Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 115–122.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1182

JLCR

Research Article

Labeled oxazaphosphorines for applications in MS studies. Synthesis of deuterium labeled cyclophosphamides and ifosfamides

JAMES B. SPRINGER, O. MICHAEL COLVIN and SUSAN M. LUDEMAN*

Duke Comprehensive Cancer Center and Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA

Received 17 July 2006; Revised 1 August 2006; Accepted 20 November 2006

Abstract: A variety of deuterated 3-amino-1-propanols were made by the $LiAlD_4$ or AlD_3 reduction of nitrile or ester precursors. The labeled aminopropanols and/or deuterated bis(2-chloroethyl)amines were used to synthesize [4,4-$^2H_2$]-, [6,6-$^2H_2$]-, [α,α.α′,α′-$^2H_4$]-, and [4,4,β,β,β′,β′-$^2H_6$]-cyclophosphamide. The labeled aminopropanols were also used to synthesize deuterated 3-(2′-benzyloxyethylamino)-1-propanols [$BnOCH_2CH_2NH(CH_2)_3OH$] which, along with labeled and unlabeled 2-chloroethylamines, led to [4,4-$^2H_2$]-, [6,6-$^2H_2$]-, [α,α-$^2H_2$]-, [α′,α′-$^2H_2$]-, [α,α.α′,α′-$^2H_4$]-, and [6,6,α,α,α′,α′-$^2H_6$]-ifosfamide. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: cyclophosphamide; ifosfamide; deuterium labeling; synthesis

Introduction

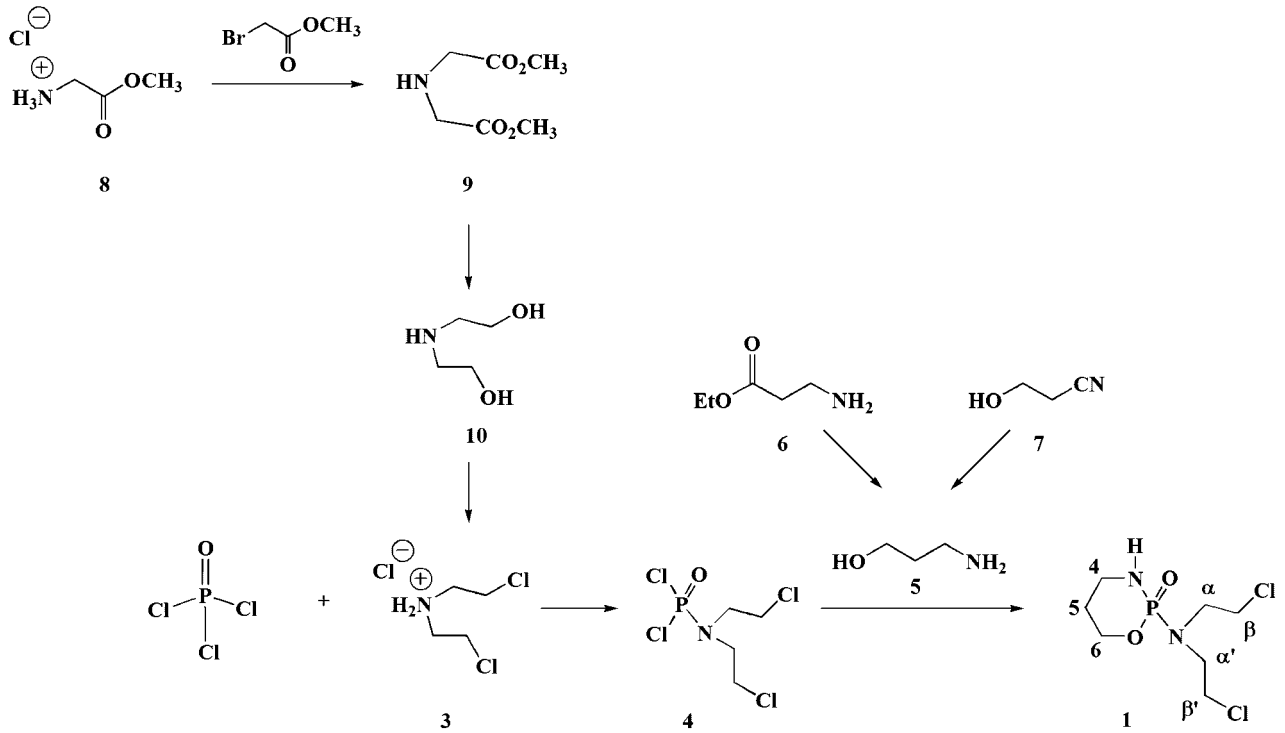
The anticancer agents cyclophosphamide (**1**) and ifosfamide (**2**) metabolize to active alkylating agents through cytochrome P450-mediated oxidation at the C-4 position.[1–4] Competing with this reaction in **1** and **2** are oxidations at the α and α′ positions, each of which leads to a dechloroethylation and the formation of the neurotoxic chloroacetaldehyde. Dechloroethylation is a minor (~10%) pathway in the metabolism of **1**[3,5] but can constitute up to 50% of the metabolism of **2**.[3,4] For MS studies of the enzymatic, kinetic, mechanistic, and stereochemical factors that influence the oxidation of **1** and **2** at one site relative to another, the following were synthesized: [4,4-$^2H_2$]-(**1**) and –(**2**); [6,6-$^2H_2$]-(**1**) and –(**2**); [α,α,α′,α′-$^2H_4$]-(**1**) and –(**2**); [4,4,β,β,β′,β′-$^2H_6$]-(**1**); [α,α-$^2H_2$]-(**2**); [α′,α′-$^2H_2$]-(**2**); and [6,6,α,α,α′,α′-$^2H_6$]-(**2**).

Results and discussion

As shown in Scheme 1, the synthesis of **1** is generally accomplished in two steps. Bis(2-chloroethyl)amine hydrochloride (**3**) is reacted with phosphorus oxychloride to give bis(2-chloroethylamino)phosphoramidic dichloride (**4**).[6] Reaction of **4** with 3-amino-1-propanol (**5**) leads to **1**.[7] Thus, strategies for incorporating isotopes into the exocyclic and/or endocyclic portions of **1** include the synthesis of labeled precursors, **3/4** and/or **5**.[8–15]

Portions of many of the published syntheses for **3/4/5** were adapted and modified for the work reported herein. Some improvements in yield and purity resulted, with the following being the most significant. Published syntheses of deuterated **5** have commonly utilized $LiAlD_4$ to reduce an ester (e.g. **6**) or a nitrile (e.g. **7**) precursor.[11–15] While such reductions of esters have generally afforded products in high yield and good purity (e.g. **6** → **5**, ~90%), $LiAlH(D)_4$ reactions with nitriles have given products that were low in yield and difficult to purify. Relative to $LiAlH_4$, AlH_3 is a better Lewis acid and reacts quickly with nitriles under mild conditions[16]; thus, AlD_3 was used to reduce nitriles in this work. Products were obtained in nearly quantitative yield and were of much higher purity than those generated from $LiAlD_4$. The higher quality of these difficult-to-purify amines led to improved yields when they were incorporated into subsequent reactions. For example, in the synthesis of [4,4-$^2H_2$]-(**1**), use of deuterated **4** generated by $LiAlD_4$ gave a net yield of ~20%[15] while use of **4** given by AlD_3 provided a net yield of ~70%.

*Correspondence to: S. M. Ludeman, DUMC Box 2638, Duke University Medical Center, Durham, NC 27710, USA.
E-mail: s.ludeman@duke.edu
Contract/grant sponsor: PHS; contract/grant number: CA16783



Scheme 1 Pathway used to incorporate deuterium labels into cyclophosphamide (**1**).

As shown in Scheme 2, compound **2** can be synthesized from three major starting materials: 3-amino-1-propanol (**5**), benzyloxyacetyl chloride and 2-chloroethylamine hydrochloride (**12**). Incorporating deuterium into these three compounds provides for **2** with labels in the oxazaphosphorine ring, endocyclic chloroethyl chain and/or the exocyclic chloroethyl chain, respectively. The synthesis of target compound [α,α.α′,α′-$^2H_4$]-(**2**) as purified enantiomers (6% yield) has been reported using a different pathway[17]; Scheme 2 provides for racemic material in fewer steps and higher yield (average 25%).

Experimental

Reactions were carried out under N_2. Generally, chemicals were purchased from Aldrich, Fisher, or VWR. THF was distilled from potassium/benzophenone ketyl; CH_2Cl_2, CH_3CN and Et_3N were distilled from CaH_2. Flash chromatography used Merck 230–400 mesh silica gel (~50 ml silica gel per gram crude material with a column height of 6 in). Analytical TLC plates were from Merck (250-microns). NMR spectra were recorded on a Varian Inova-400 or a GE QE-300 spectrometer. Chemical shifts are reported relative to TMS (0 ppm, ^1H), $CDCl_3$ (77 ppm, ^{13}C), or 25% H_3PO_4 (0 ppm, ^{31}P, external reference). NMR solvent $CDCl_3$ was washed with $NaHCO_3/D_2O$ prior to use. Deuterium incorporation was measured with CI-MS using the average *m/z* values of eight injections. Error analyses were calculated at the 95% confidence level.

Bis((1,1-$^2H_2$)-2-chloroethyl)amine hydrochloride {(1,1,1′,1′-$^2H_4$)-(3)}

Freshly distilled $SOCl_2$ (12 ml, 165 mmol) was added dropwise to a solution of [1,1,1′,1′-$^2H_4$]-(**10**) (0.89 g, 8.1 mmol) in CH_3CN (30 ml). The mixture was stirred at RT (36 h); product was isolated according to literature procedures.[8,18] The product was obtained as a flocculent, light brown solid (0.87 g, 59%). ^1H NMR (DMSO-d_6) δ 3.92 (s, 4H, two CH_2Cl).

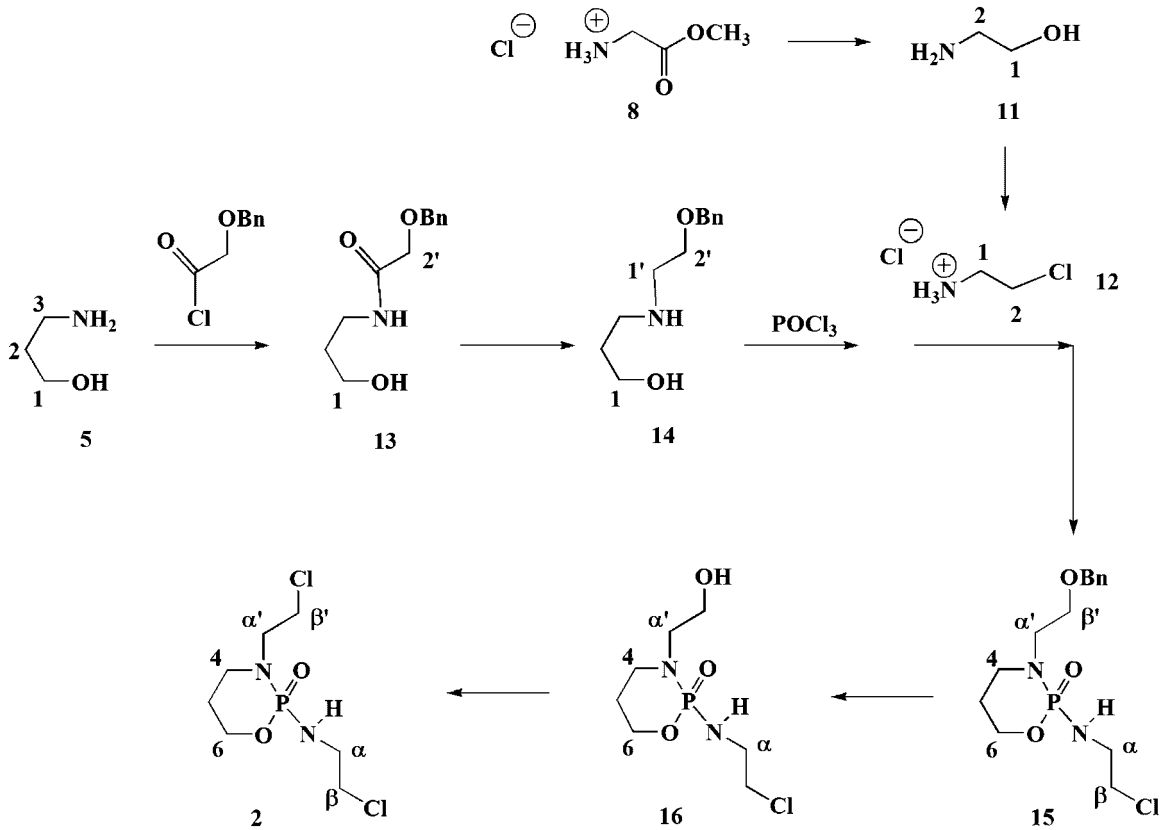
N,N-bis((1,1-$^2H_2$)-2-chloroethyl)phosphoramidic dichloride {(1,1,1′,1′-$^2H_4$)-(4)}

Prepared (60% yield) using reported procedures[8,10] but with [1,1,1′,1′-$^2H_4$]-(**3**) as a starting material. ^1H NMR ($CDCl_3$) δ 3.72 (s, 4H, two CH_2Cl). ^{13}C NMR ($CDCl_3$) δ 40.61 (d, $^3J_{CP} = 2.7$ Hz, CH_2Cl). ^{31}P NMR ($CDCl_3$) δ 18.0.

N,N-bis((2,2-$^2H_2$)-2-chloroethyl)phosphoramidic dichloride {(2,2,2′,2′-$^2H_4$)-(4)}

Prepared (58% yield) using reported procedures.[8,10] ^1H NMR ($CDCl_3$) δ 3.66 (d, $^3J_{HP} = 16.4$ Hz, 4H, two

DOI: 10.1002.jlcr



Scheme 2 Pathway used to incorporate deuterium labels into ifosfamide (**2**).

NCH_2). ^{13}C NMR ($CDCl_3$) δ 49.28 (d, $^2J_{CP}$ = 10.5 Hz, NCH_2). ^{31}P NMR ($CDCl_3$) δ 18.0.

(1,1-$^2H_2$)-3-amino-1-propanol {(1,1-$^2H_2$)-(5)}

Compound **6** (9.98 g, 65 mmol) was added slowly to a cooled (ice bath) suspension of $LiAlD_4$ (3.57 g, 81 mmol, 98 at % D, Aldrich) in THF (152 ml). The mixture was stirred at RT (2 h) and the reaction was then quenched with the sequential, dropwise addition of 50% water in THF (20 ml) and 40% NaOH (6.5 ml). The mixture was filtered and the clear filtrate was concentrated at reduced pressure. The filter cake was subjected to an overnight Soxhlet extraction with THF and this extract was combined with the residue from the initial filtrate and co-evaporated with CH_3CN. The residue was dissolved in CH_2Cl_2, dried (Na_2SO_4), filtered, and concentrated to give the product (4.51 g, 90%). ^1H NMR ($CDCl_3$) δ 2.98 (t, J = 6.0 Hz, 2H, CH_2N), 2.39 (bs, 3H, OH and NH_2), and 1.67 (t, J = 6.0 Hz, 2H, CH_2CH_2N).

(3,3-$^2H_2$)-3-amino-1-propanol {(3,3-$^2H_2$)-(5)}

AlD_3 (17 mmol) was freshly generated in dry THF (29 ml) from $LiAlD_4$ (752 mg, 17 mmol, 98 at % D, Aldrich) and double distilled H_2SO_4 (453 µl, 8.5 mmol, Aldrich) according to a literature procedure.[19] The mixture was then cooled (ice bath) and a solution of 3-hydroxypropionitrile (**7**; 0.66 ml, 9.7 mmol) in THF (3 ml) was added dropwise. The mixture was stirred at RT (2 h) and then worked up as described above for [1,1-$^2H_2$]-(**5**). Product was obtained as an oil (686 mg, 92%). ^1H NMR ($CDCl_3$) δ 3.78 (t, 2H, J = 5.6 Hz, CH_2O), 2.56 (bs, 3H, OH and NH_2), and 1.67 (t, 2H, J = 5.6 Hz, CH_2CH_2O).

β-Alanine ethyl ester hydrochloride (6)

β-Alanine (8.9 g, 0.1 mol) was esterified with abs. ethanol (228 ml) and HCl (gas) as reported for glycine.[18] The product crystallized from EtOAc (15.1 g, 98%). ^1H NMR ($CDCl_3$) δ 8.17 (bs, 3H, H_3N^+), 4.17 (q, J = 7.3 Hz, 2H, CH_2CH_3) 3.39 (m, 2H, CH_2N), 2.95 (t, J = 6.9 Hz, 2H, CH_2CO_2), and 1.27 (t, J = 7.3 Hz, 3H, CH_2CH_3).

(2,2-$^2H_2$)-glycine methyl ester hydrochloride {(2,2-$^2H_2$)-(8)}

Glycine-d_5 (5.14 g, 64 mmol, 98 at % D, Cambridge Isotopes) was esterified as described in the literature

DOI: 10.1002.jlcr

but using CH_3OD (132 ml, 99.5 at % D, Aldrich).[18] The product crystallized in quantitative yield from EtOAc. ^1H NMR (DMSO-d_6) δ 8.46 (bs, 3H, H_3N^+) and 3.72 (s, 3H, CH_3).

Dimethyl (2,2,2′,2′-$^2H_4$)-iminodiacetate {(2,2,2′,2′-$^2H_4$)-(9)}

Based on a similar synthesis,[10] Et_3N (1.8 ml, 12.9 mmol), methyl [2,2-$^2H_4$]-2-bromoacetate (1.31 ml, 13.7 mmol, 98 at % D, Cambridge Isotopes), and Et_3N (1.80 ml, 12.9 mmol) were added sequentially to [2,2-$^2H_2$]-(**8**) (1.31 g, 10.4 mmol) in THF (11 ml). Purification employed flash chromatography using EtOAc–hexanes (1:1) to elute impurities and then EtOAc to elute the product [1.24 g, 73%, R_f 0.16 in EtOAc–hexanes (1:1)].

Bis((1,1-$^2H_2$)-2-hydroxyethyl)amine {(1,1,1′,1′-$^2H_4$)-(10)}

With minor modifications to a literature procedure for the synthesis of ^{15}N-labeled material,[10] a solution of [2,2,2′,2′-$^2H_4$]-(**9**) (0.88 g, 5.3 mmol) in THF (2 ml) was added dropwise to a cooled (ice bath) suspension of $LiAlH_4$ (0.54 mg, 14 mmol) in THF (10 ml). The mixture was stirred at RT (2.25 h) and then worked up as described above for [1,1-$^2H_2$]-(**5**) to give the product (88%). ^1H NMR ($CDCl_3$) δ 3.85 (bs, 3H, NH and two OH) and 3.69 (s, 4H, two CH_2O).

(4,4-$^2H_2$)-cyclophosphamide monohydrate {(4,4-$^2H_2$)-(1) monohydrate}

A solution of [3,3-$^2H_2$]-(**5**) (1.0 g, 13.0 mmol) and Et_3N (5.4 ml, 38.7 mmol) in THF (11 ml) was added to a cooled (ice bath) solution of unlabeled **4** (3.7 g, 14.4 mmol, can be purchased from Aldrich) in THF (13 ml). The mixture was stirred at RT (2 days). Water (50 ml) was added and the mixture was extracted with EtOAc (4 × 50 ml). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residual oil was flash chromatographed using CH_3OH–CH_2Cl_2 (2:98) as eluent. Impure material was obtained and then rechromatographed using CH_3OH–CH_2Cl_2 (1:9). [4,4-$^2H_2$]-(**1**) was obtained as a colorless oil [2.71 g, 79%, R_f 0.33 in CH_3OH–CH_2Cl_2 (1:9)]. ^1H NMR ($CDCl_3$) δ 4.50–4.39 (m, 1H, C_6-H), 4.32–4.20 (m, 1H, C_6-H), 3.64 (t, J = 6.9 Hz, 4H, two CH_2Cl), 3.51–3.35 (m, 4H, two C***H***$_2$$CH_2Cl$), 3.02 (bs, 1H, NH), and 1.98–1.75 (m, 2H, C***H***$_2$$CH_2O$). *Not observed:* ($C_4$)-H [$\delta_H$ 3.50–3.20 (m)]. ^{13}C NMR ($CDCl_3$) δ 67.59 (d, $^2J_{CP}$ = 6.5 Hz, C_6), 48.64 (d, $^2J_{CP}$ = 4.6 Hz, ***C***H_2CH_2Cl), 42.19 (CH_2Cl), and 25.47 (d, $^3J_{CP}$ = 6.1 Hz, C_5). *Not observed:* C_4 (δ_C 41.37). ^{31}P NMR ($CDCl_3$) δ 12.5.

The monohydrate was made with minor modifications to a literature preparation.[12] A solution of H_2O (0.2 ml, 11.3 mmol), [4,4-$^2H_2$]-(**1**) (2.71 g, 10.3 mmol) and Et_2O (31 ml) was stored overnight at −20°C. A white precipitate was observed. Petroleum ether (1–2 ml, low boiling) was added until the supernatant was cloudy and the flask again stood at −20°C until precipitation ended, as evidenced by a clear supernatant. This procedure of adding petroleum ether until the solution was cloudy, followed by allowing the mixture to stand at −20°C until clear, was repeated several times until no amount of added petroleum ether could effect cloudiness. The supernatant was decanted and the precipitate was dried under vacuum to afford [4,4-$^2H_2$]-(**1**) monohydrate as a white solid (2.66 g, 92%). MS analysis: 98.7 ± 1.8% **1**-d_2 and 1.3 ± 0.7% **1**-d. ^1H NMR ($CDCl_3$) δ 4.48–4.40 (m, 1H, C_6-H), 4.32–4.22 (m, 1H, C_6-H), 3.63 (t, J = 6.8 Hz, 4H, two CH_2Cl), 3.53–3.35 (m, 4H, two C***H***$_2$$CH_2Cl$), 2.82 (bs, 1H, NH), 2.16 (bs, 2H, H_2O), and 1.93–1.78 (m, 2H, C***H***$_2$$CH_2O$). ^{13}C NMR ($CDCl_3$) δ 67.65 (d, $^2J_{CP}$ = 6.5 Hz, C_6), 48.65 (d, $^2J_{CP}$ = 4.2 Hz, ***C***H_2CH_2Cl), 42.24 (CH_2Cl), and 25.48 (d, $^3J_{CP}$ = 6.1 Hz, C_5). ^{31}P NMR ($CDCl_3$) δ 12.6.

(6,6-$^2H_2$)-cyclophosphamide {(6,6-$^2H_2$)-(1)}

As for [4,4-$^2H_2$]-(**1**) but with [1,1-$^2H_2$]-(**5**) as precursor. Flash chromatography used CH_3OH–CH_2Cl_2 (2:98). Yield: 91% (colorless oil). Attempts to form a crystalline hydrate were unsuccessful. MS analysis: 98.7 ± 1.9% **1**-d_2 and 1.3 ± 0.8% **1**-d.

(α,α,α′,α′-$^2H_4$)-cyclophosphamide {(α,α,α′,α′-$^2H_4$)-(1)}

As for [4,4-$^2H_2$]-(**1**) but with [1,1,1′,1′-$^2H_4$]-(**4**) and unlabeled **5** as precursorss. Flash chromatography used CH_3OH–CH_2Cl_2 (2:98). Yield: 83% (colorless oil). Attempts to form a crystalline hydrate were unsuccessful. MS analysis: 92.8 ± 1.8% **1**-d_4, 6.7 ± 0.7% **1**-d_3 and 0.5 ± 0.5% **1**-d_2.

(4,4,β,β,β′,β′-$^2H_6$)-cyclophosphamide {(4,4,β,β,β′,β′-$^2H_6$)-(1)}

As for [4,4-$^2H_2$]-(**1**) but with [2,2,2′,2′-$^2H_4$]-(**4**) and [3,3-$^2H_2$]-(**5**) as precursors. Flash chromatography used an eluent of CH_3OH–CH_2Cl_2 with increasing amounts of CH_3OH (from 2 to 10%). Yield: 54% (colorless oil). Attempts to form a crystalline hydrate were not successful. MS analysis: 97.1 ± 1.9% **1**-d_6, 2.1 ± 0.7% **1**-d_5 and 0.8 ± 0.6% **1**-d_4.

DOI: 10.1002.jlcr

(2,2-$^2H_2$)-2-aminoethanol {(2,2-$^2H_2$)-(11)}

[2,2-$^2H_2$]-(**8**) (9.16 g, 72 mmol) was reduced with $LiAlH_4$ (3.63 g, 91 mmol) by analogy to a literature procedure for the synthesis of [1,1-$^2H_2$]-2-aminoethanol (misnamed in the paper as 2-amino-**2**,**2**-dideuterioethanol HCl).[20] The product was obtained in 58% yield (2.62 g). ^1H NMR ($CDCl_3$) δ 3.6 (s, 2H, CH_2O) and 2.3 (bs, 3H, OH and NH_2).

(1,1-$^2H_2$)-2-chloroethylamine hydrochloride {(1,1-$^2H_2$)-(12)}

The title compound was synthesized from [2,2-$^2H_2$]-(**11**) and $SOCl_2$ based on literature methods for other labeled chloroethylamines.[18–20] The product was obtained as a flocculent, off-white solid (1.55 g, 32%). ^1H NMR (DMSO-d_6) δ 8.43 (bs, 2H, NH_2) and 3.84 (s, 2H, CH_2Cl).

3-(2′-Benzyloxyacetylamido)-1-propanol (13)

Benzyloxyacetyl chloride (6.0 ml, 38 mmol) was added dropwise to a solution of 3-amino-1-propanol (unlabeled **5**, 3.05 ml, 40 mmol) and Et_3N (5.80 ml, 42 mmol) in THF (400 ml) at −20°C (ice/MeOH bath). The reaction mixture was stirred for 20 h at RT and then concentrated. The residue was diluted with water (100 ml) and extracted with EtOAc (4 × 75 ml). The combined organic layers were dried ($MgSO_4$), filtered, concentrated, and flash chromatographed using EtOAc-hexanes (3:1) to elute impurities and then EtOAc to elute product as a colorless oil which solidified on standing [4.88 g, 58%, R_f 0.27 (EtOAc)]. Material tentatively identified (^1H NMR) as the *N*,*O*-bisacetylation product was also obtained [726 mg, 10%, R_f 0.68 (EtOAc)]. ^1H NMR ($CDCl_3$) δ 7.39–7.31 (m, 5H, aromatic), 6.97 (bs, 1H, OH), 4.56 (s, 2H, CH_2Ph), 3.99 (s, 2H, $CH_2C{=}O$), 3.60 (apparent q, $J = 6$ Hz, 2H, CH_2OH), 3.43 (m, 3H, $NHCH_2$), and 1.68 (quint, $J = 6$ Hz, 2H, $CH_2CH_2CH_2$). ^{13}C NMR ($CDCl_3$) δ 170.6 (C=O), 136.7, 128.5, 128.2, and 127.9 (aromatic), 73.49 ($CH_2C{=}O$), 69.22 (CH_2Ph), 59.09 (CH_2OH), 35.52 (NCH_2), and 32.06 ($CH_2CH_2CH_2$).

(1,1-$^2H_2$)-3-(2′-benzyloxyacetylamido)-1-propanol {(1,1-$^2H_2$)-(13)}

As for **13** with [1,1-$^2H_2$]-3-amino-1-propanol[6] {[1,1-$^2H_2$]-(**5**)} as precursor. Yield: 60%.

(3,3-$^2H_2$)-3-(2′-benzyloxyacetylamido)-1-propanol {(3,3-$^2H_2$)-(13)}

As for **13** but with [3,3-$^2H_2$]-3-amino-1-propanol[6] {[3,3-$^2H_2$]-(**5**)} as precursor. Yield: 58%.

3-(2′-Benzyloxyethylamino)-1-propanol (14)

AlH_3 (7.8 mmol) was generated in THF (15 ml) from $LiAlH_4$ (0.310 g, 7.8 mmol) and conc H_2SO_4 (0.21 mL, 3.8 mmol) according to the literature.[19] The mixture was cooled (water bath) and a solution of **13** (1.06 g, 4.7 mmol) in THF (1.8 ml) was added dropwise. Additional THF (2 × 2 ml) was used to rinse down the flask and syringe. The mixture was stirred at RT (2.5 h) and the reaction was then quenched at ice bath temperature by the dropwise addition of THF-H_2O (1:1, 3 ml) and then 40% NaOH (1 ml). The mixture was diluted with water (100 ml) and extracted with ether (6 × 50 ml). The combined ether layers were extracted with 1 M HCl (2 × 50 ml) and the combined acidic layers, which contained desired product, were set aside. The ether layer from this step was washed with saturated $NaHCO_3$ (50 ml), dried ($MgSO_4$), filtered, and concentrated to afford recovered starting material (**13**) (0.19 g, 18%). The pH of the set-aside, combined acidic layers was adjusted to ⩾14 (pH paper) with 40% NaOH and the resultant basic solution was extracted with ether (6 × 50 ml). The combined ether layers were dried (Na_2SO_4), filtered, concentrated and flash chromatographed using CH_3OH–CH_2Cl_2 (1:9) to elute impurities and then NH_3-saturated CH_3OH in CH_2Cl_2 (1:9) to elute product (R_f 0.50) which was isolated as an oil (0.53 g, 53%). ^1H NMR ($CDCl_3$) δ 7.37–7.28 (m, 5H, aromatic), 4.51 (s, 2H, CH_2Ph), 3.79 (t, $J = 6$ Hz, 2H, CH_2OH), 3.59 (t, $J = 5$ Hz, 2H, OCH_2CH_2N), 3.53 (bs, 2H, NH and OH), 2.87 (t, $J = 6$ Hz, 2H, $NCH_2CH_2CH_2O$), 2.82 (t, $J = 5$ Hz, 2H, OCH_2CH_2N), and 1.70 (quint, $J = 6$ Hz, 2H, $CH_2CH_2CH_2$). ^{13}C NMR ($CDCl_3$) δ 138.0 (1C), 128.4 (2C), and 127.7 (3C) (aromatic), 73.20 (CH_2Ph), 69.06 (OCH_2CH_2N), 64.04 (CH_2OH), 49.54 (OCH_2CH_2N), 49.05 ($NCH_2CH_2CH_2O$), and 30.43 ($CH_2CH_2CH_2$).

(1,1-$^2H_2$)-3-(2′-benzyloxyethylamino)-1-propanol {(1,1-$^2H_2$)-(14)}

As for **14** but with [1,1-$^2H_2$]-(**13**) (1.0 g, 4.5 mmol) as precursor and stirring overnight at RT. The reaction was quenched as described for **14** and the concentrated residue was diluted with water (100 ml) and extracted with CH_2Cl_2 (6 × 50 ml). The combined organic layers were dried (Na_2SO_4), filtered, and

DOI: 10.1002.jlcr

concentrated. Kugelrohr distillation (110°C/0.05 mm) gave impure material which was then chromatographed as for **14**. Yield: 60%.

3-((1′,1′-$^2H_2$)-2′-benzyloxyethylamino)-1-propanol {(1′,1′-$^2H_2$)-(14)}

As for **14** but using AlD_3 generated from $LiAlD_4$ (0.5 g, 11 mmol, 98 at % D, Aldrich). The reaction mixture was stirred at RT overnight before quenching with THF-H_2O (1:1, 3.5 ml) and then 40% NaOH (1.2 ml). The mixture was vacuum filtered and the filter cake was washed consecutively with THF (3×30 ml) and CH_2Cl_2 (3×30 ml). The filtrate was dried (Na_2SO_4), filtered, concentrated, and flash chromatographed as for **14**. Yield: 81%.

(3,3-$^2H_2$)-3-(2′-benzyloxyethylamino)-1-propanol {(3,3-$^2H_2$)-(14)}

As for **14** but using [3,3-$^2H_2$]-(**13**) as starting material. Yield: 69%.

(1,1-$^2H_2$)-3-(2′-benzyloxy-(1′,1′-$^2H_2$)-ethylamino)-1-propanol {(1,1,1′,1′-$^2H_4$)-(14)}

As for [1′,1′-$^2H_2$]-(**14**) but with [1,1-$^2H_2$]-(**13**) as precursor. Yield: 55%.

(4,4-$^2H_2$)-2-(β-chloroethylamino)-3-(β′-benzyloxyethyl)-2H-1,3,2-oxaza-phosphorinane-2-oxide {(4,4-$^2H_2$)-(15)}

A solution of [3,3-$^2H_2$]-(**14**) (0.68 g, 3.2 mmol) in CH_2Cl_2 (3 ml) was added dropwise to a cooled (ice bath) solution of freshly distilled $POCl_3$ (0.3 ml, 3.3 mmol) in CH_2Cl_2 (10 ml). Additional CH_2Cl_2 (2×3 ml) was used to rinse down the flask and syringe. Et_3N (0.91 ml, 6.5 mmol) was added and the mixture was stirred for 5 h (ice bath). 2-Chloroethylamine hydrochloride (**12**, 0.43 g, 3.7 mmol) was added as a solid, followed by Et_3N (0.91 ml, 6.5 mmol). After being stirred at RT overnight, the reaction mixture was washed with water (2×50 ml). The combined aqueous layers were back-extracted with CH_2Cl_2 (2×15 ml) and then the organic reaction solution and extracts were combined, dried (Na_2SO_4), filtered, concentrated and flash chromatographed using CH_3OH–CH_2Cl_2 (2.5: 97.5). The product was obtained as an oil [0.82 g, 76%, R_f 0.47 in CH_3OH–CH_2Cl_2 (1:9)]. ^1H NMR ($CDCl_3$) δ 7.38–7.29 (m, 5H, aromatic), 4.56 (d, $J = 12$ Hz, 1H, one benzylic), 4.51 (d, $J = 12$ Hz, 1H, one benzylic), 4.39–4.25 (m, 1H, one C_6H), 4.24–4.15 (m, 1H, one C_6H), 3.71–3.38 (m, 6H, NCH_2CH_2O and CH_2Cl), 3.18–2.92 (m, 3H, NCH_2CH_2Cl and NH), 2.00–1.80 (m, 2H, C_5H). *Not observed:* (C_4)-H [δ_H 3.30–3.11 (m)]. ^{13}C NMR ($CDCl_3$) δ 137.8, 128.5, and 127.8 (aromatic), 73.16 (CH_2Ph), 68.00 (d, $^3J_{CP} = 3$ Hz, NCH_2CH_2O), 67.17 (d, $^2J_{CP} = 7$ Hz, C_6), 47.77 (NCH_2CH_2O), 45.63 (d, $^3J_{CP} = 5.5$ Hz, NCH_2CH_2Cl), 42.85 (NCH_2CH_2Cl), and 26.26 (d, $^3J_{CP} = 4$ Hz, C_5). *Not observed:*C_4 (δ_C 47.01). ^{31}P NMR ($CDCl_3$) δ 12.4.

(6,6-$^2H_2$)-2-(β-chloroethylamino)-3-(β′-benzyloxyethyl)-2H-1,3,2-oxaza-phosphorinane-2-oxide {(6,6-$^2H_2$)-(15)}

As for [4,4-$^2H_2$]-(**15**) but using [1,1-$^2H_2$]-(**14**) as starting material. Yield: 83%.

2-(β-Chloroethylamino)-3-(β′-benzyloxy-(α′,α′-$^2H_2$)-ethyl)-2H-1,3,2-oxaza-phosphorinane-2-oxide {(α′,α′-$^2H_2$)-(15)}.

As for [4,4-$^2H_2$]-(**15**) but using [1′,1′-$^2H_2$]-(**14**) as starting material. Yield: 84%.

2-(β-Chloro–(α,α-$^2H_2$)-ethylamino)-3-(β′-benzyloxyethyl)-2H-1,3,2-oxaza-phosphorinane-2-oxide {(α,α-$^2H_2$)-(15))

As for [4,4-$^2H_2$]-(**15**) but using [1,1-$^2H_2$]-(**12**) and unlabeled **14** as starting materials. Yield: 87%.

2-(β-Chloro-(α,α-$^2H_2$)-ethylamino)-3-(β′-benzyloxy-(α′α′-$^2H_2$)-ethyl)-2H-1,3,2-oxazaphosphorinane-2-oxide {(α,α,α′,α′-$^2H_4$)-(15))

As for [4,4-$^2H_2$]-(**15**) but using [1,1-$^2H_2$]-(**12**) and [1′,1′-$^2H_2$]-(**14**) as precursors. Yield: 85%.

(6,6-$^2H_2$)-2-(β-chloro-(α,α-$^2H_2$)-ethylamino)-3-(β′-benzyloxy-(α′α′-$^2H_2$)-ethyl)-2H-1,3,2-oxazaphosphorinane-2-oxide {(6,6,α,α,α′,α′-$^2H_6$)-(15))

As for [4,4-$^2H_2$]-(**15**) but using [1,1-$^2H_2$]-(**12**) and [1,1,1′,1′-$^2H_4$]-(**14**) as starting materials. Yield: 82%.

(4,4-$^2H_2$)-2-(β-chloroethylamino)-3-(β′-hydroxyethyl)-2H-1,3,2-oxazaphos-phorinane-2-oxide {(4,4-$^2H_2$)-(16)}

A solution of [4,4-$^2H_2$]-(**15**) (0.79 g, 2.4 mmol) in absolute EtOH (18 ml) with added 10% Pd/C (0.18 g) was hydrogenated at 60 psi for 1 h. The mixture was vacuum filtered through a pad (2″) of Celite and the filtrate was concentrated to afford the product with high purity [0.57 g, 98%, R_f 0.22 in CH_3OH–CH_2Cl_2

DOI: 10.1002.jlcr

(1:9)]. ^{1}H NMR ($CDCl_3$) δ 4.55 (bs, 1H, OH), 4.41–4.33 (m, 1H, one C_6H), 4.30–4.21 (m, 1H, one C_6H), 3.90–3.60 (m, 5H, CH_2Cl, C***H***$_2$OH and NH), 3.35–3.05 (m, 4H, two NCH_2), and 1.90–2.01 (m, 2H, C_5H). *Not observed*: (C_4)-H [δ_H 3.39–3.04 (m)]. ^{13}C NMR ($CDCl_3$) δ 67.24 (d, $^2J_{CP} = 7$ Hz, C_6), 58.95 (CH_2OH), 51.57 (N***C***H_2CH_2O), 45.17 (d, $^3J_{CP} = 5$ Hz, CH_2Cl), 43.03 (***C***H_2CH_2Cl), and 26.04 (d, $^3J_{CP} = 4$ Hz, C_5). *Not observed*: C_4 (δ_C 46.82). ^{31}P NMR ($CDCl_3$) δ 14.9.

(6,6-$^2H_2$)-2-(β-chloroethylamino)-3-(β′-hydroxyethyl)-2H-1,3,2-oxazaphos-phorinane-2-oxide {(6,6-$^2H_2$)-(16)}

As for [4,4-$^2H_2$]-(**16**) but with [6,6-$^2H_2$]-(**15**) as starting material. Yield: 100%.

2-(β-Chloroethylamino)-3-(β′-hydroxy-(α′, α′-$^2H_2$)-ethyl)-2H-1,3,2-oxazaphosphorinane-2-oxide {(α′, α′-$^2H_2$)-(16)

As for [4,4-$^2H_2$]-(**16**) but with [α′,α′-$^2H_2$]-(**15**) as starting material. Yield: 97%.

2-(β-Chloro-(α, α-$^2H_2$)-ethylamino)-3-(β′-hydroxyethyl)-2H-1,3,2-oxaza-phosphorinane-2-oxide {(α, α-$^2H_2$)-(16)

As for [4,4-$^2H_2$]-(**16**) but with [α,α-$^2H_2$]-(**15**) as starting material. Yield: 95%.

2-(β-Chloro-(α, α-$^2H_2$)-ethylamino)-3-(β′-hydroxy-(α′, α′-$^2H_2$)-ethyl)-2H-1,3,2-oxazaphosphorinane-2-oxide {(α, α, α′, α′-$^2H_4$)-(16)}

As for [4,4-$^2H_2$]-(**16**) but with [α,α,α′,α′-$^2H_4$]-(**15**) as starting material. Yield: 100%.

(6,6-$^2H_2$)-2-(β-chloro-(α, α-$^2H_2$)-ethylamino)-3-(β′-hydroxy-(α′, α′-$^2H_2$)-ethyl)-2H-1,3,2-oxazaphosphorinane-2-oxide {(6,6,α, α, α′, α′-$^2H_6$)-(16)}

As for [4,4-$^2H_2$]-(**16**) but with [6,6,α,α,α′,α′-$^2H_6$]-(**15**) as precursor. Yield: 97%.

(4,4-$^2H_2$)-ifosfamide {(4,4-$^2H_2$)-(2)}

A solution of triphenylphosphine (0.67 g, 2.6 mmol) in THF (3 ml) was added dropwise to a solution of freshly recrystallized *N*-chlorosuccinimide (0.34 g, 2.6 mmol) in THF (15 ml). Additional THF (3 ml) was used to rinse the flask and syringe. The turbid mixture was stirred vigorously for several minutes and then a solution of [4,4-$^2H_2$]-(**16**) (0.57 g, 2.3 mmol) in THF (3 ml) was added quickly with another 3 ml THF being used to rinse the flask and syringe. The mixture was stirred at RT for 20 h. Absolute ethanol (10 ml) was then added to react with excess *N*-chlorosuccinimide and the solvents were evaporated and the residue was purified by flash chromatography using EtOAc to elute impurities and then EtOH–EtOAc (1:9) to elute product [0.37 g, 61%, R_f 0.38 in CH_3OH–CH_2Cl_2 (1:9)]. ^{1}H NMR ($CDCl_3$) δ 4.42–4.34 (m, 1H, one C_6H), 4.29–4.19 (m, 1H, one C_6H), 3.66 [t, $J = 6$ Hz, 2H, CD_2NCH_2C***H***$_2$Cl], 3.61 (t, $J = 6$ Hz, 2H, $NHCH_2C$***H***$_2$Cl], 3.52–3.18 (m, 5H, CD_2NCH_2 and $NHCH_2$), and 2.05–1.92 (m, 2H, C_5H). *Not observed*: (C_4)-H [δ_H 3.30–3.20 (m)]. ^{13}C NMR ($CDCl_3$) δ 67.08 (d, $^2J_{CP} = 7$ Hz, C_6), 49.94 (d, $^2J_{CP} = 3$ Hz, CD_2N***C***H_2), 45.54 (d, $^3J_{CP} = 5$ Hz, CD_2NCH_2***C***H_2Cl), 43.03 (NH***C***H_2), 42.02 (d, $^3J_{CP} = 4$ Hz, $NHCH_2$***C***H_2Cl), and 26.06 (d, $^3J_{CP} = 5$ Hz, C_5). *Not observed*: C_4 (δ_C 47.56). ^{31}P NMR ($CDCl_3$) δ 12.1.

(6,6-$^2H_2$)-ifosfamide {(6,6-$^2H_2$)-(2)}

As for [4,4-$^2H_2$]-(**2**) but using [6,6-$^2H_2$]-(**16**) as starting material. Yield: 56%.

(α, α-$^2H_2$)-ifosfamide {(α, α-$^2H_2$)-(2)}

As for [4,4-$^2H_2$]-(**2**) but using [α,α-$^2H_2$]-(**16**) as starting material. Yield: 68%.

(α′, α′-$^2H_2$)-ifosfamide {(α′, α′-$^2H_2$)-(2)}

As for [4,4-$^2H_2$]-(**2**) but using [α′,α′-$^2H_2$]-(**16**) as starting material. Yield: 65%.

(α, α, α′, α-$^2H_4$)-ifosfamide {(α, α, α′, α′-$^2H_4$)-(2)}

As for [4,4-$^2H_2$]-(2) but with [α,α,α′,α′-$^2H_4$]-(**16**) as starting material and a 42 h *rxn* time. Yield: 54%.

(6,6,α, α, α′, α′-$^2H_6$)-ifosfamide {(6,6,α, α, α′, α′-$^2H_6$)-(1)}

As for [4,4-$^2H_2$]-(**2**) but using [6,6,α,α,α′,α′-$^2H_4$]-(**16**) as starting material. Yield: 66%.

Conclusion

This describes the synthesis of various deuterium labeled cyclophosphamides and ifosfamides that can be used for LC-MS and GC-MS investigations of kinetics, mechanism and enzyme specificity. These syntheses are readily adapted to tritium incorporation at specific sites through the use of [^{3}H]-$LiAlH_4$.

DOI: 10.1002.jlcr

Acknowledgements

This work was supported in part by Public Health Service Grant CA16783 (OMC/SML) awarded by the National Cancer Institute (Department of Health and Human Services). NMR and MS data were obtained through the Shared Instrumentation Facility of the Duke University Medical Center and the Department of Chemistry.

REFERENCES

1. Colvin M, Chabner BA. Alkylating agents. In *Cancer Chemotherapy: Principles and Practice* (3rd edn). Chabner BA, Collins JM (eds). JL Lippincott: Philadelphia, 1991; 276–313.
2. Ludeman SM. *Current Pharm Design* 1999; **5**: 627–643.
3. Williams ML, Wainer IW. *Current Pharm Design* 1999; **5**: 665–672.
4. Wang JJ-H, Chan KK. *J Chromatog B* 1995; **674**: 205–217.
5. Yu LJ, Drewes P, Gustafsson K, Brain EGC, Hecht JED, Waxman DJ. *J Pharmacol Exper Ther* 1999; **288**: 928–937.
6. Friedman OM, Seligman AM. *J Am Chem Soc* 1954; **76**: 655–658.
7. Arnold H, Bourseaux F. *Angew Chem* 1958; **70**: 539–544.
8. Ludeman SM, Shulman-Roskes EM, Wong KK, Han SY, Anderson LW, Strong JM, Colvin OM. *J Pharm Sci* 1995; **84**: 393–398.
9. Griggs LJ, Jarman M. *J Med Chem* 1975; **18**: 1102–1106.
10. Ludeman SM, Shulman-Roskes EM, Gamcsik MP, Hamill TG, Chang YH, Koo KI, Colvin OM. *J Label Compd Radiopharm* 1993; **33**: 313–326.
11. Cox PJ, Farmer PB, Foster AB, Griggs LJ, Jarmin M, Kinas R, Pankiewicz K, Stec WJ. *Biomed Mass Spectrom* 1977; **4**: 371–375.
12. Walsh SP, Shulman-Roskes EM, Anderson LW, Chang YH, Ludeman SM. *J Label Compd Radiopharm* 1995; **36**: 1193–1198.
13. Cox PJ, Farmer PB, Foster AB, Gilby ED, Jarman M. *Curr Treat Rep* 1976; **60**: 483–491.
14. Jarman M, Taylor GN. *J Label Compd Radiopharm* 1981; **18**: 463.
15. Connors TA, Cox PJ, Farmer PB, Foster AB, Jarman M, Macleod JK. *Biomed Mass Spectrom* 1974; **1**: 130–136.
16. Paquette LA. *Encyclopedia of Reagents for Organic Synthesis* (vol. A-BRU). Wiley: New York, 1995.
17. Misiura K, Kinas RW, Kuśnierczyk H. *Bioorg Med Chem Lett* 2002; **12**: 427–431.
18. Shulman-Roskes EM, Gamcsik MP, Colvin OM, Chang YH, Koo KI, Ludeman SM. *J Label Compd Radiopharm* 1994; **34**: 231–237.
19. Yoon NM, Brown HC. *J Am Chem Soc* 1968; **90**: 2927–2938.
20. Springer JB, Colvin ME, Colvin OM, Ludeman SM. *J Org Chem* 1998; **63**: 7218–7222.