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Journal of Labelled Compounds and Radiopharmaceuticals

Received 7 August 2013, Revised 11 October 2013, Accepted 21 October 2013 Published online 5 December 2013 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3142

Labeled oxazaphosphorines for applications in mass spectrometry studies. 2. Synthesis of deuterium-labeled 2-dechloroethylcyclophosphamides and 2- and 3-dechloroethylifosfamides

James B. Springer,[a] O. Michael Colvin,[a] and Susan M. Ludeman[b]*

The prodrugs cyclophosphamide (CP) and ifosfamide (IF) each metabolize to an active alkylating agent through a cytochrome P450-mediated oxidation at the C-4 position. Competing with this activation pathway are enzymatic oxidations at the exocyclic α and α′ carbons, which result in dechloroethylation of CP and IF. The incidence of oxidation at one position relative to another is believed to be at least one factor underlying the high degree of interpatient variability in both CP and IF pharmacokinetics. As standards for the mass spectrometry quantification of dechloroethylation, the following were synthesized: (1) [4,4,5,5-$^2H_4$]-2-dechloroethylcyclophosphamide (equivalent to [4,4,5,5-$^2H_4$]-3-dechloroethylifosfamide); (2) [α,α,4,4,5,5-$^2H_6$]-2-dechloroethylcyclophosphamide (equivalent to [α,α,4,4,5,5-$^2H_6$]-3-dechloroethylifosfamide); and (3) [α,α,4,4,5,5-$^2H_6$]-2-dechloroethylifosfamide. The common precursor to all of the target compounds was [2,2,3,3-$^2H_4$]-3-aminopropanol. A one-pot reaction of this compound with $POCl_3$ and unlabeled or labeled 2-chloroethylamine hydrochloride gave the d_4 and d_6 labeled 2-dechloroethylcyclophosphamides. The construction of the 2-dechloroethylifosfamide from the aminopropanol required five discreet steps. Optimization of the synthetic pathways and stability studies are discussed.

Keywords: dechloroethylcyclophosphamide; dechloroethylifosfamide; deuterium; synthesis

Introduction

The prodrugs cyclophosphamide (**1**, CP; Scheme 1) and ifosfamide (**2**, IF; Scheme 2) each metabolize to an active alkylating agent through a cytochrome P450-mediated oxidation at the C-4 position. Competing with this activation pathway are enzymatic oxidations at the exocyclic α and α′ carbons of CP and IF.[1,2] Each reaction at a side chain produces a hemiaminal (as shown for CP in Scheme 1); rearrangement results in a fragmentation of the parent drug to give a dechloroethyl oxazaphosphorine and chloroacetaldehyde. No toxicities have been linked unambiguously to the dechloroethyl metabolites; however, chloroacetaldehyde is associated with neurotoxicity, and this side effect, especially during IF treatment, can be dose-limiting.[2]

The incidence of oxidation at one position relative to another is believed to be at least one factor underlying the high degree of interpatient variability in both CP and IF pharmacokinetics. As a result, there is much interest in determining the influence of different variants, such as race, gender, age, and polymorphisms, on the extent of C-4 and side chain oxidations in CP and IF.[2–7] There are multiple reports of applications of mass spectrometry (MS) to such studies, particularly those relating to the quantification of C-4 oxidation.[4,5,7,8] Typically, deuterated analogs of the C-4 oxidized analytes are used as internal standards.[4,8] The availability of labeled dechloroethyl metabolites for use as internal standards would facilitate extensions of such work to the reliable quantification of each dechloroethylation reaction.

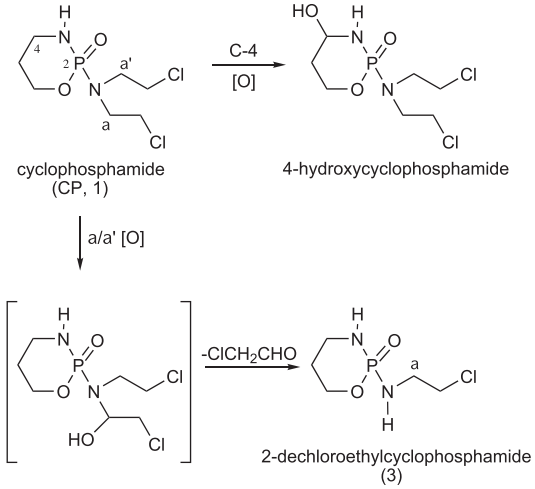
P-450 Oxidation of either the α or α′ carbon in CP yields the same product: 2-dechloroethylcyclophosphamide (**3**) (Scheme 1). Similar oxidation of the nonequivalent α and α′ carbons in IF give 2-dechloroethylifosfamide (**4**) and 3-dechloroethylifosfamide (**5**), respectively (Scheme 2). Note that the product of 2-dechloroethylation in CP is identical to that for 3-dechloroethylation in IF.

As standards for the MS quantification of dechloroethylation, the following three compounds were synthesized (Schemes 3 and 4): [4,4,5,5-$^2H_4$]-2-dechloroethylcyclophosphamide ([4,4,5,5-$^2H_4$]-**3**); [α,α,4,4,5,5-$^2H_6$]-2-dechloroethylcyclophosphamide ([α,α,4,4,5,5-$^2H_6$]-**3**); and [α,α,4,4,5,5-$^2H_6$]-2-dechloroethylifosfamide ([α,α,4,4,5,5-$^2H_6$]-**4**). Depending on one's frame of reference, the **3**-d_4 and **3**-d_6 structures can also be named

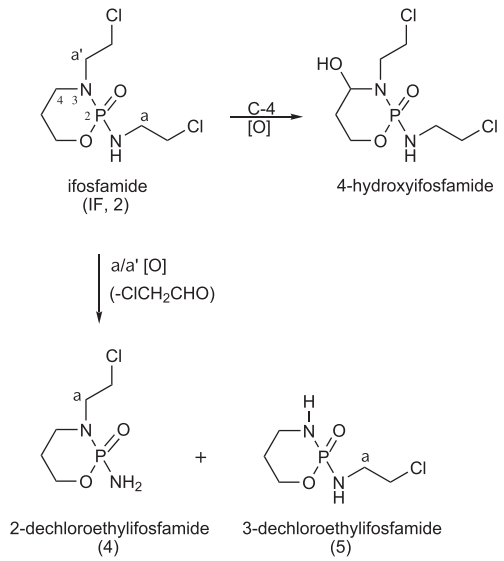
[a]*Duke Comprehensive Cancer Center and Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA*

[b]*Department of Basic and Social Sciences, Albany College of Pharmacy and Health Sciences, Albany, NY 12208, USA*

**Correspondence to: Susan M. Ludeman, Albany College of Pharmacy and Health Sciences, 106 New Scotland Ave., Albany, NY 12208, USA. E-mail: susan.ludeman@acphs.edu*



Scheme 1. Competing C-4 and side chain oxidations in cyclophosphamide.



Scheme 2. Competing C-4 and side chain oxidations in ifosfamide.

3-dechloroethylifosfamides [4,4,5,5-$^{2}H_4$]-**5** and [α,α,4,4,5,5-$^{2}H_6$]-**5**, respectively.

Experimental

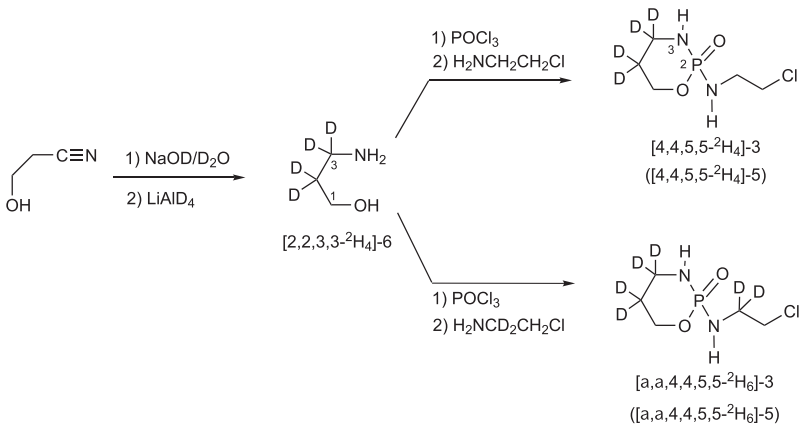
All glassware were dried before use, and reactions were carried out under N_2. Generally, chemicals were purchased from Sigma-Aldrich Corp., St. Louis, MO, USA, Thermo Fisher Inc., Hampton, NH, USA, or VWR, Bridgeport, NJ, USA. THF was distilled from potassium/benzophenone ketyl; CH_2Cl_2, CH_3CN and Et_3N were distilled from CaH_2. Flash chromatography was carried out on 230–400 mesh silica gel (Merck). In general, approximately 50 mL of dry silica gel was used per gram crude material, and columns were 6″ in height. Analytical thin layer chromatography plates were hard-coated with a 250-micron layer of silica gel 60 (Merck). Radial chromatography was performed on a chromatotron (Harrison and Harrison). NMR spectra were recorded on a Varian Inova-400 (Agilent Technologies, Santa Clara, CA, USA) or a GE QE-300 spectrometer (GE NMR Instruments, Freemont, CA, USA). Chemical shifts are reported relative to TMS (0 ppm, ^{1}H), $CDCl_3$ (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.

[2,2,3,3-$^{2}H_4$]-3-Amino-1-propanol ([2,2,3,3-$^{2}H_4$]-6).

As per a literature report,[9] 3-hydroxypropionitrile was reacted with NaOD/D_2O (≥98 atom %D, MSD Isotopes, now Cambridge Isotope Laboratories, Tewksbury, MA, USA) to give [2,2-$^{2}H_2$]-3-deuterioxypropionitrile ($DOCH_2CD_2CN$) in 65% yield [R_f 0.52, EtOAc-hexanes (3:1); ^{13}C NMR ($CDCl_3$) δ 118.4 (CN) and 57.22 (OCH_2)]. This nitrile was then reduced with $LiAlD_4$ (98 atom %D, Aldrich) according to the literature[9] to give the final product [96% yield; ^{13}C NMR ($CDCl_3$) δ 61.82 (CH_2O)].

[4,4,5,5-$^{2}H_4$]-2-Dechloroethylcyclophosphamide ([4,4,5,5-$^{2}H_4$]-3). Equivalent to [4,4,5,5-$^{2}H_4$]-3-dechloroethylifosfamide ([4,4,5,5-$^{2}H_4$]-5).

A solution of [2,2,3,3-$^{2}H_4$]-**6** (461 mg, 5.8 mmol) in CH_2Cl_2 (6 mL) was added dropwise to a cooled (ice bath) solution of freshly distilled $POCl_3$ (0.55 mL, 5.9 mmol) in CH_2Cl_2 (17 mL). Et_3N (1.66 mL, 11.9 mmol) was then added, and the mixture was stirred for 5 h at ice bath temperature. 2-Chloroethylamine hydrochloride (770 mg, 6.6 mmol) was added as a solid, in one portion, followed by Et_3N (1.66 mL, 11.9 mmol). The reaction mixture was stirred at room temperature overnight and was then filtered through a pad (2″) of Celite 545 (Thermo Fisher Scientific, Hampton, NH). The filtrate was concentrated, and the residue was flash-chromatographed using CH_3OH-CH_2Cl_2 (5:95). The product was still contaminated, and therefore, the material was flash-chromatographed again using EtOH-EtOAc (1:9). Pure product was obtained [527 mg, 45%, R_f 0.19 in EtOH-EtOAc (1:9)]. ^{1}H NMR ($CDCl_3$) δ 4.40 (dd, $^{2}J_{HH}$ = 11 Hz and $^{3}J_{HP}$ = 11 Hz, 1H, one C_6H), 4.26 (dd, $^{2}J_{HH}$ = 11 Hz and $^{3}J_{HP}$ = 11 Hz, 1H, one C_6H), 3.62 (t, J = 6 Hz, 2H, CH_2Cl), 3.33–3.25 (m, 2H, C***H***$_2CH_2Cl$), 3.16 (bs, 1H, one NH), and 2.81 (bs, 1H, one NH).



Scheme 3. Synthesis of deuterated 2-dechloroethylcyclophosphamides (structurally equivalent to 3-dechloroethylifosfamides).

^{13}CNMR ($CDCl_3$) δ 67.85 (d, $^2J_{CP}$ = 7 Hz, C_6), 45.88 (d, $^3J_{CP}$ = 5 Hz, CH_2Cl), and 42.98 (**C**H_2CH_2Cl). ^{31}P NMR ($CDCl_3$) δ 10.8.

[α,α,4,4,5,5-$^2H_6$]-2-Dechloroethylcyclophosphamide ([α,α,4,4,5,5-$^2H_6$]-3). Equivalent to [α,α,4,4,5,5-$^2H_6$]-3-dechloroethylifosfamide ([α,α,4,4,5,5-$^2H_6$]-5).

The title compound was synthesized using the same procedure as that given above for [4,4,5,5-$^2H_4$]-**3** but instead used [1,1-$^2H_2$]-2-chloroethylamine hydrochloride[10] instead of unlabeled 2-chloroethylamine hydrochloride as a starting material. The reaction mixture was concentrated to ~1/3 of its original volume, and this turbid mixture was loaded onto a 9″ silica gel column made with a 50-mL silica gel for flash chromatography. Elution with EtOH-EtOAc (1:9) gave the product (0.19 g, 33%, R_f 0.19). ^{1}H NMR ($CDCl_3$) δ 4.39 (dd, $^2J_{HH}$ = 11 Hz and $^3J_{HP}$ = 11 Hz, 1H, one C_6H) 4.26 (dd, $^2J_{HH}$ = 11 Hz and $^3J_{HP}$ = 11 Hz, 1H, one C_6H), 3.61 (s, 2H, CH_2Cl), and 3.30 and 3.05 (bm, 1H each, two NH). ^{13}C NMR ($CDCl_3$) δ 67.88 (d, $^2J_{CP}$ = 7 Hz, C_6) and 45.59 (d, $^3J_{CP}$ = 5 Hz, CH_2Cl). ^{31}P NMR ($CDCl_3$) δ 11.2.

[2,2,3,3-$^2H_4$]-3-(2′-Benzyloxyacetylamido)-1-propanol ([2,2,3,3-$^2H_4$]-7).

Using a literature procedure for unlabeled material,[10] [2,2,3,3-$^2H_4$]-**6** was incorporated as a starting material to give the title compound in 60% yield. The product was obtained as an oil, which solidified upon standing [R_f 0.27 (EtOAc)]. ^{1}H 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), and 3.60 (s, 2H, C**H**$_2$OH); N-H not visible above the baseline. ^{13}C NMR ($CDCl_3$) δ 170.6 (C=O), 136.7, 128.5, 128.2, and 127.9 (aromatic), 73.49 (**C**H_2C=O), 69.22 (CH_2Ph), and 59.09 (CH_2OH).

1′,1′,2,2,3,3-[$^2H_6$]-3-(2′-Benzyloxyethylamino)-1-propanol ([1′,1′,2,2,3,3-$^2H_6$]-8).

With minor modification to a literature preparation of unlabeled material,[10] AlD_3 [from $LiAlD_4$ (98 atom %D, 1.0 g, 22.6 mmol)] was reacted with [2,2,3,3-$^2H_4$]-**7** (1.5 g, 6.4 mmol) for ~12 h. The flask was then cooled (ice bath), and the reaction was quenched with the dropwise addition of 1 *M* sodium potassium tartrate (5.0 mL). The mixture was diluted with water (100 mL) and CH_2Cl_2 (50 mL); 40% NaOH (~1.0–1.5 mL) was added to insure a basic pH. The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). All organic layers were combined, dried (Na_2SO_4), filtered, and evaporated to afford the product (86%) as a pure, colorless oil [R_f 0.50 in NH_3-saturated CH_3OH : CH_2Cl_2 (1:9)]. ^{1}H NMR ($CDCl_3$) δ 7.37–7.28 (m, 5H, aromatic), 4.51 (s, 2H, CH_2Ph), 3.79 (s, 2H, C**H**$_2$OH), 3.59 (s, 2H, OCH_2CD_2N), and 3.53 (bs, 2H, NH and OH). ^{13}C NMR ($CDCl_3$) δ 138.0, 128.4 and 127.7 (aromatic), 73.20 (CH_2Ph), 69.06 (O**C**H_2CD_2N), and 64.04 (CH_2OH).

[α,α,4,4,5,5-$^2H_6$]-2-Amino-3-(2′-benzyloxyethyl)-2H$_2$-1,3,2-oxazaphosphorinane-2-oxide ([α,α,4,4,5,5-$^2H_6$]-9).

A solution of [1′,1′,2,2,3,3-$^2H_6$]-**8** (1.21 g, 5.6 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of freshly distilled $POCl_3$ (0.53 mL, 5.7 mmol) in CH_2Cl_2 (17 mL) at ice bath temperature. Additional CH_2Cl_2 (3 mL) was used to rinse down the flask and syringe. Et_3N (1.57 mL, 11.3 mmol) was then added, and the mixture was stirred in an ice bath (3 h). The mixture was allowed to warm for ~1–2 min and was then cooled again. Anhydrous NH_3 was bubbled through the mixture at ice bath temperature for 15 min, and then the flask was capped and parafilmed, the bath was removed, and the mixture was stirred overnight. (*Note*: The cap blew off overnight as a result of the pressure, and the solvent evaporated.) The crude reaction mixture was taken up in minimal CH_2Cl_2 and loaded onto a 6″ flash chromatography column, which had been made with 100-mL silica gel and EtOH-EtOAc (1:9) eluent. The product was obtained as a colorless solid [1.36 g, 88%, R_f 0.37 in CH_3OH-CH_2Cl_2 (1:9)]. ^{1}H NMR ($CDCl_3$) δ 7.38–7.27 (m, 5H, aromatic), 4.58 (d, *J* = 12 Hz, 1H, one CH_2Ph), 4.51 (d, *J* = 12 Hz, 1H, one CH_2Ph), 4.35–4.21 (m, 2H, CH_2OP), 3.67 (d, *J* = 10 Hz, 1H, one NCD_2CH_2O) 3.51 (d, *J* = 10 Hz, 1H, one NCD_2CH_2O), and 2.88 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$) δ 137.8, 128.4, 127.8, and 127.7 (aromatic), 73.08 (CH_2Ph), 68.09 (NCD_2**C**H_2O), and 67.44 (d, $^2J_{CP}$ = 7 Hz, C_6). ^{31}P NMR ($CDCl_3$) δ 13.2.

[α,α,4,4,5,5-$^2H_6$]-2-Amino-3-(2′-hydroxyethyl)-2H$_2$-1,3,2-oxazaphosphorinane-2-oxide ([α,α,4,4,5,5-$^2H_6$]-10).

A solution of [α,α,4,4,5,5-$^2H_6$]-**9** (1.14 g, 4.2 mmol) in 11.8% (v/v) solution of 1,4-cyclohexadiene in absolute ethanol (85 mL) was passed through a 1 × 6 cm column of freshly prepared palladium black[11] at a rate of 1–2 mL/min. Concentration of the eluent gave a residue that was flash-chromatographed (CH_3OH-CH_2Cl_2, 1:9) to isolate the product (0.36 g, 47%, R_f 0.15). Unreacted starting material was also recovered (0.63 g, 56%, R_f 0.37). ^{1}H NMR ($CDCl_3$) δ 4.60 (bs, 1H, OH), 4.38–4.24 (m, 2H, CH_2OP), 3.80–3.60 (m, 2H, NCD_2CH_2O), and 3.55 (bs, 2H, NH_2). ^{31}P NMR ($CDCl_3$) δ 15.7.

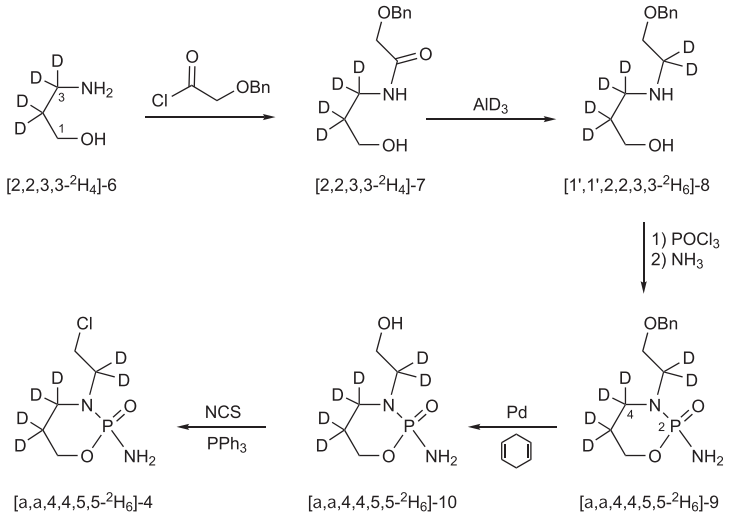
[α,α,4,4,5,5-$^2H_6$]-2-Dechloroethylifosfamide ([α,α,4,4,5,5-$^2H_6$]-4).

A solution of triphenylphosphine (0.62 g, 2.4 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of freshly recrystallized (benzene) *N*-chlorosuccinimide (0.33 g, 2.4 mmol) in CH_2Cl_2 (27 mL). Additional CH_2Cl_2 (2 mL) was used to rinse the flask and syringe. The turbid mixture was stirred vigorously for several minutes, and then a solution of [α,α,4,4,5,5-$^2H_6$]-**10** (0.37 g, 2.0 mmol) in CH_2Cl_2 (5 mL) was added quickly with another 2-mL CH_2Cl_2 being used to rinse the flask and syringe. The mixture was stirred at room temperature overnight and then concentrated. The residue was passed through two flash chromatography columns, each 6″ tall and made with 30-mL silica gel [first column, EtOH-EtOAc (1:9) eluent; second column, EtOH-benzene (1:9) eluent]. The still impure product [R_f 0.33 in CH_3OH-CH_2Cl_2 (1:9)] was then successfully purified using radial chromatography on a 2-mm silica gel plate with EtOH-EtOAc (1:9) as eluent [0.23 g, 56%, R_f 0.19 in EtOH-EtOAc (1:9)]. ^{1}H NMR ($CDCl_3$) δ 4.37–4.25 (m, 2H, CH_2O), 3.65 (s, 2H, CH_2Cl), and 3.05 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$) δ 67.23 (d, $^2J_{CP}$ = 7 Hz, C_6) and 42.34 (CH_2Cl). ^{31}P NMR δ 12.7.

Results and Discussion

Tetratedeuterated 3-aminopropanol ([2,2,3,3-$^2H_4$]-**6**) was the common precursor to all of the desired dechloroethyl compounds (Schemes 3 and 4).[9] For the 2-dechloroethylcyclophosphamides, [2,2,3,3-$^2H_4$]-**6** was reacted with $POCl_3$ followed by either unlabeled or [1,1-$^2H_2$]-2-chloroethylamine hydrochloride[10] to give tetradeuterated or hexadeuterated products (Scheme 3, [4,4,5,5-$^2H_6$]- **3** or [α,α,4,4,5,5-$^2H_6$]- **3**, respectively). Initially, the work-up included a water wash of the reaction mixture; however, 2-dechloroethylcyclophosphamide proved to be quite hydrophilic, and even multiple back-extractions were not successful in removing all products from the water layer. The water washes were dropped from subsequent syntheses, and yields improved by 10–50%. As mentioned previously, the **3**-d_4 and **3**-d_6 structures can also be named 3-dechloroethylifosfamides [4,4,5,5-$^2H_4$]-**5** and [α,α,4,4,5,5-$^2H_6$]-**5**, respectively.

As shown in Scheme 4, construction of the remaining chloroethyl chain in 2-dechloroethylifosfamide [α,α,4,4,5,5-$^2H_6$]-**4** required multiple steps; the pathway was a modified version of the one used to make labeled ifosfamides.[10] Variations tried during optimization of the synthetic scheme included the following: (1) the oxygen in **6** was protected as an acetal to prevent the formation of any *N*- and *O*-bisalkylated product during synthesis of **7** (no advantage obtained); (2) in place of the benzyloxy group in **7**, **8** and **9**, *t*-butyldimethylsilyl and *t*-butyldiphenylsilyl ethers were investigated as moieties, which might be easily converted to the alcohol group in **10**, and synthesis of these compounds added many steps to the pathway without any net benefits; (3) catalytic transfer hydrogenations of **9** with 10% Pd/C and formic acid[12] or ammonium formate[13] resulted in sluggish reactions at room temperature and decomposition upon heating; (4) reduction of **9** with hydrogen and 10% Pd/C at 60 psi[10] was incomplete



Scheme 4. Synthesis of deuterated 2-dechloroethylifosfamide.

after 4 h, and allowing the reaction to continue overnight resulted in decomposition as well as the persistence of unreacted **9**; and (5) direct conversion of **9** to final product **4** was tried using trimethylsilyl chloride and sodium iodide, and extensive decomposition resulted.

Similar to all CP-related and IF-related compounds, the stabilities of dechloroethyl metabolites **3**/**5** and **4** vary significantly depending on conditions. For **3**/**5** in buffered solutions at 20°C, Gilard *et al*. report half-lives of 2.8 h at pH 3.0 and 49 days at pH 5.5; under the same conditions, **4** exhibits half-lives of 25 min at pH 3.0, 3.8 days at pH 5.5, and 36 days at pH 6.8.[14] According to Kaijser *et al*., the dechloroethyl metabolites are unchanged after 8 weeks at 4°C in water or ethyl acetate but undergo 10% loss after 4 weeks (4°C) in plasma or urine (no loss at −20°C).[15]

Using ^{31}P NMR, we monitored the stabilities of the dechloroethyl compounds under conditions that have been used for the gas chromatography (GC)–MS–MS and liquid chromatography–MS–MS quantifications of 4-hydroxy-CP, the C-4 oxidation product derived from CP (Scheme 1).[4,8] In this procedure, reaction samples are added to solutions containing a derivitizing agent, which stabilizes the reactive 4-hydroxy-CP; the solvent is composed of 2 *M* ammonium diphosphate (pH ~4.8), methanol, and acetonitrile (20:25:55). Samples may be stored for months at −80°C prior to analysis. For the GC method, the derivitized 4-hydroxy-CP is extracted from the sample and treated with a silylating agent in acetonitrile for 2 h at 70°C. To determine the suitability of these conditions for isolation and analysis of the dechloroethyl metabolites, each compound was dissolved in the ammonium phosphate/methanol/acetonitrile solution and allowed to sit at room temperature. No changes were observed in ^{31}P NMR spectra taken of **4** periodically over 10 months. During a 14-month observation time, an unidentified but prominent ^{31}P signal appeared in spectra of **3**/**5**; however, extraction of the solution yielded near quantitative recovery of **3**/**5**. Additional studies performed using liquid chromatography–MS–MS techniques demonstrated that both labeled metabolite and its unlabeled counterpart had the same stability: their relative concentrations remained the same in the derivitizing solution over the length of the experiment (several days at −80°C).

Under the silylation reaction conditions,[8] each dechloroethyl metabolite was found to react rapidly and to give multiple products (^{31}P NMR). Mono- or bis-silylated derivatives were anticipated, but the product mixture was much more complex. In the absence of the silylating agent, the metabolites were unchanged (acetonitrile, 70°C, 2 h). Samples for analysis by the cited GC–MS–MS method would best be split into aliquots, one for silylation and detection of C-4 oxidation products and one for separate analysis of dechloroethylation products.

Conclusion

There is ongoing interest in determining the influence of C-4 versus side chain oxidations on the interpatient variability in response to treatment with the anticancer agents CP and IF. MS has been applied to both clinical and *in vitro* studies, particularly those relating to the quantification of C-4 oxidation. The syntheses of deuterium-labeled internal standards have been reported for use in C-4 oxidation studies[16]; deuterated standards for companion investigations of dechloroethylation reactions were the synthetic goals of this work. Oxidative dechloroethylation of CP gives one possible product (**3**), whereas the same reaction of IF leads to two possible products (**4** and **5**; metabolite **5** is structurally equivalent to **3**). Tetra- and/or hexadeuterated analogs of each of these metabolites were synthesized by multistep but otherwise generally straightforward pathways.

Acknowledgements

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

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] R. J. Hansen, S. M. Ludeman, A. E. Pegg, M. E. Dolan, *DNA Repair*. **2007**, *6*, 1145.
[2] Z. Huang, P. Roy, D. J. Waxman, *Biochem. Pharmacol.*. **2000**, *59*, 961.
[3] N. Pinto, S. M. Ludeman, M. E. Dolan, *Pharmacogenomics*. **2009**, *10*, 1897.
[4] C. M. Walko, A. J. Combest, I. Spasojevic, A. Y. C. Yu, S. Bushan, J. H. Hull, J. Hoskins, D. Armstrong, L. Carey, F. Collicio, E. C. Dees, *Cancer Chemother. Pharmacol.*. **2012**, *69*, 1189.
[5] C. Ekhart, V. D. Doodeman, S. Rodenhuis, P. H. M. Smits, J. H. Beijnen, A. D. R. Huitema, *Pharmacogenet. Genom.*. **2008**, *18*, 515.
[6] M. Nakajima, S. Komagata, Y. Fujiki, Y. Kanada, H. Ebi, K. Itoh, H. Mukai, T. Yokoi, H. Minami, *Pharmacogenet. Genom.*. **2007**, *17*, 431.
[7] K. Aleksa, A. Nava-Ocampo, G. Koren, *Chirality*. **2009**, *21*, 674.
[8] L. W. Anderson, S. M. Ludeman, O. M. Colvin, L. B. Grochow, J. M. Strong, *J. Chromatogr. B Biomed. Appl.*. **1995**, *667*, 247.
[9] S. P. Walsh, E. M. Shulman-Roskes, L. W. Anderson, Y.-H. Chang, S. M. Ludeman, *J. Label. Compd. Radiopharm.* **1995**, *36*, 1193.
[10] J. B. Springer, O. M. Colvin, S. M. Ludeman, *J. Label. Compd. Radiopharm.* **2007**, *50*, 115.
[11] J. B. Springer, M. E. Colvin, O. M. Colvin, S. M. Ludeman, *J. Org. Chem*. **1998**, *63*, 7218.
[12] B. ElAmin, G. M. Anantharamaiah, G. P. Royer, G. E. Means, *J. Org. Chem.*, **1979**, *44*, 3442.
[13] T. Bieg, W. Szeja, *Synthesis*, **1985**, 76.
[14] V. Gilard, R. Martino, M. Malet-Martino, U. Niemeyer, J. Pohl, *J. Med. Chem.* **1999**, *42*, 2542.
[15] G. P. Kaijser, J. H. Beijnen, A. Bult, G. Wiese, J. de Kraker, H. J. Keizer, W. J. Underberg, *Chromatogr*. **1992**, *583*, 175.
[16] S. M. Ludeman, E. M. Shulman-Roskes, K. K. T. Wong, S. Y. Han, L. W. Anderson, J. M. Strong, O. M. Colvin, *J. Pharm. Sci.*, **1995**, *84*, 393.