SYNTHESIS OF [2-^{13}C, 2-^{14}C]2-AMINOETHANOL, [1-^{13}C, 1-^{14}C]2-CHLOROETHYLAMINE, N,N'-BIS([1-^{13}C, 1-^{14}C]2-CHLOROETHYL)-N-NITROSOUREA(BCNU) AND N-([1-^{13}C, 1-^{14}C]2-CHLOROETHYL)-N-NITROSOUREA(CNU)

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SUMMARY

[2-^{13}C, 2-^{14}C]2-Aminoethanol hydrochloride was prepared in good yield from Na*CN in a two step sequence by first converting the Na*CN to $OHCH_2$*CN and then reducing the nitrile directly with a solution of borane-tetrahydrofuran complex. The reaction procedure was simple and the pure product could be obtained readily. Using this specifically labelled precursor, the synthesis of [1-^{13}C, 1-^{14}C]2-chloroethylamine hydrochloride, N-([1-^{13}C, 1-^{14}C]2-chloroethyl)-N-nitrosourea(CNU) and N,N'-bis([1-^{13}C, 1-^{14}C]2-chloroethyl)-N-nitrosourea(BCNU) in good yield without isotope scrambling was also reported.

Key words: N,N'-Bis([1-^{13}C, 1-^{14}C]2-chloroethyl)-N-nitrosourea, N-([1-^{13}C, 1-^{14}C]2-Chloroethyl)-N-nitrosourea, [1-^{13}C, 1-^{14}C]2-Chloroethylamine, [2-^{13}C, 2-^{14}C]2-Aminoethanol, Borane-tetrahydrofuran complex, Nitrosation, Isotope Scrambling.

In our continuing studies on the application of a direct ^{13}C NMR method to determine the molecular basis of the interaction between alkylating agents and nucleic acids[1,2] it is essential to synthesize specifically ^{13}C(^{14}C)-labelled alkylating agents: 2-chloroethylamine(1), N,N'-bis(2-chloroethyl)-N-nitrosourea(BCNU)(11) and N-(2-chloroethyl)-N-nitrosourea(CNU)(13). The addition of the ^{14}C label can be used in quantitation of the percentage of nucleic acid alkylation. The immediate precursor for the synthesis of these agents is [2-^{13}C, 2-^{14}C]2-aminoethanol hydrochloride(6).

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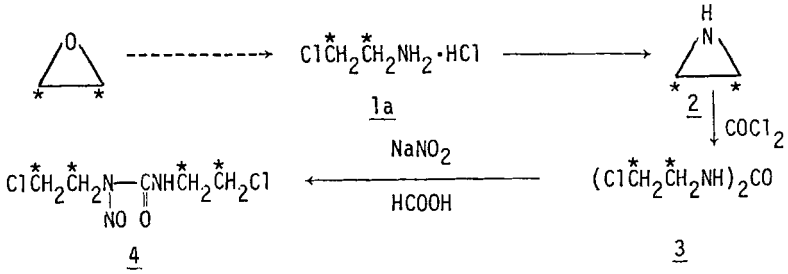
The preparation of 2-aminoethanol hydrochloride(8) from Na*CN has been reported by treatment with formaldehyde, followed by benzoyl chloride and then reduction of the cyanomethyl benzoate with lithium aluminum hydride (LAH)[3]. The yield is poor (30.9% based on Na*CN), and the work-up of the reaction is elaborate. The poor yield is probably due to the hydrogenolysis of the C-OH bond by LAH. A milder reducing agent may, therefore, be desirable.

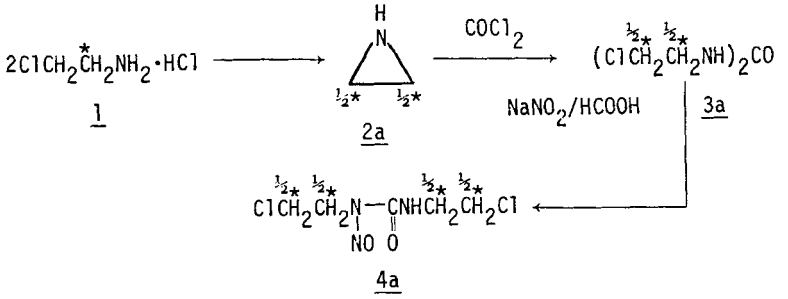
We successfully used a solution of borane-tetrahydrofuran complex[4] to reduce directly the glyconitrile(7) which can be readily obtained by reaction of formaldehyde(5) with Na*CN(6) in aqueous solution[5]. The final product is precipitated from the reaction mixture by passing HCl gas. No further purification is necessary.. This approach provided the desired compound in 60% yield based on Na*CN. This method offers a simpler procedure to perform the reduction without protection of the hydroxy group. Further treatment with thionyl chloride gave [1-^{13}C, 1-^{14}C]2-chloroethylamine hydrochloride(1) in quantitative yield.[6]

BCNU was previously synthesized from 2-chloroethylamine hydrochloride by conversion of 2-chloroethylamine hydrochloride (1) to aziridine[7](2), which then reacted with phosgene in acetone solution[8] to yield the N,N'-bis(2-chloroethyl)urea(3). Nitrosation[9] with $NaNO_2$/HCOOH gave the desired product(4) with ^{14}C in the chloroethyl groups.[10] (Schemes 1 and 2). However, the synthesis via scheme 1 would result in second-order ^{13}C spectra due to the near equivalence of the two methylene carbon resonances, while synthesis following scheme 2 would result in isotope scrambling, which reduces the NMR sensitivity and makes the ^{13}C spectrum of the nucleic acid adduct too complicated.

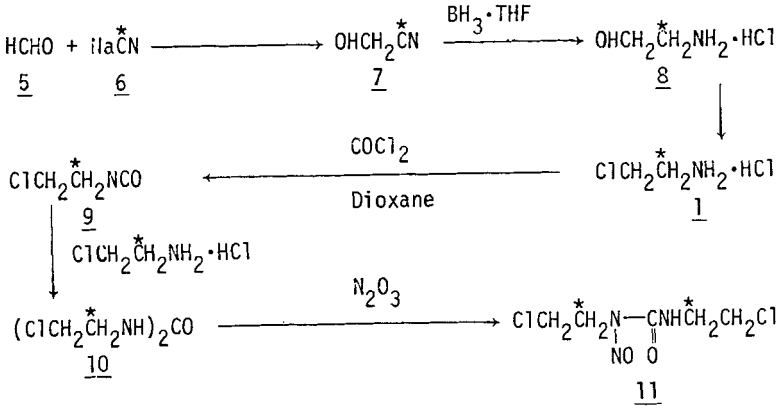
Scheme 1



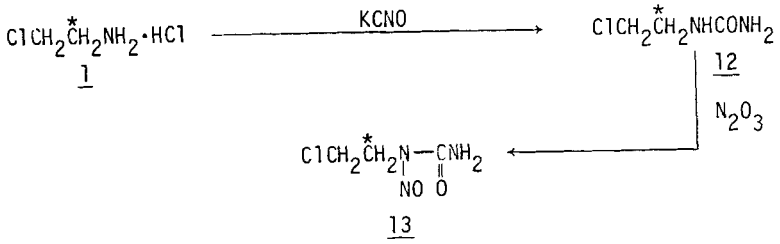
Scheme 2



Scheme 3



Scheme 4



Brundrett et al. synthesized BCNU-α-d_4 and BCNU-β-d_4 via the oxazolidone intermediate to prevent isotope scrambling[11], but the yield was low (12%).

The synthesis of BCNU was therefore modified to make specifically carbon-labelled compounds without isotope scrambling or second-order spin-spin couplings and in good yield [an overall yield of 50% based on 2-aminoethanol hydrochloride (8)]. The method involved generation of the 2-chloroethyl isocyanate(9) by bubbling phosgene gas into a slurry of 2-chloroethylamine hydrochloride in dioxane at 65-70°C followed by dropwise addition of the above solution to an ice cold aqueous solution of 2-chloroethylamine hydrochloride(1) previously neutralized with triethylamine. The precipitated N,N'-bis(2-chloroethyl)urea(10) was then nitrosated with N_2O_3 in dichloromethane[12] to yield the labelled N,N'-bis(2-chloroethyl)-N-nitrosourea(11). The ^{13}C NMR spectrum of the product confirmed that no isotope scrambling occurred during the synthesis.

The other alkylating agent N-([1-^{13}C, 1-^{14}C]2-chloroethyl)-N-nitrosourea(13) was also obtained from [1-^{13}C, 1-^{14}C]2-chloroethylamine hydrochloride(1) by reaction with KCNO[13] followed by nitrosation of the resulting urea derivative 12 with N_2O_3 in dichloromethane which gave better yields (50%) as compared to the literature method[13] (27%).

EXPERIMENTAL

[2-^{13}C, 2-^{14}C]2-Aminoethanol hydrochloride(8): [^{13}C]Sodium cyanide ($Na^{13}CN$, 95%, 500 mg, 10 mmol, from Prochem U.S. Service) was taken in an Erlenmeyer flask to which 500 μCi $Na^{14}CN$ (specific activity 60 mCi/mmol, from Amersham Inc.) was transferred with water, the total volume being made to 1.2 ml, then 0.9 ml of 37% formaldehyde solution was added dropwise with stirring. An additional 0.7 ml H_2O was added afterwards. The

temperature of the reaction mixture was maintained between -5 to 0°C. After stirring for 10 min. 1.15 ml of cold H_2SO_4 solution (2.85 ml conc. H_2SO_4 in 8.65 ml H_2O) was slowly added. A 5% solution of NaOH was then added dropwise until the pH was about 3. The solution was centrifuged and the residue washed with 5 ml portions of ether five times. The ether solutions and the centrifugate were combined and continuously extracted with ether for 24 hr.

The ether extract was dried for 3-4 hr over Drierite, then transferred to a 50 ml two-neck flask to which a septum and a distillation unit were attached. The flask was flushed with nitrogen, 15 ml of dry THF (distilled over LAH and stored under nitrogen) was injected into the flask and most of the ether distilled. The distillation unit was replaced with a water condenser and, under nitrogen, 5.7 ml of 1.76 M BH_3·THF (10 mmol)[14,15] was injected into the reaction vessel slowly with cooling. After the addition was completed the reaction mixture was refluxed for 2 hr and then stirred at room temperature for 4 hr. The excess diborane was destroyed by the slow addition of 2 ml of methanol and vigorous stirring for 1 hr. A stream of dry HCl gas was then passed through the reaction mixture and the precipitated hydrochloride filtered, washed with ether and dried to yield 591 mg of 2-aminoethanol hydrochloride (60% based on Na*CN), specific activity 48.5 μCi/mmol; m.p. 80-82°C (lit.[3] m.p. 79.8-82°C); cochromatographed with an authentic sample on sil G/UV_{254} TLC plate (Brinkmann Instrument, Inc.) developed with n-BuOH : AcOH : H_2O-3 : 1 : 1, Rf 0.26; 95% ^{13}C-enrichment based on ^{1}HNMR calculation; ^{13}CNMR (D_2O) (δ) : 41.6 (C_2) ppm.

[1-^{13}C, 1-^{14}C]2-Chloroethylamine hydrochloride(1): [2-^{13}C, 2-^{14}C]2-Aminoethanol hydrochloride(8) (591 mg, 6.0 mmol) was added

into 1 ml of toluene in a 15 ml round-bottom flask fitted with water condenser and $CaCl_2$ drying tube. Purified thionyl chloride (0.5 ml, 6.5 mmol) was then added to the reaction flask and gradually heated to 60-65°C. After 3 hr an additional amount of thionyl chloride (0.2 ml) was added and heated for another 2 hr. The reaction mixture was cooled to room temperature, and methanol added dropwise to decompose excess thionyl chloride. The solvent was evaporated. This procedure was repeated until all thionyl chloride was removed. A white product was obtained and dried at 60°C under vacuum, 698 mg (99.4%); m.p. 148-150°C (lit.[6] m.p. 148.5-150°C); specific activity: 48.4 μCi/mmol. The radiochromatogram (sil G/UV_{254}, Brinkmann Instrument Inc.) showed a single product, identical with an authentic sample, Rf 0.37 (n-BuOH : AcOH : H_2O-3 : 1 : 1), ^{13}CNMR (D_2O) (δ) : 42.6 ppm.

[1-^{13}C]2-Chloroethyl isocyanate(9): [1-^{13}C]2-Chloroethylamine hydrochloride(1), (292 mg, 2.5 mmol, obtained from Na^{13}CN, 95%, following the procedure for the synthesis of [1-^{13}C, 1-^{14}C]-2-chloroethylamine hydrochloride) was slurried in 5 ml of dioxane. Phosgene gas was cautiously bubbled through it while the temperature of the reaction mixture was slowly brought to 65-70°C. When a clear solution resulted, the bubbling of phosgene was stopped, and the reaction mixture heated at 65-70°C for 1 hr. The excess phosgene was then removed by flushing with nitrogen. The clear solution of [1-^{13}C]2-chloroethyl isocyanate was employed for the next stage without further purification.

N,N'-Bis([1-^{13}C, 1-^{14}C]2-chloroethyl)urea(10): The solution of [1-^{13}C]2-chloroethyl isocyanate(9) in dioxane obtained from the previous reaction was added dropwise to a stirred solution of 292 mg (2.5 mmol) of [1-^{13}C, 1-^{14}C]2-chloroethylamine hydrochloride(1, specific activity 5 μCi/mmol, prepared by adding

30 mg of [1-^{13}C, 1-^{14}C]2-chloroethylamine hydrochloride, specific activity 48.4 μCi/mmol, to 262 mg of [1-^{13}C]2-chloroethylamine hydrochloride) in 5 ml water containing 0.35 ml (2.5 mmol) of triethylamine at 0°C. It was stirred for two hours and the precipitated N,N'-bis ([1-^{13}C, 1-^{14}C]2-chloroethyl)urea(10) was filtered, washed well with water and dried, yield 233 mg (50% based on [1-^{13}C]2-chloroethylamine hydrochloride), m.p. 126-127°C (lit.[8] m.p. 127°C), specific activity 4.8 μCi/mmol.

N,N'-Bis([1-^{13}C, 1-^{14}C]2-chloroethyl)-N-nitrosourea(11): The above bisalkylurea(10) (233 mg, 1.25 mmol) was slurried in 15 ml of dry dichloromethane, the temperature being maintained between 0-5°C. N_2O_3 gas was slowly bubbled into the reaction mixture till a clear pale yellow solution was obtained. The dichloromethane was evaporated and the yellow oil left behind was shaken with 30 ml pet. ether (b.p 50-60°C fraction) and chilled in the freezer for 24 hr when a yellow crystalline solid separated. The solid BCNU was filtered in the cold room and washed well with cold pet. ether to yield 239 mg (89%) of product, m.p. 31-32°C (lit.[9] m.p. 30-32°C); Specific activity: 4.97 μCi/mmol. The radiochromatogram (sil G/UV$_{254}$, Brinkmann Instruments Inc.) revealed a pure product identical with an authentic sample, Rf 0.67 (benzene: chloroform 1:1). ^{13}C NMR ($CDCl_3$) (δ): 29.97 ($ClCH_2$-$CH_2N(NO)$—); 42.25 ($ClCH_2CH_2NH$—).

N-([1-^{13}C, 1-^{14}C]2-Chloroethyl)urea(12): [1-^{13}C, 1-^{14}C]2-Chloroethylamine hydrochloride(1) (388 mg, 3.32 mmol, specific activity 12.4 μCi/mmol, prepared by adding 100 mg of [1-^{13}C, 1-^{14}C]2-chloroethylamine hydrochloride, specific activity 48.4 μCi/mmol, to 288 mg of [1-^{13}C]2-chloroethylamine hydrochloride) was dissolved in 10 ml water, and 269 mg (3.32 mmol) of potassium cyanate added. The solution was evaporated to dryness

in vacuo (bath temp. 30°C). The residue was extracted repeatedly with hot absolute ethanol and filtered. The filtrate was evaporated to dryness and the solid obtained was crystallized from ethanol to yield 305 mg (75%) of product, m.p. 100-101°C (lit.[13] m.p. 99-101°C), specific activity 12.3 μCi/mmol.

N-[1-^{13}C, 1-^{14}C]2-Chloroethyl-N-nitrosourea(13): In a slurry of 305 mg (2.5 mmol) of 12 in 20 ml dry dichloromethane, maintained at 10°C, in a moisture free atmosphere, N_2O_3 gas was slowly bubbled till the slurry became homogenous and a pale yellow solution resulted. The solution was filtered and the dichloromethane evaporated leaving behind a pale yellow crystalline solid, 190 mg (50% yield) m.p. 77-78°C (lit.[13] m.p. 75-78°C) specific activity 12.3 μCi/mmol. The radiochromatogram (sil G/UV$_{254}$, Brinkmann Instruments Inc.) showed a single product, Rf 0.54 (chloroform: ethylacetate 8:2). ^{13}CNMR ($CDCl_3$) (δ): 39.65 ($ClCH_2\underline{C}H_2N$-) ppm.

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15. Standard solutions of the borane-tetrahydrofuran complex (1.0 M) are available from the Aldrich Chemical Company.