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Research Article

Synthesis of [$^{13}C_4$,$^{15}N_2$]pyrrolo[2,1-*f*][1,2,4]triazinone†

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Summary

[$^{13}C_4$,$^{15}N_2$]Pyrrolotriazinone, **1**, was synthesized in six steps from ethyl (1,2,3,4-$^{13}C_4$)acetoacetate and (^{15}N)ammonium hydroxide. A total of 1.3 g [$^{13}C_4$, $^{15}N_2$]pyrrolotriazinone was obtained in an overall yield of 17% based on isotopic ethyl acetoacetate. Chemical purity was determined by HPLC to be 99.5%. The percent [^{13}C,^{15}N]-isotopic abundance in [$^{13}C_4$,$^{15}N_2$]pyrrolotriazinone was determined by mass spectral analysis to be 98.0%. The fully assigned ^{1}H and ^{13}C NMR spectra of [$^{13}C_4$,$^{15}N_2$]pyrrolotriazinone were consistent with the desired structure. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: [^{13}C]; [^{15}N]; stable label; chemotype; pyrrolotriazine

Introduction

Pyrrolo[2,1-*f*][1,2,4]triazinone is a novel chemical structure utilized as a core chemotype within medicinal chemistry.[1,2,3] This chemotype scaffold was common to a number of programs and thereby presented an opportunity to support both discovery and development activities across several programs by the preparation of an isotopically labeled core pyrrolotriazinone. This strategy allows for the general utility of the isotopic label pyrrolotriazines without concern for subsequent SAR elaborations that would afford unique drug candidates. Preparation of [$^{13}C_4$,$^{15}N_2$]pyrrolotriazinone, **1**, would be most generally useful in subsequent LC-MS analysis since this M + 6 fragment would likely provide separation from the MS envelope of unlabeled drug (Figure 1).

Generation of an M + 6 stable label was envisioned by initial formation of stable-labeled α-aminocarbonylpyrrole via cyclocondensation of readily

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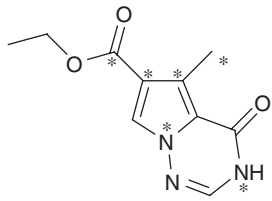


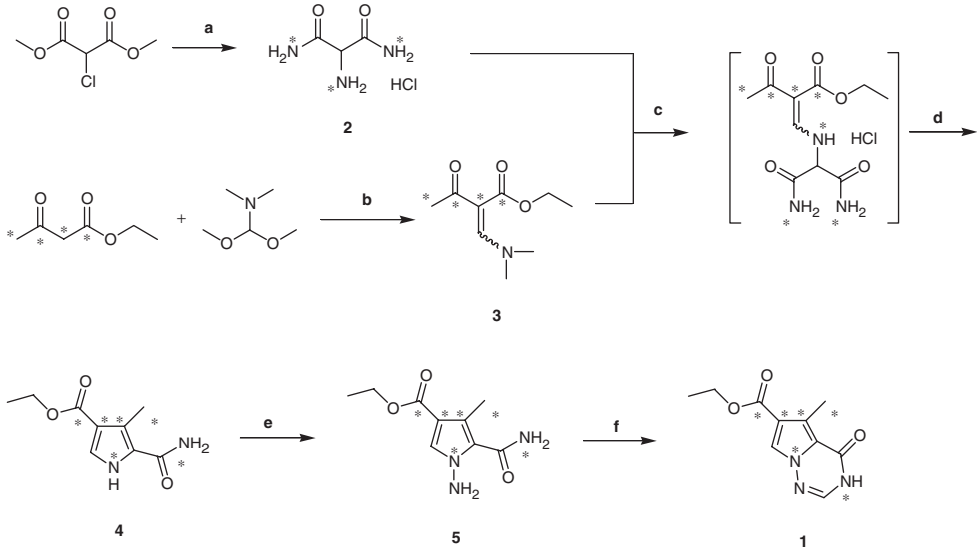
[$^{13}C_4$,$^{15}N_2$]pyrrolotriazinone, **1**

Figure 1. [$^{13}C_4$,$^{15}N_2$]pyrrolotriazinone, 1

available precursors ethyl (1,2,3,4-$^{13}C_4$)acetoacetate and (^{15}N)ammonium hydroxide.[4,5,6] Elaboration of the pyrrole to the desired [$^{13}C_4$,$^{15}N_2$]pyrrolotriazinone, **1**, could be achieved by *N*-amination, followed by cyclization of the resultant *N*-amino-α-aminocarbonylpyrrole with a formate equivalent.[1,7] The preparation and characterization of [$^{13}C_4$,$^{15}N_2$]pyrrolotriazinone, **1**, is herein detailed. Noteworthy, this synthetic strategy would also allow for the preparation of [^{14}C]pyrrolotriazinone via a [^{14}C]radiolabeled formate equivalent such as applied to the preparation of labeled nucleosides.[8,9]

Results and discussion

The first reactant for the cyclocondensation was prepared by reaction of dimethyl chloromalonate with an excess of (^{15}N)ammonium hydroxide in a pressure tube maintained at 45°C (Scheme 1). This reaction proceeded slowly



Scheme 1. Synthesis of [$^{13}C_4$,$^{15}N_2$]pyrrolo[2,1-*f*][1,2,4]triazinone, 1

at this temperature, but cleanly afforded the ($^{15}N_3$)aminodiamide hydrochloride, **2**, which precipitated from the reaction solution, and was isolated by filtration, in quantitative yield.

The second precursor to the cyclocondensation was prepared by aminomethylation of ethyl (1,2,3,4-$^{13}C_4$)acetoacetate with *N*,*N*-dimethylformamide dimethyl acetal to afford the *β*-aminoenone, **3**.[5,6] This *β*-aminoenone was isolated as a crude oil and used without further purification. The MeOH liberated by condensation did not effect transesterification of the acetoacetate ethyl ester, as determined by NMR.

The cyclocondensation reaction was achieved by reaction of aminodiamide hydrochloride **2** with the *β*-aminoenone, **3**, in glacial acetic acid at room temperature for 18 h, followed by heating at 100°C for 6 h.[4,5] Pyrrole formation proceeded by initial nucleophilic substitution of the protonated dimethylaminoenone of **3** by the primary amine of the diamide, **2**. Cyclocondensation then occurred at the more electrophilic carbonyl center, and the desired product was formed with loss of the primary amide via a decarboxylation-like reaction. The crude material was purified by silica gel chromatography, to afford the pyrrole, **4**, as a white solid in a modest yield of 30%.

Preparation of *N*-aminopyrrole was achieved by the reaction of sodium anion of the pyrrole **4** with monochloroamine in anhydrous DMF.[7] The desired *N*-aminopyrrole **5** was, in part, precipitated from the solution upon quenching with aqueous $Na_2S_2O_3$. This precipitated product was isolated by filtration. Additional *N*-aminopyrrole was isolated from the filtrate following extractive workup and chromatography.

The target [$^{13}C_4$,$^{15}N_2$]pyrrolotriazinone, **1**, was prepared by condensation of *N*-aminopyrrole with an excess of triethylorthoformate and a catalytic amount of *p*-toluenesulfonic acid monohydrate in *N*,*N*-dimethylacetamide. The reaction afforded high yield of [$^{13}C_4$,$^{15}N_2$]pyrrolotriazinone, which was isolated by filtration following quenching the reaction with water. The chemical purity of **1** was 99.5% and the isotopic abundance determined by mass spectral analysis was 98.0%. Characterization of [$^{13}C_4$,$^{15}N_2$]pyrrolotriazinone **1** was consistent with authentic unlabeled standard.

Experimental

General: E. Merck silica gel 60F plates (250 μm) were used for analytical TLC. TLC plates were visualized by UV and by iodine. Flash grade silica gel for use in gravity chromatography was obtained from EM Science. ^1H and proton decoupled ^{13}C NMR were recorded on a Bruker DRX-400 MHz or a JEOL ECL-400 MHz spectrometer. Chemical shifts are reported in ppm downfield from TMS. Mass spectra were taken on either a Sciex API3 Plus triple quadrupole mass spectrometer or a Finnigan SSQ 7000 single quadrupole

mass spectrometer. Elemental analysis and Karl–Fischer analysis were performed by Robertson Microlit Laboratories. Melting point was determined by Melt-Temp melting point apparatus. HPLC grade H_2O was obtained by deionization and reverse osmosis (Milli-Q).

Materials: (^{15}N)Ammonium hydroxide (98%+, 6 N in H_2O) and ethyl (1,2,3,4-$^{13}C_4$)acetoacetate (99%, lot PR13101) were purchased from Cambridge Isotope Laboratories, Inc. Monochloramine was prepared *in situ* by reaction of NH_4Cl and NH_4OH with NaOCl in anhydrous ether at 0°C.[7]

High-performance liquid chromatography: HPLC was performed with an Shimadzu SCL-10A system controller, dual LC-10AT pumps, SPD-10A UV-VIS detector and CLASS VP 5.0 integration software. Method A used YMC-ODS-A column (4.6 × 150 mm, 3 μm) with mobile phase A: 0.1% TFA/H_2O and B: CH_3CN and a flow rate of 1.0 ml/min. A gradient of 5% B from 0 to 5 min, 5–95% B from 5 to 25 min and 95–5% B from 25 to 30 min was used. UV detection was monitored at 210 and 254 nm.

2-($^{15}N_3$)Aminomalonamide hydrochloride **2**

A pressure tube containing (^{15}N)ammonium hydroxide (6 N, 25 ml, 150 mmol) at room temperature was slowly charged with dimethyl chloromalonate (3.0 g, 18.0 mmol). The sealed reaction solution was stirred at room temperature for 15 min, then heated at 40–45°C for 96 h. During this time a light-yellow solid precipitated from the solution. The mixture was cooled to room temperature, evaporated and dried under vacuum to afford 3.20 g (100% yield) of the crude product as a friable HCl salt. This crude salt **2** was used without further purification. ^{1}H-NMR (dimethyl sulfoxide-d_6): δ 3.50 (bs, 3H), 3.82 (s, 1H), 7.25 (m, 2H), 7.48 (m, 2H).

2-(Dimethylaminomethylene)-3-oxo-(1,2,3,4-$^{13}C_4$)butanoic acid ethyl ester **3**

N,*N*-dimethylformamide dimethyl acetal (1.95 g, 16.4 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate (40 mg, 0.21 mmol) were added into a reaction flask containing ethyl (1,2,3,4-$^{13}C_4$)acetoacetate (2.0 g, 14.9 mmol) maintained under N_2. The reaction mixture was stirred at 80°C. The reaction progress was monitored by TLC (5% MeOH/EtOAc, $R_f = 0.5$) and by HPLC (method A) RT = 18.5 min. After a total of 70 min, the dark red mixture was evaporated to a residue and dried under vacuum to give 2.78 g of a crude dark red oil (90% yield by HPLC). This crude oil **3** was used without further purification. ^{1}H-NMR (dimethyl sulfoxide-d_6): δ 1.25 (t, 3H, $J = 7.1$ Hz), 2.01 (m, 1.5H), 2.50 (m, 1.5H), 2.80 (s, 3H), 2.95 (s, 3H), 4.15–4.20 (m, 2H), 8.02 (s, 1H).

5-(^{15}N)Carbamoyl-4-(^{13}C)methyl-1H-(3,4-$^{13}C_2$,1-$^{15}N_1$)pyrrole-3-(^{13}C)carboxylic acid ethyl ester **4**

A suspension of aminomalonamide **2** (3.20 g, 20.4 mmol) and dimethylaminoenone **3** (2.70 g, 14.3 mmol) in glacial HOAc (50 ml) was stirred at room temperature for 18 h. At this time the reaction had yielded a solution. The solution was heated to 106°C for 6 h, during which time a precipitate formed, which was identified by TLC as an undesired by-product. The reaction was cooled to room temperature, and the suspension was filtered under vacuum. The filtrate was evaporated to dryness, and the crude mixture was chromatographed on silica gel, eluting with CH_2Cl_2 (300 ml) and then CH_2Cl_2/MeOH (97:3) (0.5 l), to afford 850 mg (30%) of product **4** as a white solid. TLC (EtOAc/MeOH (95:5)) R_f 0.5. HPLC (method A) RT 14.0 min. MS (ES^+) 203.08. MP 208–210°C. ^{1}H-NMR (dimethyl sulfoxide-d_6): δ 1.08 (t, 3H, J = 7.1 Hz), 2.12 (m, 1.5H), 2.32 (m, 1.5H), 3.98 (m, 2H), 6.85 (bs, 1H), 7.1 (bs, 1H), 7.25 (m, 1H), 11.4 (s, 0.5H), 11.6 (s, 0.5H).

1-Amino-5-(^{15}N)Carbamoyl-4-(^{13}C)methyl-1H-(3,4-$^{13}C_2$,1-$^{15}N_1$)pyrrole-3-(^{13}C)carboxylic acid ethyl ester **5**

Pyrrole **4** (2.06 g, 10.2 mmol) was suspended in anhydrous DMF (8 ml) and cooled to 0°C under nitrogen. Solid NaH (317.9 mg, 13.2 mmol) was added in portions to this reaction mixture at 0°C. The mixture was stirred at 0°C for 15 min and at room temperature for 1 h. After this time, the reaction resulted in a clear solution. The reaction was cooled to 0°C and an ice-cold solution of ethereal 0.15 M NH_2Cl (13.2 mmol, 88 ml), mixed with anhydrous cold DMF (10 ml), was added slowly (8). The resulting reaction solution was stirred at 0°C for 10 min and at then at room temperature for 3.5 h. The reaction was cooled to 0°C, and saturated $Na_2S_2O_3$ (50 ml) was added, followed by water (40 ml). The desired product, which was partially precipitated during this step, was isolated by vacuum filtration to yield 0.80 g of pure product. The DMF/aqueous filtrate was reduced in volume and extracted with EtOAc (3 × 60 ml). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated to afford crude product. The crude product was chromatographed by silica gel, eluting with EtOAc/Hexane (1:1) to EtOAc/MeOH (95:5), to afford an additional 0.42 g of product **5** (total yield = 1.22 g, 55%). R_f0.45 (5% MeOH/EtOAc); HPLC (method A) RT 13.53 min; mp 170–172°C. ^{1}H-NMR (dimethyl sulfoxide-d_6): δ 1.22 (t, 3H, J = 7.1 Hz), 2.26 (m, 1.5H), 2.60 (m, 1.5H), 4.15 (m, 2H), 6.46 (s, 2H), 7.20–7.34 (m, 1.5H), 7.5 (bs, 0.5H), 8.08 (bs, 0.5H), 8.30 (bs, 0.5H). ^{13}C-NMR (dimethyl sulfoxide-d_6): δ 11.49 (m, $^*CH_3$), 58.93 (CH_3-$\underline{C}H_2$-O), 110.17 (m, $^*C$=$^*\underline{C}$-$^*CH_3$), 123.77 (m, $^*C$=$^*\underline{C}$-$^*CH_3$), 163.43 (m, $^*C$=O).

5-(Methyl-^{13}C)methyl-4-oxo-3,4-dihydro-(5,6-$^{13}C_2$,1,8-$^{15}N_2$)pyrrolo[2,1–f][1,2,4]triazine-6-(carbonyl-^{13}C)carboxylic acid ethyl ester **1**

N-aminopyrrole **5** (1.22 g, 5.6 mmol) was dissolved in *N*,*N*-dimethylacetamide (7 ml), and then triethyl orthoformate (6.24 g, 42.1 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate (40 mg, 0.21 mmol) were added. The mixture was heated to 73°C for 40 min. The reaction was then reduced in volume under vacuum to approximately 8 ml, and the resulting DMA mixture was stirred at room temperature while water (17 ml) was added slowly. The resulting precipitate was collected by vacuum filtration, then washed with ether (25 ml), to give 1.17 g of the desired product as a white solid. The filtrate was extracted with EtOAc (3×30 ml) and the combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated to dryness. This crude product was chromatographed on silica gel eluting with 25–50% EtOAc/hexanes to afford an additional 100 mg of product **1** (total 1.27 g, 99.5% yield). R_f 0.35 (EtOAc/Hexane, 50:50); mp 195–197°C; HPLC (method A) RT 2.41 min, purity 99.5%; MS (ES^+) 228.1. ^{1}H-NMR (dimethyl sulfoxide-d_6): δ 1.27 (t, 3H, $J = 7.1$ Hz), 2.50 (m 1.5H), 2.73 (m, 1.5H), 4.21 (m, 2H), 7.83 (m, 1H), 7.91 (m, 1H), 11.6 (bm, 1H). ^{13}C-NMR (dimethyl sulfoxide-d_6): δ 11.07 (m, $^*CH_3$), 114.05 (m, $^*C{=}\underline{^*C}\text{-}^*CH_3$), 123.01 (m, $^*\underline{C}{=}^*C\text{-}^*CH_3$), 163.33 (m, $^*C{=}O$). Analytically calculated for $C_6 13C_4 H_{11} 15N_3 O_3$: C, 54.62; H, 4.88; N 19.37. Found: C, 54.45; H, 4.77; N, 19.48. Karl–Fisher: <0.10.

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