative integral areas. The letter designates the multiplicity of the signal: s, singlet; bs, broad singlet; d, doublet; dd, doublet of doublet; t, triplet, q, quartet; m, multiplet. ^{13}C NMR spectra are proton decoupled spectra unless otherwise mentioned. Dry solvents were either freshly distilled over calcium hydride under nitrogen or bought from commercially available sources.

4.1.1. 3-(3-Methyl-pyrrol-1-yl)-propionic acid ethyl ester (5). A total of 9.23 mL (62 mmol) of dimethyl methylsuccinate was added to a 250 mL 3-necked round-bottomed flask fitted with a mechanical stirrer and flushed with nitrogen. A total of 20 mL of freshly distilled dichloromethane was added to the reaction flask, which was then cooled to −78°C. A total of 217 mL of DIBAL (217 mmol, 1 M solution in dichloromethane) was then added to the reaction flask. The reaction mixture was stirred for about 40 min. A solution of 19.0 g (124 mmol) of β-alanine ethyl ester hydrochloride in 150 mL of deionized water was then added slowly to the reaction flask. It was further stirred for 3 h at rt. The reaction mixture was yellow in color with a white fluffy emulsion-like solid floating on the top. The white solid was filtered off and washed with dichloromethane. The combined organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated in vacuo. The product was purified by flash chromatography using neutral alumina in 80:20 petroleum ether/ethyl acetate as the solvent system (significant loss of product occurred if purified on silica gel). A total of 4.91 g (44%) of **2** was obtained a pale yellow oil. It can be stored for up to a maximum of 2 months in the freezer without decomposition. IR: 2985, 2253, 1730, 1267, 1161 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.26 (t, 3H, J=7.1 Hz), 2.09 (s, 3H), 2.74 (t, 2H, J=7.0 Hz), 4.14 (t, 2H, J=7.0 Hz), 4.17 (q, 2H, J=7.1 Hz), 5.96 (d, 1H, J=2 Hz), 6.44 (s, 1H), 6.56 (d, 1H, J=2 Hz). ^{13}C NMR ($CDCl_3$): δ 12.3, 14.6, 37.1, 45.3, 61.3, 110.0, 119.0, 119.5, 120.9, 171.6. MS (EI): m/z 181 (M^+), 108 ($M^+-CO_2CH_2CH_3$), 94 ($M^+-CH_2CO_2CH_2CH_3$). HRMS, m/z (M^+, $C_{10}H_{15}NO_2$) calcd 181.1103, obsd 181.1104.

4.1.2. 7-Methyl-2,3-dihydro-1*H*-pyrrolizin-1-one (Danaidone). A total of 0.320 g (1.76 mmol) of **2** was placed in a 50 mL round-bottomed flask, which was flushed with nitrogen. A total of 15 mL of freshly distilled dichloromethane was added and the reaction flask was cooled to 0°C. A total of 2.5 mL of BBr_3 (2.5 mmol, 1 M solution in dichloromethane) was added and stirred at rt overnight. The reaction was then quenched with 1 mL of deionized water. Saturated sodium bicarbonate solution was added until pH=8. The phases were separated and the aqueous layer was extracted with dichloromethane (2×10 mL). The combined organic layer was then dried over anhydrous sodium sulfate, and the solvent was evaporated in vacuo. The product was purified by flash chromatography using 1:1 petroleum ether/ethyl acetate as the solvent system. A total of 0.179 g (75%) of Danaidone was obtained as pale yellow crystals. Mp 68–70°C. (lit.[8a] 72–74°C). IR: 3053, 2926, 1681, 1389 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.33 (s, 3H), 3.05 (t, 2H, J=6.3 Hz), 4.23 (t, 2H, J=6.3 Hz), 6.28 (d, 1H, J=2 Hz), 6.90 (d, 1H, J=2 Hz).

4.1.3. 7-Methyl-5-nitro-2,3-dihydro-1*H*-pyrrolizin-1-one (6). Compound **6** is obtained as a side product from the synthesis of **7** in <5% yield. It is a pale yellow crystalline solid. Mp 132–134°C. R_f 0.67 (1:1 petroleum ether/ethyl acetate). IR: 3064, 2248, 1721 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 3H), 3.11 (t, 2H, J=6.0 Hz), 4.66 (t, 2H, J=6.0 Hz), 7.04 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 11.2, 38.9, 44.9, 116.4, 116.6, 121.1, 132.3, 190.5. MS (EI): m/z 180 (M^+). Anal. calcd for $C_8H_8N_2O_3$: C, 53.33; H, 4.44; N, 15.55. Found: C, 53.48; H, 4.43; N, 15.55.

4.1.4. 7-Methyl-6-nitro-2,3-dihydro-1*H*-pyrrolizin-1-one (7). A total of 0.100 g (0.741 mmol) of Danaidone was placed in a 10 mL round-bottomed flask with a stir bar and the reaction vessel was flushed with nitrogen. To this, 0.187 g (1.85 mmol) of potassium nitrate was added and the reaction flask was cooled to 0°C. A total of 2 mL of trifluoroacetic acid was added dropwise and the temperature was maintained between 4 and 6°C. The reaction was monitored by TLC (1:1 petroleum ether/ethyl acetate). After 45 min, the reaction was complete and was quenched with 2 mL of deionized water, then the solvent was evaporated in vacuo and purified by flash chromatography using 1:1 petroleum ether/ethyl acetate (R_f 0.15) as the solvent system. A total of 0.080 g (60%) of **7** was obtained as yellow crystalline solid. Mp 72–74°C. IR: 3071, 2250, 1726 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.60 (s, 3H), 3.08 (t, 2H, J=6.6 Hz), 4.35 (t, 2H, J=6.6 Hz), 7.79 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 11.4, 39.0, 43.4, 119.0, 122.4, 122.7, 146.2, 191.7. MS (EI): m/z 180 (M^+). Anal. calcd for $C_8H_8N_2O_3$: C, 53.33; H, 4.44; N, 15.55. Found: C, 53.46; H, 4.42; N, 15.53.

4.1.5. 7-Bromomethyl-6-nitro-2,3-dihydro-1*H*-pyrrolizin-1-one (8). A total of 0.081 g (0.45 mmol) of **7** was placed in a 50 mL round-bottomed flask, which was fitted with a reflux condenser and flushed with nitrogen. A total of 20 mL of anhydrous carbon tetrachloride and 160 mg (1.01 mmol) of *N*-bromosuccinimide were added to the reaction flask. It was heated to reflux by irradiating with a 660 W sunlamp. A total of 0.030 g (catalytic amount) of AIBN was added quickly when refluxing. It was then refluxed for 3 h. At the end of 3 h, succinimide was found floating in the reaction mixture as a white fluffy solid. It was filtered, washed with carbon tetrachloride (succinimide is soluble in dichloromethane and hence carbon tetrachloride is used for the work-up). Isobutyronitrile was formed from AIBN and was removed from the product by azeotroping with toluene. The product was then purified by flash chromatography using 99.5:0.5 CH_2Cl_2/CH_3OH. A total of 0.094 g (80%) of **8** was obtained as a pale yellow oil. IR: 3054, 2305, 1721, 1422, 1265 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.14 (t, 2H, J=6.5 Hz), 4.42 (t, 2H, J=6.5 Hz), 4.93 (s, 2H), 7.82 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 19.1, 38.8, 43.8, 117.2, 120.0, 122.3, 128.9, 190.0. MS (CI): m/z 261 (M^++3), 259 (M^++1), 165 (M^+-CH_2Br). Anal. calcd for $C_8H_7N_2O_3Br$: C, 37.06; H, 2.70; N, 10.81; Br, 30.88. Found: C, 37.15; H, 2.47; N, 10.83; Br, 31.03.

4.1.6. 7-Hydroxymethyl-6-nitro-2,3-dihydro-1*H*-pyrrolizin-1-one (9). A total of 0.0207 g (0.0799 mmol) of **8** was dissolved in 10 mL of deionized water and refluxed for 3 h under nitrogen. A total of 0.0156 g (100%) of **9** was obtained as a colorless oil and is used without further purification. IR: 3427, 2900, 2250, 1280 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.16 (t, 2H, J=6.5 Hz), 4.46 (t, 2H, J=6.5 Hz), 5.07 (s, 2H), 7.84 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 37.6, 44.5, 68.2, 116.2, 120.8, 125.4, 139.6, 191.0. MS: m/z 196 (M^+). Anal. calcd for $C_8H_8N_2O_4$: C, 48.97; H, 4.02; N, 14.28. Found: C, 48.73; H, 4.02; N, 14.20.

4.1.7. 7-Hydroxymethyl-6-nitro-2,3-dihydro-1*H*-pyrrolizin-1-ol (10). A total of 0.0204 g (0.104 mmol) of **9** was placed in a 50 mL round-bottomed flask with a stir bar under nitrogen. A total of 0.030 mg (0.938 mmol) of $NaBH_4$ and 1 mL of anhydrous methanol was syringed into the reaction flask. The reaction mixture was stirred at 0°C for 1 h. The solvent was evaporated in vacuo and the product was purified by flash chromatography using 98:2 CH_2Cl_2/ CH_3OH. A total of 0.0179 g (87%) of **10** was obtained as a pale yellow oil. IR: 3480, 2965, 2249, 1279 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.35–2.50 (m, 1H), 2.7–2.9 (m, 1H), 3.90–4.05 (m, 1H), 4.15–4.30 (m, 1H), 4.79 (d, 1H, J= 14 Hz), 4.97 (d, 1H, J=14 Hz), 5.2–5.3 (m, 1H), 7.49 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 34.3, 46.0, 67.8, 68.9, 112.5, 113.6, 117.9, 135.2. MS: m/z 198 (M^+). Anal. calcd for $C_8H_{10}N_2O_4$: C, 48.48; H, 5.05; N, 14.14. Found: C, 49.08; H, 4.95; N, 13.85.

4.1.8. 1-Acetoxy-7-acetoxymethyl-6-nitro-2,3-dihydro-1*H*-pyrrolizine (11). A total of 0.0179 g (0.0904 mmol) of **10** was placed in a 25 mL round-bottomed flask under nitrogen. A total of 2 mL of a pre-mixed solution of acetic anhydride and pyridine (1:1) was added to the reaction flask. The reaction mixture was stirred at rt for 3 h and then quenched with 1:1 water/ethyl acetate (2 mL), after which, it was stirred for another hour to hydrolyze any acetic anhydride present. The reaction mixture was washed with deionized water and ethyl acetate, followed by 1N HCl (to remove traces of pyridine), then neutralized with saturated sodium bicarbonate solution until pH=7 and finally washed with saturated brine solution. The combined organic layer was then dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated in vacuo. The product was purified by flash chromatography using dichloromethane as the eluent. A total of 0.018 g (70%) of **11** was obtained as a pale yellow oil. ^1H NMR ($CDCl_3$): δ 2.06 (s, 3H), 2.07 (s, 3H), 2.45–2.55 (m, 1H), 2.75–3.0 (m, 1H), 4.0–4.3 (m, 2H), 5.35 (s, 2H), 6.15–6.25 (m, 1H), 7.62 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 30.0, 34.3, 46.2, 46.5, 64.9, 69.2, 112.2, 114.4, 117.9, 120.0, 170.1, 172.1. MS: m/z 282 (M^+). Anal. calcd for $C_{12}H_{14}N_2O_6$: C, 51.06; H, 4.96; N, 9.93. Found: C, 51.45; H, 4.88; N, 9.68.

4.1.9. 7-Ethoxymethyl-6-nitro-2,3-dihydro-1*H*-pyrrolizin-1-one (12). A total of 0.030 g (0.116 mmol) of **8** was placed in a 25 mL round-bottomed flask, which was fitted with a reflux condenser and flushed with nitrogen. A total of 15 mL of absolute ethanol (200 proof) was then added to the reaction flask and the reaction mixture was refluxed for 3 h. A total of 0.024 g (93%) of **12** was obtained. The product was a colorless oil and used without further purification. IR: 2980, 2253, 1719, 1516, 1328, 1264 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.24 (t, 3H, J=7.1 Hz), 3.11 (t, 2H, J= 6.5 Hz), 3.65 (q, 2H, J=7.1 Hz), 4.39 (t, 2H, J=6.5 Hz), 4.88 (s, 2H), 7.83 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 15.6, 39.1, 43.6, 60.6, 67.0, 117.9, 122.4, 129.9, 140.2, 190.6. MS: m/z 224 (M^+). Anal. calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.36; N, 12.50. Found: C, 53.26; H, 5.72; N, 12.22.

4.1.10. 7-Ethoxymethyl-6-nitro-2,3-dihydro-1*H*-pyrrolizin-1-ol. A total of 0.020 g (0.0892 mmol) of **12** was placed in a 10 mL round-bottomed flask, which was maintained at 0°C using an ice bath. A total of 0.006 g (0.188 mmol) of $NaBH_4$ and 1 mL of anhydrous methanol were added. The reaction was stirred for 30 min at 0°C. The solvent was evaporated in vacuo and purified by flash chromatography using 98:2 CH_2Cl_2/CH_3OH. A total of 0.019 g (95%) of the product was obtained as a pale yellow oil. IR: 3459, 2983, 2253, 1506, 1267, 909 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.25 (t, 3H, J=7.0 Hz), 2.35–2.50 (m, 1H), 2.75–2.85 (m, 1H), 3.56 (q, 2H, J=7.0 Hz), 3.95–4.05 (m, 1H), 4.15–4.25 (m, 1H), 4.79 (d, 1H, J=14 Hz), 4.97 (d, 1H, J=14 Hz), 5.2–5.3 (m, 1H), 7.49 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 15.7, 35.7, 46.7, 67.6, 67.8, 68.2, 112.3, 112.8, 116.5, 137.8. MS: m/z 226 (M^+). Anal. calcd for $C_{10}H_{14}N_2O_4$: C, 53.09; H, 6.19; N, 12.39. Found: C, 52.71; H, 6.48; N, 11.97.

4.1.11. 1-Acetoxy-7-ethoxymethyl-6-nitro-2,3-dihydro-1*H*-pyrrolizine (13). A total of 0.020 g (0.0884 mmol) of the preceding alcohol was placed in a 10 mL round-bottomed flask fitted with a stir bar, to which a pre-mixed solution of 1 mL acetic anhydride and 1 mL pyridine was added at rt. The reaction mixture was stirred for 3 h and then quenched with 1:1 water/ethyl acetate (2 mL), after which, it was stirred for another hour to hydrolyze any acetic anhydride present. The reaction mixture was washed with deionized water and ethyl acetate, followed by 1N HCl (to remove traces of pyridine), then neutralized with saturated sodium bicarbonate solution until pH=7 and finally washed with saturated brine solution. The combined organic layer was then dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated in vacuo. The product was purified by flash chromatography using 98:2 CH_2Cl_2/ CH_3OH. A total of 0.023 g (97%) of **13** was obtained as a pale yellow oil. ^1H NMR ($CDCl_3$): δ 1.21 (t, 3H, J=7.0 Hz), 2.07 (s, 3H), 2.4–2.55 (m, 1H), 2.75–2.95 (m, 1H), 3.60 (q, 2H, J=7.0 Hz), 3.95–4.30 (m, 2H), 4.74 (s, 2H), 6.23 (d, 1H, J=6.3 Hz), 7.59 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 15.7, 30.2, 35.9, 46.1, 65.0, 66.9, 69.8, 114.1, 117.9, 128.7, 133.7, 170.6. MS: m/z 268 (M^+). Anal. calcd for $C_{12}H_{16}N_2O_5$: C, 53.73; H, 5.97; N, 10.45. Found: C, 54.03; H, 6.27; N, 10.67.

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