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SYNTHESIS OF ^{15}N LABELLED ISOPHOSPHORAMIDE MUSTARD

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SUMMARY

The ifosfamide metabolite isophosphoramide mustard (IPM) was synthesized with isotopic enrichment at each nitrogen site. Glycine-^{15}N was converted to 2-chloroethylamine-^{15}N hydrochloride (4 steps, 21% net yield) which was then reacted with phenyl dichlorophosphate to provide *N,N'*-bis(2-chloroethyl)phosphorodiamidic-$^{15}N_2$ acid phenyl ester [62%, PhOP(*O*)($^{15}NHCH_2CH_2Cl)_2$]. Catalytic hydrogenation of this phenyl ester followed by the addition of cyclohexylamine (CHA) provided IPM-$^{15}N_2$ as the CHA salt (63%).

Key words. $^{15}N_2$-isophosphoramide mustard; 2-chloroethylamine-^{15}N hydrochloride; ifosfamide metabolite.

INTRODUCTION

Cyclophosphamide (*Cytoxan*) is clinically effective against a broad spectrum of human cancers. Of the hundreds of analogs engendered by cyclophosphamide, its structural isomer ifosfamide (*Ifex*) is among the most notably useful.[1-4] It is generally accepted that these drugs undergo similar metabolic transformations, ultimately generating the DNA bisalkylating agents phosphoramide mustard [PM, $HOP(O)(NH_2)N(CH_2CH_2Cl)_2$] and isophosphoramide mustard [IPM, $HOP(O)(NHCH_2CH_2Cl)_2$], respectively.[2,3] Experiments have indicated that both PM and IPM alkylate nucleophiles *via* the intermediacy of electrophilic aziridines and/or aziridinium ions;[5-7] however, the reactivity of PM toward aziridinium ion formation is

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considerably greater than that of IPM at physiological pH and temperature.[5,6] While the reasons for these differences in alkylation kinetics between PM and IPM are not obvious, the differences are dramatic and ought to result in significant variations in *in vivo* DNA alkylation chemistry.

The alkylation chemistry of PM and IPM must be directed by the charge densities which exist in these molecules. In particular, electron densities at the nitrogen atoms are presumably key factors in determining the alkylating activity of these species. We have reported the use of ^{15}N isotopic labelling and NMR spectroscopy to probe the electronic characteristics of PM.[8,9] To pursue companion studies of IPM, similarly isotopically labelled materials are required. We now report the synthesis of IPM with ^{15}N incorporated at both nitrogens [*N,N'*-bis(2-chloroethyl)phosphorodiamidic-$^{15}N_2$ acid (1)].

DISCUSSION

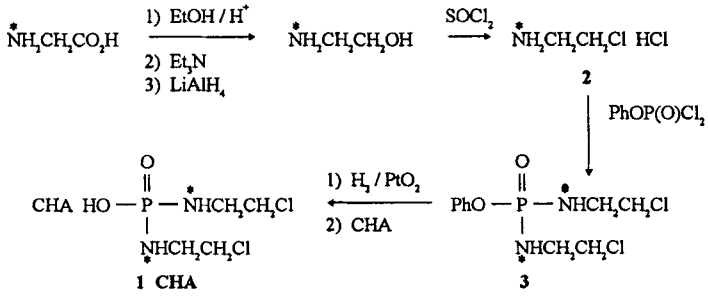
Synthesis of N,N'-Bis(2-chloroethyl)phosphorodiamidic-$^{15}N_2$ Acid (1). A key intermediate in the synthesis of ^{15}N labelled IPM was 2-chloroethylamine-^{15}N hydrochloride (2). As outlined in the given scheme, this compound was made from glycine-^{15}N (98 atom % ^{15}N) through sequential esterification, reduction, and chlorination reactions. The chlorination reaction proved to be particularly problematic. Low product yields and purity resulted when published syntheses of ^{15}N and ^{13}C labelled bis(2-chloroethyl)amine hydrochlorides[8,10] were used as models for the synthesis of 2 from ethanolamine-^{15}N. A more successful route was found by following a literature report for the synthesis of tritium labelled 2-chloroethylamine hydrochloride.[11] Use of milder conditions (room temperature) as well as acetonitrile in place of $CHCl_3$ or dimethylformamide during the reaction of ethanolamine-^{15}N with thionyl chloride provided 2 in a consistently higher yield.

With minor modifications to literature procedures,[12,13] 2 was reacted with phenyl dichlorophosphate to provide *N,N'*-bis(2-chloroethyl)phosphorodiamidic-$^{15}N_2$ acid phenyl ester (3). Hydrogenation of 3 followed by reaction with cyclohexylamine yielded the salt of *N,N'*-bis(2-chloroethyl)phosphorodiamidic-$^{15}N_2$ acid (1•CHA). This reaction gave an impurity [^{31}P NMR (D_2O) δ 8.3 (d, $^1J_{^{31}P-^{15}N}$ *ca.* 33 Hz)] which was similar to the contaminant found in the hydrogenation reaction

which produced PM[8]; the impurity could be removed by repeated fractional crystallizations. This by-product arising from the hydrogenation of 3 was not identified; however, the appearance of a doublet (rather than a triplet) in the ^{31}P NMR indicated that one of the ^{15}N moieties had been lost.

During the course of the hydrogenation reaction, aliquots of the reaction mixture were removed at hourly intervals, filtered, and analyzed by ^{31}P NMR. In comparison with unlabelled material, the hydrogenation reaction of labelled phosphorodiamidate 3 [δ_{31P} 12.8 (EtOH)] was suprisingly sluggish (1.5 h versus 7 h, respectively). The longer reaction time did not significantly increase or decrease the amount of impurity [*vide supra*, δ_{31P} 6.9 (EtOH)] formed relative to product 1 [δ_{31P} 10.8 (EtOH)].

Scheme



* = ^{15}N; CHA=cyclohexylamine

CONCLUSION

Through a series of reactions, glycine-^{15}N was converted to 2-chloroethylamine-^{15}N hydrochloride (2). This compound was then used to prepare IPM with isotopic enrichment at each nitrogen atom (1). Each individual reaction in the synthetic scheme was accomplished with a yield better than 50%; the net yield over six steps was 8%.

EXPERIMENTAL SECTION

Cyclohexylamine, tetrahydrofuran (THF), thionyl chloride, and triethylamine refer to dried and/or distilled solvents and reagents. Reaction mixtures that did not include water were carried out under nitrogen. Reactions done at 4 °C refer

to ice bath conditions. Glycine-^{15}N (98 atom % ^{15}N) was purchased from Aldrich and Sigma Chemical Companies. All other solvents and reagents were generally obtained from Aldrich Chemical Company or Fisher Scientific Company. Unlabelled IPM (as the free acid) was synthesized by reported methods[12,13] and was then verified by spectroscopic comparison with material which was a gift from Dr. Wojciech J. Stec of The Polish Academy of Sciences, Lodz, Poland.

Analytical TLC employed 2.5 cm x 10 cm plates coated with a 250 μm layer of silica gel GF (Analtech); I_2 and a 254-nm UV lamp were used for component visualization. Melting points were obtained on a Fisher-Johns Melting Point Apparatus and were uncorrected. Hydrogenations were carried out using a Parr medium-presure shaker-hydrogenator and a 50 mL pressure bottle.

NMR spectra were obtained on a Bruker MSL500 spectrometer; post-acquisition processing was accomplished with a Bruker work-station. ^1H (500 MHz) NMR chemical shifts (ppm) were referenced to TMS ($CDCl_3$) or TSP (D_2O). ^{31}P (202.5 MHz) NMR chemical shifts (ppm) were referenced to a capillary insert of 1% H_3PO_4 in D_2O.

Glycine-^{15}N Ethyl Ester Hydrochloride. A literature procedure was used to prepare the title compound from glycine-^{15}N (3.00 g, 39.4 mmol, 98 atom % ^{15}N).[8] The product was isolated as white needles [5.36 g, 97%, mp 141 °C (mp for unlabelled material: 145-146 °C)]: ^1H NMR (D_2O) δ 4.18 (q, J = 7.2 Hz, 2H, OCH_2), 3.79 (s, 2H, $^{15}NCH_2$), and 1.17 (t, J = 7.2 Hz, 3H, CH_3).

Ethyl 2-Aminoacetate-^{15}N (Glycine-^{15}N Ethyl Ester). Et_3N (8.6 mL, 62 mmol) was added dropwise to a suspension of glycine-^{15}N ethyl ester hydrochloride (7.91 g, 56 mmol) in ether (125 mL). After stirring overnight, the mixture was filtered and the filtrate was concentrated providing the desired product as a pale yellow oil (3.66 g, 64%) which was used without further purification. ^1H NMR ($CDCl_3$) δ 4.19 (q, J = 7.2 Hz, 2H, OCH_2), 3.43 (s, 2H, $^{15}NCH_2$), 1.60 - 1.35 (br s, 2H, $^{15}NH_2$), and 1.28 (t, J = 7.2 Hz, 3H, CH_3).

Ethanolamine-^{15}N. A solution of ethyl 2-aminoacetate-^{15}N (3.56 g, 34 mmol) in THF (35 mL) was added dropwise to a suspension of $LiAlH_4$ (5.16 g, 136 mmol) in THF

(150 mL). The reaction mixture was refluxed overnight and was then cooled to room temperature before the careful and sequential addition of H_2O (2.7 mL), 15% NaOH (2.7 mL), and more H_2O (8.1 mL). The mixture was stirred for 2 h and was then filtered. The filtrate was dried ($MgSO_4$) and then concentrated to afford the crude product as an oil (0.53 g, 25%). Soxhlet extraction (THF, 2 d) of the reaction solids provided additional product [R_f 0.2 ($CHCl_3$ - CH_3OH, 8:2)] which was somewhat more impure by TLC (0.71 g, 34%). Total crude yield: 1.24 g, 59%. ^1H NMR ($CDCl_3$) δ 3.62 (td, J = 5.2, 3.0 Hz, 2H, CH_2O), 2.85 (t, J = 5.2 Hz, 2H, $^{15}NCH_2$), and 2.58 - 2.21 (br s, 3H, $^{15}NH_2$, OH).

2-Chloroethylamine-^{15}N Hydrochloride (2). The title compound was synthesized using minor modifications of a literature procedure.[11] Ethanolamine-^{15}N (200 mg, 2.63 mmol) was dissolved in acetonitrile (8 mL) at room temperature. Thionyl chloride (1.92 mL, 26.4 mmol) was added dropwise over 3 min. The solution was stirred at room temperature for 24 h. Ether (60 mL) was then added and the mixture was allowed to sit at room temperature for 10 min. The precipitating product was readily observed. Following centrifugation, the isolated solid was washed with ether to remove some colored impurities. The product was isolated as a light beige solid (175 mg, 57%). ^1H NMR (D_2O) δ 3.88 (td, J = 5.5, 3.5 Hz, 2H, CH_2Cl) and 3.42 (t, J = 5.5 Hz, 2H, $^{15}NCH_2$).

N,N'-Bis(2-chloroethyl)phosphorodiamidic-$^{15}N_2$ Acid Phenyl Ester (3). 2-Chloroethylamine-^{15}N hydrochloride (2, 100 mg, 0.85 mmol) was suspended in CH_2Cl_2 (2 mL) and was cooled to 4 °C. Phenyl dichlorophosphate (63.2 μL, 0.42 mmol) was added dropwise followed by Et_3N (236 μL, 1.72 mmol). The reaction mixture was allowed to warm gradually to room temperature and stirring was continued overnight. $Et_3N{\cdot}HCl$ was then removed by filtration and the filtrate was concentrated. The crude product was purified by flash chromatography [4 g silica gel (EM Reagents, < 230 mesh), R_f 0.18 (CH_3OH - $CHCl_3$, 1:99)] yielding the product as a pale yellow oil [78.4 mg, 62%, R_f 0.66 (CH_3OH - $CHCl_3$, 1:9)]. ^1H NMR ($CDCl_3$) δ 7.38 - 7.12 (m, 5H, Ar), 3.63 - 3.56 (m, 4H, CH_2Cl), and 3.40 - 3.32 (m, 4H, $^{15}NCH_2$); the shifts of the two ^{15}NH resonances were variable. ^{31}P NMR ($CDCl_3$) δ 11.2 (t, $^1J_{31P-15N}$ = 37.8 Hz).

N,N'-Bis(2-chloroethyl)phosphorodiamidic-$^{15}N_2$ Acid Cyclohexylammonium Salt (1•CHA). A mixture of phosphorodiamidate 3 (78 mg, 0.26 mmol) and PtO_2 (15 mg, 0.66 mmol) in ethanol (3 mL) was hydrogenated at 50 psi for 7 h. The pressure was released and nitrogen was bubbled through the reaction mixture for several minutes. The mixture was diluted with ethanol (8 mL) and to this was added cyclohexylamine (70 μL, 0.52 mmol). After stirring at room temperature for 30 min, the suspension was filtered and the filtrate was concentrated at ambient temperature. The resultant solid was washed with ether (1 mL). This solid was then dissolved in a minimum amount of ethanol and ether was added dropwise to turbidity. After the mixture sat at 4 °C for 1 h, the mother liquor was removed by pipet. The residual solid (an impurity) was rinsed with ether and this ether washing was added to the mother liquor. This solution was concentrated at room temperature. The resultant solid was recrystallized a second time thus affording desired product as a white powder (60 mg) which was judged to be 88% pure by ^{31}P NMR (peak height comparisons). Corrected yield *ca.* 63%. ^1H NMR (D_2O) δ 3.63 - 3.58 (m, 4H, CH_2Cl), 3.11 (apparent dt, $^3J_{HP}$ = 9.9 Hz, $^3J_{HH}$ = 6.1 Hz, 4H, $^{15}NCH_2$), and for the cyclohexylammonium ion, 3.18 - 3.09 (m, 1H), 1.99 - 1.95 (m, 2H), 1.81 - 1.73 (m, 2H), 1.68 - 1.60 (m, 1H), 1.38 - 1.27 (m, 4H), and 1.24 - 1.12 (m, 1H). ^{31}P NMR (D_2O) δ 12.9 (t, $^1J_{31P-15N}$ = 27.5 Hz).

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REFERENCES

1. Zon G. - Progr. Med. Chem. *19:* 205 (1982).

2. Stec W.J. - Organophosphorus Chem. *13:* 145 (1982)

3. Friedman O.M., Myles A., Colvin M. - Adv. Cancer Chemother. *1:* 143 (1979).

4. Brock N. - J. Cancer Res. Clin. Oncol. *111:* 1 (1986).

5. Engle T.W., Zon G., Egan W. - J. Med. Chem. *25:* 1347 (1982).

6. Boal J.H., Williamson M., Boyd V.L., Ludeman S.M., Egan W. - J. Med. Chem. *32:* 1768 (1989).

7. Colvin M., Brundrett R.B., Kan M.-N.N., Jardine I., Fenselau C. - Cancer Res. *36*: 1121 (1976).

8. Ludeman S.M., Shulman-Roskes E.M., Gamcsik M.P., Hamill T.G., Chang Y.H., Koo K.I., Colvin O.M. - J. Labelled Compd. Radiopharm. *33*: 313 (1993).

9. Gamcsik M.P., Ludeman S.M., Shulman-Roskes E.M., McLennan I.J., Colvin M.E., Colvin O.M. - J. Med. Chem. (submitted, 1993).

10. Ho C.-K., Ludeman S.M. - J. Labelled Compd. Radiopharm. *28*: 587 (1990).

11. Madelmont J.C., Moreau M.F., Parry D., Godeneche D., Duprat J., Meyniel G., Oiry J., Imbach J.L. - J. Labelled Compd. Radiopharm. *20*: 7 (1983).

12. Struck R.F., Dykes D.J., Corbett T.H., Suling W.J., Trader M.W. - Br. J. Cancer *47*: 15 (1983).

13. Ludeman S.M., Boyd V.L., Regan J.B., Gallo K.A., Zon G., Ishii K. - J. Med. Chem. *29*: 716 (1986).