AN IMPROVED SYNTHESIS OF 5,5-DIDEUTEROCYCLOPHOSPHAMIDE

Jarman, M., and Taylor, G.N.

Chester Beatty Research Institute, Institute of Cancer Research:Royal Cancer Hospital, London SW3 6JB, England.

SUMMARY

A high yielding synthesis of 5,5-dideuterocyclophosphamide is described which involves the base catalysed deuterium exchange of 3-hydroxypropionitrile and subsequent medium pressure catalytic hydrogenation to yield 1-amino-2,2--dideuteropropan-3-ol. This compound is condensed with N,N-bis(2-chloroethyl)-phosphoramidic dichloride to yield pure 5,5-dideuterocyclophosphamide which is readily isolated as a crystalline monohydrate.

Key Words: Deuterium, Cyclophosphamide, Exchange, Catalytic Hydrogenation.

INTRODUCTION

A systematic study of the deuterated analogs of the metabolically activated alkylating agent cyclophosphamide (1, N,N-[bis(2-chloroethyl)amino]tetrahydro--2H-1,3,2-oxazaphosphorine-2-oxide) has been made with a view to assessing the scope for modifying the metabolism profile of this anticancer drug(1). As part of this study, we have examined the effect of deuterium substitution at C-5 on the rate of the step in the metabolic sequence responsible for the anticancer activity of 1, namely the β-elimination of acrolein (2) from aldophosphamide (3) to yield the phosphoramide mustard (4) (Scheme 1). Deuterium incorporation at C-5 confers a primary kinetic isotope effect (k_H/k_D >5.3) on this elimination which may be expected to decrease both the potency (as measured by the ED_{90}*) and the toxicity (as measured by the LD_{50}) of the drug. The predicted alteration in potency was demonstrated in tests against the ADJ/PC6 plasma cell tumour in mice (1). The ED_{90} values for cyclophosphamide and its 5-d_2 analog were 3.6 and 47.5 mg/Kg, respectively, showed the 5-d_2 analog to be ~13 fold less potent than the parent drug. However, the LD_{50} value (>600 mg/kg) was not attained with the quantities of the labeled analog obtainable by the published

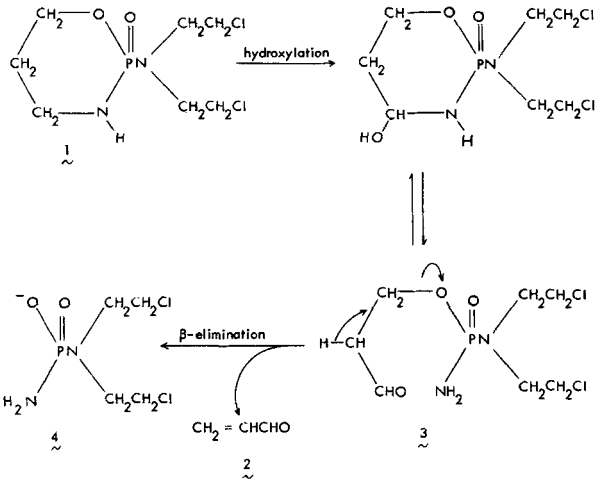
* ED_{90} = dose producing 90% tumor inhibition, LD_{50} = dose lethal to 50% of animals in the group.

0362-4803/81/040463-10$01.00

Received October 17, 1979

synthetic route. For this reason, and for other biological studies an improved synthesis was required, which we now describe.

Scheme 1

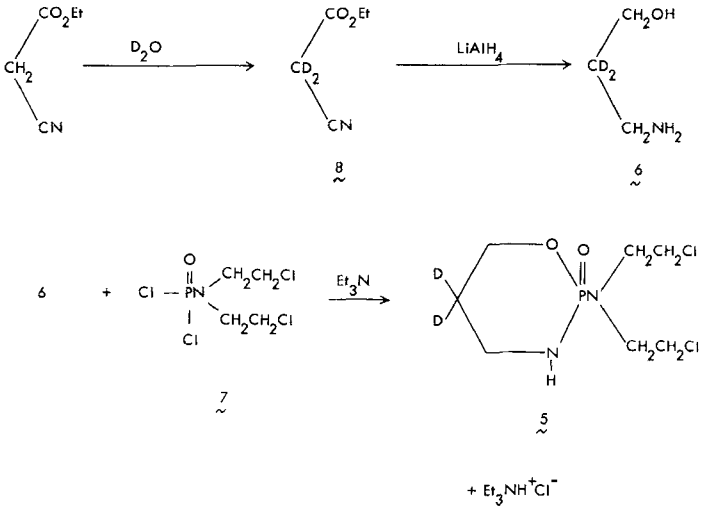


1 2 3 4

DISCUSSION

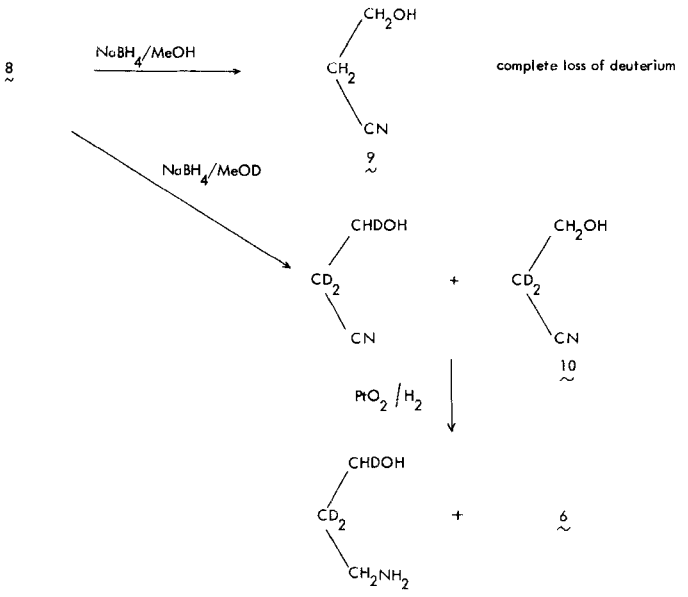
The reported synthesis of 5 (N,N-[bis(2-chloroethyl)amino]tetrahydro-5,5-dideutero-2H-1,3,2-oxazaphosphorine-2-oxide) involves the condensation of 1-amino-2,2-dideuteropropan-3-ol (6) with N,N-bis(2-chloroethyl)phosphoramidic dichloride (7) in the presence of triethylamine. Compound 6 was synthesised by the reduction of ethyl cyanodideuteroacetate (8, prepared by heating the non-deuterated material under reflux in D_2O) using lithium aluminium hydride (see Scheme 2 and ref. 1). The key step in the synthesis of 5 is the formation of 6 and the above procedure for this stage was found to be both unreliable and to give low yields; 5% yield of isolated 5 based on unexchanged ethyl cyanoacetate. Since 6 obtained from this procedure was not readily purified, the crude product was used for condensation with 7 yielding a complex mixture of products from which the desired 5 was isolated only after time-consuming column chromatography. Thus, in the reported preparation of 5 unexchanged 8 (2.0 g) yielded 5 (only 0.24 g) after chromatography and crystallisation.

Scheme 2



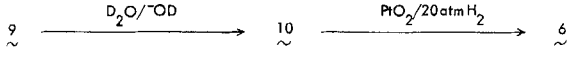
In designing a better synthesis of 5 it was thought, initially, that the stepwise reduction of 8 via 3-hydroxypropionitrile (9) would provide a more satisfactory route to 6 since the main problems with the original synthesis were the extreme hydrophilicity of 6 coupled with the undesirable inorganic residues both of which rendered the work-up difficult and inefficient. The two-stage reduction was achieved using excess sodium borohydride in methanol followed by hydrogenation (Adams' catalyst) as shown in Scheme 3. Unfortunately, reduction of the carboethoxy group of 8 with sodium borohydride did not yield the required product 6. Reduction in methanol-OD caused deuterium incorporation into the hydroxymethyl group of 9 yielding ~30% 5,6,6-trideuterocyclophosphamide after the final condensation whereas reduction in nondeuterated methanol, as expected, caused complete loss of deuterium by base-catalysed exchange with the methanol hydroxyl protons.

Scheme 3



It was discovered that 9 undergoes a base-catalysed deuterium exchange with D_2O (no exchange occurs at neutral pH) to yield the desired 2,2-dideutero-3--hydroxypropionitrile (10) which was reduced to 6 (~40% isolated yield) by medium pressure hydrogenation (Adams catalyst) (Scheme 4).

Scheme 4



The primary amine 6 was separated from the by-products [mainly di(3-hydroxy--2,2-dideuteropropyl)amine (2)] by vacuum distillation (see Experimental section) and was shown to be deuterated exclusively at C-2 by its ^1H NMR spectrum (Fig. 1a) when compared with that of 1-aminopropan-3-ol itself (Fig. 1b).

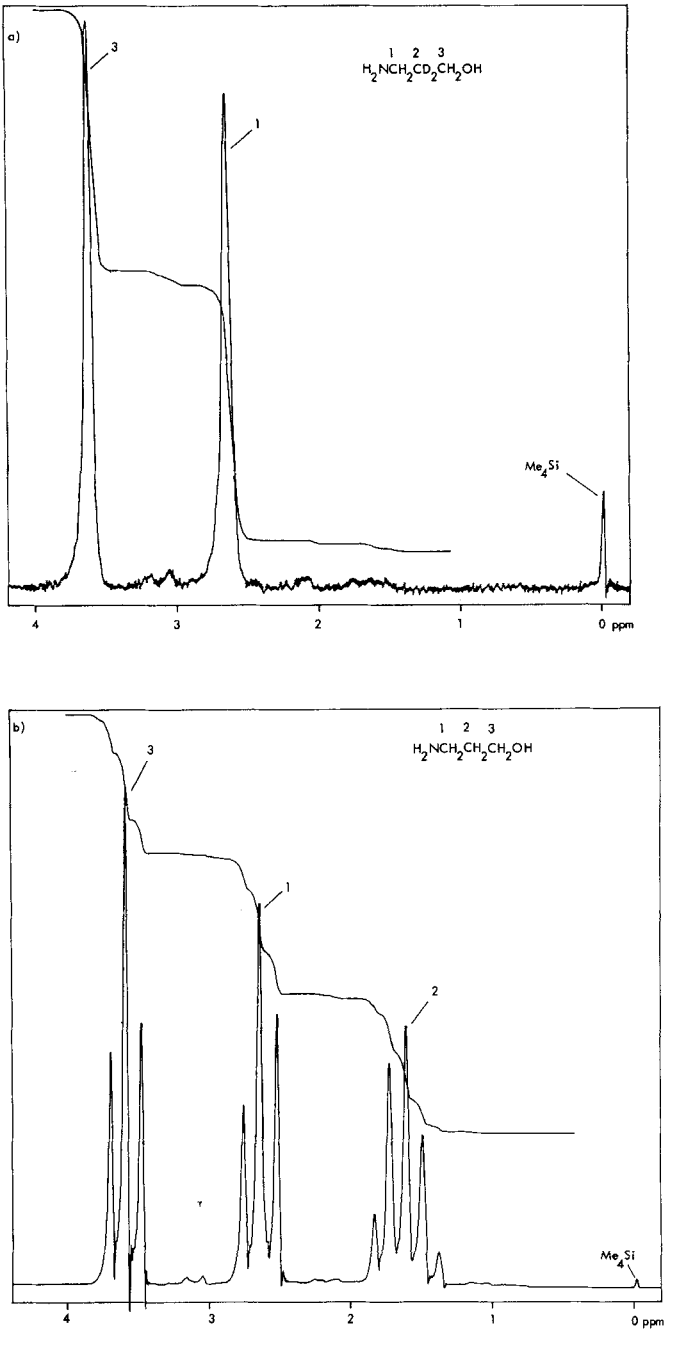


Fig. 1. 60 MHz ^{1}H NMR spectra (D_2O) of 1-amino-2,2-dideuteropropan-3-ol (a) and 1-aminopropan-3-ol (b)

The hydrogenation of 10 at atmospheric pressure yielded mainly the secondary amine even in the presence of excess ammonia, hydrogen chloride or acetic anhydride (3). The catalytic hydrogenation of 9 using Raney nickel has been described previously (2,4). However, in the presence of deuterated Raney nickel (5) all the protons in 1-aminopropan-3-ol are readily exchanged and thus deuterium loss from 6 produced during the hydrogenation would almost certainly have occurred.

Our new synthesis produced 6 reliably and in higher yield than before and it was readily isolated in contrast with the original procedure (1). The condensation of pure 6 with 7 yielded 5 as the only product mobile under the chromatographic conditions used (silica gel, chloroform-ethanol, 9:1); excess 6 and other by-products (mainly triethylamine hydrochloride) being retained during the elution of 5. The purity of 5 was shown by thin-layer chromatography and mass spectrometry (extent of isotopic substitution at C-5). The ^{1}H NMR spectrum of 5 (Fig. 2a) when compared with that of cyclophosphamide itself (Fig. 2b) demonstrates the unique dideuteration at C-5. The yield (20%) of 5 based on the starting material 9 is thus four times that obtained in the previously reported synthesis (1).

Finally, this report contains two observations of potentially general interest to investigators using nitriles as intermediates in the synthesis of deuterium labelled compounds, namely that 3-hydroxypropionitrile survived the base-catalysed H-D exchange of the C-2 protons, and that the exchanged product could be reduced catalytically without loss or scrambling of deuterium.

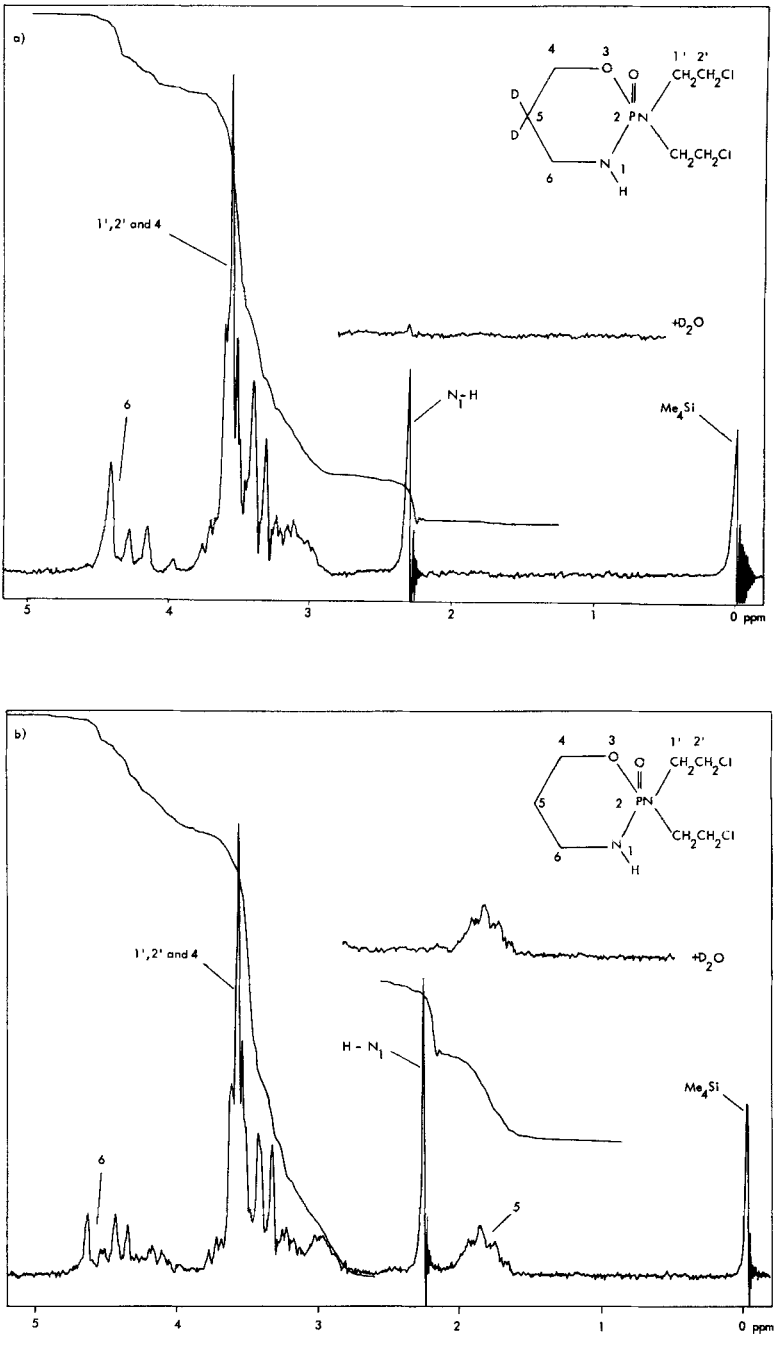


Fig. 2. 60 MHz ^1H NMR spectra ($CDCl_3$) of 5,5-dideuterocyclophosphamide (a) and cyclophosphamide (b).

EXPERIMENTAL

Melting points were determined with a Kofler hot-stage apparatus. Merck Kieselgel G was used for column chromatography and for TLC. NMR spectra (60 MH_z) were obtained with a Perkin-Elmer R-10 spectrometer. Mass spectra were determined with an AEI MS-12 spectrometer using the direct insertion method with an ionizing voltage of 70 eV, a trap current of 100 μAmp, and ion-source temperature of 120°C. IR spectra were obtained with a Perkin-Elmer 257 spectrophotometer.

2,2-Dideutero-3-hydroxypropionitrile (10)

3-Hydroxypropionitrile (9) was distilled (110°/15 mm Hg) before use. A solution of 5 (11 g, 154.9 mmol) in D_2O (110 ml) to which was added 30% NaOD in D_2O (150 μl) was heated under reflux for 25 min (the exchange was monitored by NMR spectroscopy), cooled and adjusted to pH 7 with DCl in D_2O. The D_2O was evaporated under reduced pressure and the residual oil (10 g) was distilled (62-63°/0.7 mm Hg) to yield 10 (8.2 g, 112.3 mmol, 72%): IR(film) 3430(s), 2960(m), 2540(s), 2260(s), 1380(m), 1070(s)cm^{-1} ; NMR, δ Me_4Si(10% v/v, $CDCl_3$), 10, 3.80 (s, 3-CH_2), 4.05 (s, OH), 5 , 2.60 (t, 2-CH_2), 3.80 (t, 3-CH_2), 4.50 (s, OH). The NMR spectrum of 10 showed a small resonance (4.7% of the total integration) at δ 2.6 which was analysed as a triple 1:1:1 triplet arising from the small amount (9%) of 2-deutero-3-hydroxypropionitrile (d_1 species) with $J_{2,3}$ = 7 Hz and $J_{2,D}$ = 2.5 Hz.

1-Amino-2,2-dideuteropropan-3-ol (6)

To a solution of 10 (5 g, 68.5 mmol) in distilled ethanol (150 ml), platinum oxide (1 g) was added. The solution was hydrogenated at 290 psi at room temperature overnight. The mixture was filtered, concentrated and the residual oil (5.1 g) was distilled (58-60°C/1.5 mm Hg) to yield 6 (1.95 g, 25.3 mmol, 37%). The by-products [mainly di(3-hydroxy-2,2-dideuteropropyl)amine] did not distil at elevated temperatures but polymerised in the distillation vessel.

IR (film) 3300(s), 2870(s), 2200(w), 2120(w), 1050(s); NMR, see Fig. 1a.

5,5-Dideuterocyclophosphamide (5)

N,N-Bis(2-chloroethyl)phosphoramidic dichloride (6) (7) was purified by distillation (110°/0.5 mm Hg) and two crystallisations from acetone-light petroleum (b.p. 40-60°) (1:1). To a stirred solution of 7 (4.51 g, 17.4 mmol) in anhydrous 1,2-dichloroethane (10.6 ml) at 0°C under anhydrous conditions was added dropwise at 0°C during ~ 15 min a solution of 6 (1.95 g, 25.26 mmol) in anhydrous 1,2-dichloroethane (10.6 ml) and anhydrous triethylamine (6.6 ml, 47.35 mmol). The mixture was then stirred for a further 96 h at room temperature. Ice-water (20 ml) was added, the organic layer was separated, and the aqueous phase was extracted with dichloromethane (4 x 20 ml). The combined organic phases were dried (Na_2SO_4), filtered and concentrated to yield an oil (4.93 g) which contained 5 as the only mobile component (TLC). Elution from a column (4 x 10 cm) of Kieselgel Merck, 7734) with chloroform-ethanol, 9:1 (250 ml) gave 5 as an oil (4.12 g, 15.7 mmol) which was dissolved in acetone (7 ml) and distilled water (22 ml) was added. The acetone was removed under reduced pressure whereupon 5 crystallised. Repetition of the procedure with the material in the mother liquor gave a second crop. The crystals were air dried (dessicant causes loss of water of crystallisation leaving an anhydrous oil) to yield 5 (3.3 g, 12.5 mmol, 72% based on 7) with m.p. 36-38° (cf. 38-41.5° for cyclophosphamide under the same heating conditions): IR (film) 3220(s). 2960(m), 2940(sh), 2880(m), 2230(vw), 2150(vw), 1450(m), 1220(s)cm^{-1}; NMR spectrum, see Fig. 2a.

Mass spectrum: m/e 213/212 $(M-CH_2Cl)^+$ for d_2 and d_1 species respectively was 100:10.3.

Anal. Calcd. for $C_7H_{15}Cl_2D_2N_2O_3P$ (monohydrate): C, 29.91; N, 9.97; Cl, 25.23; P, 11.02. Found: C, 30.07; N, 10.06; Cl, 24.99; P, 10.95.

ACKNOWLEDGMENTS

This investigation was supported by grants (G973/786 and G969/189C) to the Institute of Cancer Research from the Medical Research Council. The authors thank Professor A.B.Foster for his interest and Mr M.H.Baker for skilled technical assistance.

REFERENCES

1. Cox, P.J., Farmer, P.B., Foster, A.B., Gilby, E.D., and Jarman, M. Cancer Treat. Rep. 60: 483 (1976)
2. Sasa, T., J.Soc.Org. Synthet. Chem., Japan, 12: 24 (1954); C.A. 51 2780H.
3. 'Catalytic Hydrogenation over Platinum Metals' Rylander, P.N. Academic Press, New York and London, pp. 205-209 (1967).
4. Volkava, L.D., and Sokol'skii, D.V. Izv. Akad. Nauk Kaz. SSR, Ser. Khim. 17: 60(1967), C.A. 67, 53293n.
5. Unpublished observations by Jarman, M. and Taylor, G.N., also see Koch, H.J. and Stuart, R.S., Carbohydr. Res., 59:C1-C6 (1977).
6. Friedman, P.M., and Seligman, A.M. J. Amer. Chem.Soc., 76:655 (1954).