# Synthesis of 4-$^{13}$C-4-Hydroperoxycyclophosphamide and Alpha-$^{13}$C-4-Hydroperoxycyclophosphamide

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## SUMMARY

New synthetic routes were developed for incorporating $^{13}$C into the oxazaphosphorine ring and nitrogen mustard functionality of cyclophosphamide metabolites. 1,2-$^{13}C_2$-Vinylbromide and ethylene oxide were used to synthesize 3,4-$^{13}C_2$-3-butenyl N,N-bis(2-chloroethyl)phosphorodiamidate via the intermediacy of 3,4-$^{13}C_2$-3-buten-1-ol. Ozonolysis of the phosphorodiamidate gave 4-$^{13}$C-4-hydroperoxycyclophosphamide with an overall yield of 16% which was 27-times higher than a previously reported synthesis. As an extension of this synthetic pathway, 2-$^{13}$C-ethyl bromoacetate was used to incorporate $^{13}$C into the $C_1$ position of bis(2-chloroethyl)amine hydrochloride which was used as a precursor to alpha-$^{13}$C-4-hydroperoxycyclophosphamide (obtained in 16% overall yield). Also discussed are applications of these pathways to the synthesis of diversely labelled ($^{14}$C, $^{13}$C, $^{2}$H) 4-hydroperoxycyclophosphamides, chirally-labelled oxazaphosphorines, phosphoramide mustards, and related alkylating agents.

**KEY WORDS:** 4-$^{13}$C-4-Hydroperoxycyclophosphamide; alpha-$^{13}$C-4-hydroperoxycyclophosphamide; 3,4-$^{13}C_2$-3-buten-1-ol; 1-$^{13}$C-bis(2-chloroethyl)amine hydrochloride; labelled oxazaphosphorines.

## INTRODUCTION

The therapeutic efficacy of the prodrug cyclophosphamide (1) in the treatment of many cancers is well established (1). As shown in Scheme I, the pertinent aspects of cyclophosphamide metabolism include an initial enzyme-mediated oxidation to give 4-hydroxycyclophosphamide (2) followed by a spontaneous ring-opening reaction to provide aldophosphamide (3). Metabolite 3 may undergo fragmentation to form acrolein and the ultimate alkylating agent phosphoramide mustard (4) and/or 3 may be enzymatically oxidized to the non-toxic carboxyphosphamide (5). A second detoxification pathway is the enzymatic oxidation of 2 to ketocyclophosphamide (6) (2).

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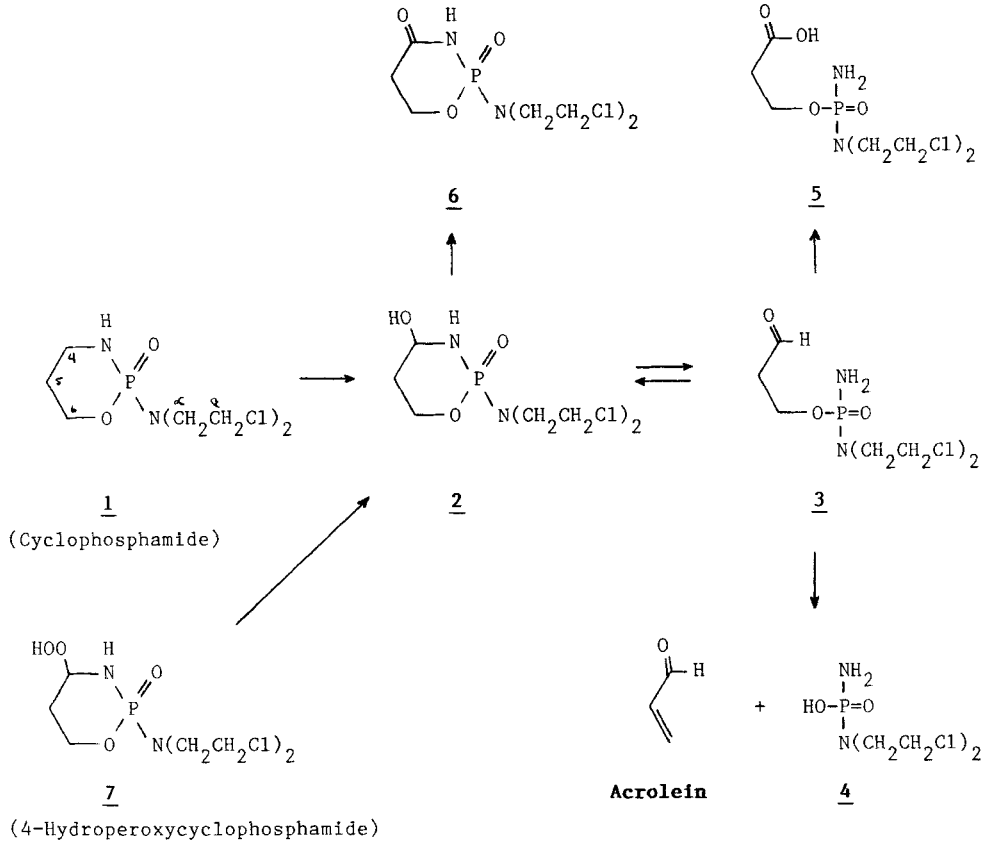
While the general features of the metabolism of 1 have been detailed, specific causal relationships between this chemistry and the oncostatic selectivity of 1 have not been established (2,3). NMR spectroscopy has been increasingly utilized as an analytical tool for the study of selectivity mechanisms as well as for related investigations of metabolite reactivity (5-10). $^{31}P$ NMR has proven extremely useful in this regard, but there are limitations (e.g. isochronous metabolite signals and the obvious inability to monitor metabolites which do not contain phosphorus) which can be overcome through the use of $^{13}C$ NMR and appropriately labelled metabolites.

**Scheme I**




Access to the metabolites of 1 is conveniently provided by the reduction of 4-hydroperoxycyclophosphamide (7, Scheme I) (4,6); thus, incorporation of $^{13}C$ into 7 was the target of the work described herein. Among the various metabolites, the carbon atom which originates from the $C_4$ position in 7 is the most

distinctive; the $^{13}C$ NMR chemical shifts of this carbon in each of the metabolites (including various others not shown in Scheme I) have been reported (4,5). The unique spectroscopic character of this carbon provided the basis for our synthesis of 4-$^{13}$C-4-hydroperoxycyclophosphamide (4-$^{13}$C-7). For the purpose of monitoring the reactions of 4, alpha-$^{13}$C-4-hydroperoxycyclophosphamide (alpha-$^{13}$C-7) was also synthesized.

## SYNTHESIS OF 4-$^{13}$C-4-HYDROPEROXYCYCLOPHOSPHAMIDE

A synthesis of 4-$^{13}$C-7 was previously reported (4); however, the overall yield of the multi-step route was only 0.6%. In our hands, the most effective synthesis of 7 involves the ozonolysis of 3-butenyl N,N-bis(2-chloroethyl)phosphorodiamidate (8), of which 3-buten-1-ol is a precursor (4). For the synthesis of 4-$^{13}$C-7, 3-$^{13}$C-3-buten-1-ol was thus a key intermediate to be prepared (eq. 1).

$$H_2C=^{13}CHCH_2CH_2OH \longrightarrow H_2C=^{13}CHCH_2CH_2O-\underset{\underset{N(CH_2CH_2Cl)_2}{|}}{\overset{\overset{NH_2}{|}}{P}}=O \xrightarrow{O_3} 4\text{-}^{13}C\text{-}7 \qquad \text{(eq. 1)}$$

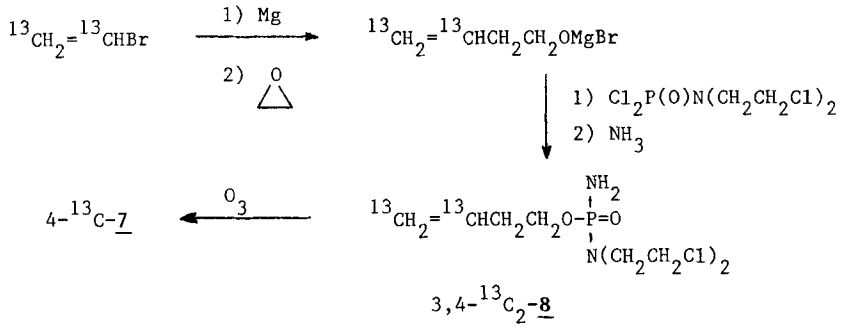
3-$^{13}$C-8

The initial synthetic scheme considered would have used $^{13}C$-sodium cyanide as the source of the carbon label. In trial reactions, ethylene glycol was treated with sodium hydride followed by benzyl bromide to give 2-benzyloxyethanol (81%). Chlorination of this alcohol with thionyl chloride provided 2-benzyloxyethylchloride (92%) which was then converted to the iodide (78%) with sodium iodide. 2-Benzyloxyethyliodide was reacted with sodium cyanide in DMSO to yield 3-benzyloxypropionitrile (91%). The nitrile was reduced with diisobutylaluminum hydride (DIBAL-H) and then hydrolysis with 10% $H_2SO_4$ (11) provided 3-benzyloxypropanal (53%). Multiple attempts to convert this aldehyde to 4-benzyloxy-1-butene (precursor to 3-buten-1-ol) via a Wittig reaction (12,13) were unsuccessful. This route was therefore abandoned in favor of a more direct synthesis of labelled 3-buten-1-ol, as outlined in Scheme II.

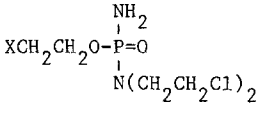
In initial trials using unlabelled materials, the intermediate 3-buten-1-ol (as generated by a Grignard reaction) was isolated by distillation (14,15). However, it was subsequently determined that 8 could be more efficiently synthesized through a 'one-pot' reaction which did not include the isolation of

the alcohol. On the other hand, the one-pot reaction led to a product mixture contaminated with a compound tentatively identified by $^{1}H$ and $^{13}C$ NMR as **9**.

**Scheme II**




Formation of **9** could be accounted for by the reaction of ethylene oxide with halide(s) to give $XCH_2CH_2O^-$. In a variety of solvent systems, **8** and **9** had nearly identical $R_f$ values which, therefore, necessitated multiple chromatographic separations in order to obtain pure **8** (attempts to ozonate crude mixtures of **8** and **9** led to poor recovery of hydroperoxide **7**). The formation of **9** could be minimized by decreasing the amount of ethylene oxide and for our purposes it was determined that use of 0.75 equivalents of ethylene oxide (relative to starting material vinyl bromide) provided optimal conditions for generating **8** in a relatively efficient and cost-effective manner. By this method, a mixture of **8** and **9** was obtained in a ratio of 2:1, respectively. It can be noted that isolation of the intermediate 3-buten-1-ol obviated formation of **9** in subsequent reactions; however, the ultimate yield of **8** which was achieved was still lower than in the one-pot reaction. The yield of **8** also did not improve when vinyl lithium was prepared by substituting magnesium with lithium in Scheme II (16).




**9**

Incorporation of 1,2-$^{13}C_2$-vinylbromide into Scheme II provided purified 3,4-$^{13}C_2$-**8** in 37% yield which was comprable to that achieved when unlabelled **8** was synthesized from commercially available 3-buten-1-ol (40%)(4). Ozonolysis of 3,4-$^{13}C_2$-**8** gave 4-$^{13}$C-4-hydroperoxycyclophosphamide (4-$^{13}$C-**7**) in 42% yield,

which was a marked improvement over yields reported for the synthesis of unlabelled **7** (27%)(4). The overall yield, then, of 4-$^{13}$C-**7** from 1,2-$^{13}C_2$-vinylbromide was 16%, a 27-fold increase in efficiency over the previously reported synthesis of the same compound by an alternative pathway (4).

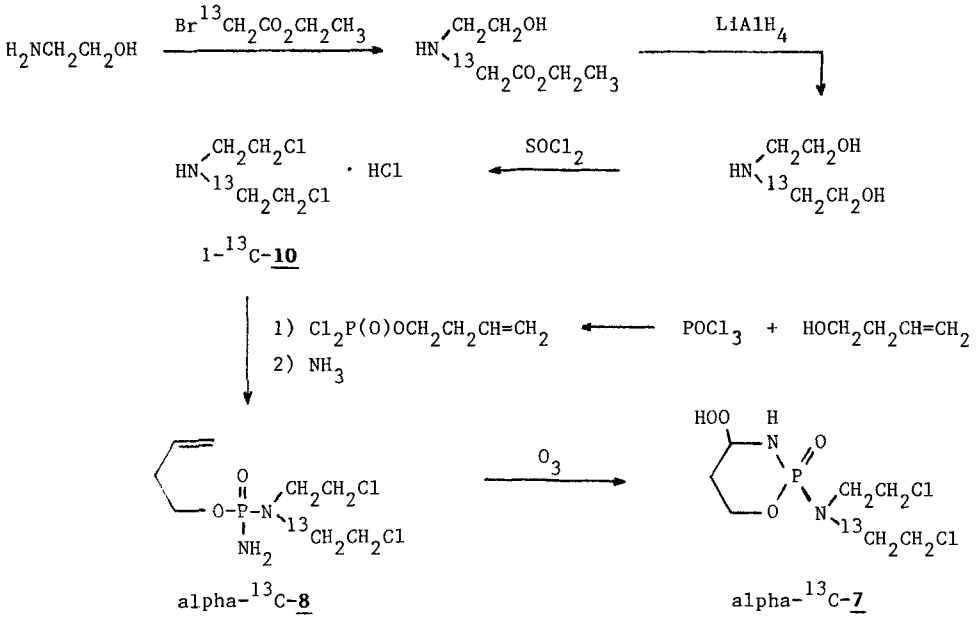
## ALPHA-$^{13}$C-4-HYDROPEROXYCYCLOPHOSPHAMIDE

For incorporation of $^{13}$C into the nitrogen mustard functionality of **7**, the synthesis of 1-$^{13}$C-bis(2-chloroethyl)amine hydrochloride (1-$^{13}$C-**10**) was a key target. Since both glycine and chloroacetyl chloride labelled with $^{13}$C were commercially available, the synthesis of **10** was originally investigated as shown in eq. 2. However, this route gave various intermediates which were difficult to isolate and purify, and led to unacceptably low yields of **10**.

$$H_2NCH_2CO_2H \xrightarrow[2)\ SOCl_2]{1)\ LiAlH_4} H_2NCH_2CH_2Cl \xrightarrow[2)\ (BH_3)_2]{1)\ ClCH_2C(O)Cl} \underset{\mathbf{10}}{HN(CH_2CH_2Cl)_2\cdot HCl} \quad \text{(eq. 2)}$$

Therefore, an alternative approach to the synthesis of 1-$^{13}$C-**10** and, ultimately, alpha-$^{13}$C-**7**, was pursued as outlined in Scheme III.

**SCHEME III**




For the synthesis of butenyl phosphorodiamidate **8**, typically 3-buten-1-ol is reacted with N,N-bis(2-chloroethyl)phosphorodiamidate dichloride (formed from

$POCl_3$ and **10**), as shown in Scheme II. However, a more efficient use of labelled **10** was achieved by first synthesizing 3-butenyl dichlorophosphate and then reacting this intermediate with **10**, followed by ammonia, to form **8**. Thus, incorporation of 2-$^{13}$C-ethyl bromoacetate into the synthesis shown in Scheme III provided alpha-$^{13}$C-**7** in an overall yield of 16%.

## CONCLUSION

Schemes II and III outline specific syntheses of 4-$^{13}$C-4-hydroperoxycyclophosphamide (4-$^{13}$C-**7**) and alpha-$^{13}$C-4-hydroperoxycyclophosphamide (alpha-$^{13}$C-**7**), respectively. These synthetic pathways are very versatile, however, and could be applied to the synthesis of any number of variously labelled oxazaphosphorines and phosphoramide mustards. For example, ethylene oxide is commercially available labelled with $^{14}$C, $^{13}$C, or $^{2}$H and thus could be used to generate **7** with labels at the $C_5$ and $C_6$ positions. Incorporation of 2*R*,3*R*- or 2*S*,3*S*-2,3-$^{2}H_2$-ethylene oxide (17,18) into Scheme II would yield **7** chirally-labelled at the $C_5$ and $C_6$ positions. Such chiral compounds would allow for stereochemical mechanistic studies of the fragmentation of **3** to acrolein and **4** (Scheme I).

Ethanolamine and ethyl bromoacetate (Scheme III) are commercially available or are readily synthesized from other compounds with $^{14}$C, $^{13}$C, or $^{2}$H enrichment at various positions. Thus, $^{14}$C and/or $^{13}$C and/or $^{2}$H could be incorporated into **10** at positions $C_1$ and/or $C_2$ and, furthermore, one or both of the chloroethyl groups could be labelled. These nor-nitrogen mustards could then be used to synthesize diversely labelled phosphoramide mustards, 4-hydroperoxycyclophosphamides, and related alkylating agents.

A particularly intersting extension of the syntheses described herein would be the synthesis of labelled 4-hydroperoxyifosfamide; ifosfamide is a clinically useful, structural isomer of cyclophosphamide which undergoes a similar metabolism. Substitution of $ClP(O)(HNCH_2CH_2Cl)_2$ for $Cl_2P(O)N(CH_2CH_2Cl)_2$ and ammonia in Scheme II would provide 4-$^{13}$C-4-hydroperoxyifosfamide (19).

This work not only describes an improved synthesis of 4-$^{13}$C-**7** and a new synthesis of alpha-$^{13}$C-**7**, but it also provides versatile synthetic pathways for making a wide variety of diversely labelled compounds related to the anticancer agent cyclophosphamide.

## EXPERIMENTAL

Tetrahydrofuran (THF), $Et_2O$, benzene, $P(O)Cl_3$, and pyridine refer to freshly distilled solvents/reagents. Reaction mixtures that do not include water were carried out under nitrogen. Ozone (ca. 5-10 g/h) was produced by a Model 03V10-0 ozone generator (Ozone Research and Equipment Corp.). Analytical TLC employed 2.5 or 5 x 10 cm plates coated with a 250 micrometer layer of silica gel GF (Analtech). Flash chromatography employed 230-400 mesh silica gel (EM reagents). 89.55-MHz $^1H$, 22.49-MHz $^{13}C$, and 36.23-MHz $^{31}P$ NMR spectra were obtained with a JEOL FX-90Q broad-banded spectrometer. Unless specified otherwise, NMR samples were prepared using $CDCl_3$ and $^1H$ and $^{13}C$ chemical shifts (ppm) refer to $Me_4Si$ as an internal reference. $^{31}P$ ppm values refer to external 25% $H_3PO_4$ in $D_2O$. In $D_2O$, $^1H$ chemical shifts refer to TSP as a reference.

**3,4-$^{13}C_2$-3-BUTENYL N,N-BIS(2-CHLOROETHYL)PHOSPHORODIAMIDATE (3,4-$^{13}C_2$-8).** THF (3.5 mL) and Mg (545 mg, 22.4 mmol) were added to a dry 50-mL 3-neck round-bottomed flask equipped with a liquid nitrogen condensor and septa. The mixture was cooled to -108 °C (THF/liquid $N_2$ bath) and then 1,2-$^{13}C_2$-vinylbromide (2 g, 18.7 mmol, MSD Isotopes, 90 atom % $^{13}C$) was transferred as a gas to this flask from the manufacturer's glass vessel (which we equipped with a septum) via a double-ended needle using reduced pressure. The Grignard reaction was initiated by refluxing the reaction mixture (30 min); a spontaneous reflux was then allowed to continue for 10 min. An oil bath was then used to heat the reaction mixture at 85 °C for an additional 80 min. During the latter heating stage, THF was added at various stages (1 mL after 40 min and 5 mL after 50 min). After stirring at room temperature for 60 min, the reaction was cooled to -23 °C ($CCl_4$/dry ice bath) and more THF (5 mL) was added followed by chilled ethylene oxide (576 mg, 13.1 mmol) in THF (5 mL). The mixture was stirred at -23 °C for 30 min, at 0 °C for 1.5 h, and at room temperature for 3 h. After cooling again to -23 °C, N,N-bis(2-chloroethyl)phosphoramidic dichloride (5.7 g, 22.4 mmol) in THF (18 mL) was added and the reaction stirred at low temperature for 2 h. After warming to 5 °C (ice bath), the reaction mixture was diluted with THF (10 mL) and $NH_3$ was then bubbled through the mixture for 15 min. The reaction flask was then stoppered and allowed to sit at room temperature overnight before filtration

and concentration at reduced pressure. The residual oil was flash chromatographed (3 cm x 50 cm column; $CH_3OH$ - $CH_2Cl_2$, 1:50) giving 3.13 g of crude product ($R_f$ 0.4 - 0.65, $CH_3OH$ - $CH_2Cl_2$, 1:9). In ca. 1 g portions, this material was then repeatedly (2-3 times) chromatographed under pressure (20 psi) on silica gel (less than 230 mesh, EM Reagents, 3 cm x 50 cm column, $CH_3OH$ - $CH_2Cl_2$, 1:50). Pure 3,4-$^{13}C_2$-**8** ($R_f$ 0.5, $CH_3OH$ - $CH_2Cl_2$, 1:9) was obtained as a colorless oil in 37% yield (1.93 g, 7 mmol) based on the amount of 1,2-$^{13}C_2$-vinylbromide used in the initial reaction. $^1H$ NMR (ppm): 6.9 - 4.6 (doubled m, $^1J_{CH}$ = 151 Hz, 1H, $^{13}CH$=), 5.13 - 4.12 (doubled m, $^1J_{CH}$ = 154 Hz, 2H, $^{13}CH_2$=), 4.17 - 3.90 (m, 2H, $CH_2O$), 3.77 - 3.09 (m, 10 H, $NH_2$ and 2 $NCH_2CH_2$), and 2.4 (apparent q, $J$ = 6.6 Hz, 2H, $CH_2CH_2O$). $^{13}C$ NMR (ppm): 133.8 (d, $^1J_{CC}$ = 69.6 Hz, $^{13}CH$=) and 117.2 (d, $^1J_{CC}$ = 69.6 Hz, $^{13}CH_2$=). $^{31}P$ NMR (ppm): 15.62.

**4-$^{13}$C-4-HYDROPEROXYCYCLOPHOSPHAMIDE (4-$^{13}$C-7).** Ozone was bubbled through a solution of 3,4-$^{13}C_2$-**8** (663 mg, 2.4 mmol) in acetone-water (2:1, 18 mL) at 5 °C (ice bath) for 15 min. The volume of the solution was adjusted to 18 mL with more acetone, aqueous $H_2O_2$ (0.90 mL of a 30% solution) was added, and the reaction flask was then stoppered and kept at room temperature overnight. The reaction mixture was concentrated on a rotary evaporator at ambient temperature and the residual aqueous solution was extracted with $CH_2Cl_2$ (6 x 25 mL). The combined extracts were dried ($MgSO_4$), concentrated at ambient temperature, and the residual material was then dissolved in minimal $Et_2O$ and stored overnight at -20 °C. The crystalline product was separated and the mother liquor was purified by flash chromatography (2 cm x 15 cm column, $CH_3OH$ - $CH_2Cl_2$, 4:96) and the fractions containing desired product ($R_f$ 0.30) were concentrated; the residue was taken up in minimal $Et_2O$ for crystallization at -20 °C. The combined yield of 4-$^{13}$C-**7** as a white microcrystalline solid was 42% (297 mg, 1 mmol). The $^1H$ NMR spectrum was consistant with cis stereochemistry (4, 20, 21). $^1H$ NMR (ppm): 5.15 (doubled, doubled q, $^1J_{CH}$ = 170 Hz, $^3J_{HH}$ = 2 Hz, $^3J_{HP}$ = 26 Hz, 1 H, $^{13}C$-H), 5.10 - 3.90 (m, 3H, OOH and $CH_2O$), 3.80 - 3.20 (m, 8H, 2 $NCH_2CH_2Cl$), and 2.20 - 1.91 (m, 3H, NH and $CH_2CH_2O$). $^{13}C$ NMR (ppm): 86.45 (d, $^2J_{CP}$ = 3.7 Hz). $^{31}P$ NMR (ppm): 9.91 (d, $^2J_{CP}$ = 3.7 Hz).

**2-$^{13}$C-ETHYL 2-(2-HYDROXYETHYLAMINO)ACETATE.** 2-$^{13}$C-Ethyl bromoacetate (2 g, 11.8 mmol, MSD Isotopes, 90 atom % $^{13}$C) was added dropwise to a solution of ethanolamine (2.18 g, 35.7 mmol) in $CHCl_3$ (12 mL). After stirring at room temperature for 20 min, the reaction mixture was concentrated and purified by flash chromatography (5 cm x 15 cm column, $CH_3OH$ - $CHCl_3$, 8:92) giving the product as a colorless oil ($R_f$ 0.35, 1.23 g, 8.3 mmol, 70% yield). $^1$H NMR (ppm): 4.20 (q, $J$ = 7.3 Hz, 2H, $CO_2\underline{CH_2}CH_3$), 3.66 (t, $J$ = 5.9 Hz, 2H, $\underline{CH_2}OH$), 3.43 (d, $^1J_{CH}$ = 136.7 Hz, 2H, $^{13}\underline{CH_2}CO_2CH_2$), 2.80 (t, $J$ = 5.9 Hz, 2H, $\underline{CH_2}CH_2OH$), 2.42 (broad s, 2H, NH and OH), and 1.29 (t, $J$ = 7.3 Hz, 3H, $CH_3$). $^{13}$C NMR (ppm): 50.1.

**1-$^{13}$C-2,2'-IMINODIETHANOL.** 2-$^{13}$C-Ethyl 2-(2-hydroxyethylamino)acetate (1.20 g, 8.1 mmol) in THF (15 mL) was added dropwise to an ice-cooled suspension of $LiAlH_4$ (459 mg, 12.1 mmol) in THF (13 mL). Following complete addition, the reaction mixture was refluxed overnight and then cooled to room temperature. Following the sequential addition of $H_2O$ (0.2 mL), 15% NaOH (0.2 mL), $H_2O$ (0.8 mL), and THF (50 mL), the reaction mixture stirred overnight at room temperature. Filtration followed by concentration gave the product as a colorless oil which was used without further purification (737 mg, 6.9 mmol, 85%). $^1$H NMR (ppm): 3.80 (s, 3H, NH and 2 OH), 3.68 (t, $J$ = 5.9 Hz, 4H, two $CH_2O$), 2.73 (t, $J$ = 5.9 Hz, 2H, $NCH_2$), and 2.73 (doubled t, $^1J_{CH}$ = 131.8 Hz, $^3J_{HH}$ = 5.9 Hz, 2H, $N^{13}CH_2$). $^{13}$C NMR (ppm): 51.1.

**1-$^{13}$C-N,N-BIS(2-CHLOROETHYL)AMINE HYDROCHLORIDE (1-$^{13}$C-10).** A solution of 1-$^{13}$C-2,2'-iminodiethanol (737 mg, 6.9 mmol) and thionyl chloride (4.14 g, 34.8 mmol) in $CHCl_3$ (8 mL) stirred at room temperature overnight and was then refluxed for 4 h. The reaction mixture was concentrated and the residual solid was purified by crystallization using $Et_2O$. Product 1-$^{13}$C-**10** was obtained in 81% yield (1.0 g). $^1$H NMR ($D_2O$) (ppm): 3.82 (t, $J$ = 5.9 Hz, 4H, 2 $CH_2O$), 3.43 (t, $J$ = 5.9 Hz, 2H, $NCH_2$), and 3.43 (doubled t, $^1J_{CH}$ = 147 Hz, $^3J_{HH}$ = 5.9 Hz, 2H, $N^{13}CH_2$).

**3-BUTENYLPHOSPHORYL DICHLORIDE.** $POCl_3$ (3.06 g, 20 mmol) was added dropwise to an ice-cooled solution of 3-buten-1-ol (1.73 mL, 20 mmol) in benzene (20 mL). This was followed by the dropwise addition of pyridine (1.58 g, 20 mmol). The reaction mixture stirred overnight at room temperature and was then filtered and

concentrated. The residue was purified by flash chromatography (4 cm x 15 cm column, $CHCl_3$) and the product was obtained as a colorless oil ($R_f$ 0.70, 2.55 g, 13.5 mmol, 68%). $^1H$ NMR (ppm): 5.05 - 4.56 (m, 1 H, $CH_2$=C<u>H</u>), 4.23 - 4.04 (m, 2H, $CH_2$=), 3.52 - 3.21 (m, 2H, $CH_2O$), 3.20 - 2.40 (m, 2H, $CH_2CH_2O$). $^{31}P$ NMR (ppm): 6.87.

**ALPHA-$^{13}$C-3-BUTENYL N,N-BIS(2-CHLOROETHYL)PHOSPHORODIAMIDATE (ALPHA-$^{13}$C-8).** Pyridine (569 mg, 5.6 mmol) was added dropwise to a suspension of 1-$^{13}$C-**10** (505 mg, 2.8 mmol) in $CH_2Cl_2$ (10 mL) at ice-bath temperature. This was followed (after ca. 5 min) by the dropwise addition of 3-butenylphosphoryl dichloride (1.57 g, 8.3 mmol) in $CH_2Cl_2$ (2 mL). After stirring at room temperature for 4 days, the reaction mixture was filtered, concentrated, and purified by flash chromatography (1.5 cm x 15 cm column, $Et_2O$). Fractions containing the desired intermediate ($R_f$ 0.80) were concentrated at ambient temperature and then immediately diluted with THF (30 mL) and cooled to 5 °C. $NH_3$ was bubbled through this cooled solution for 15 min and the reaction flask was then stoppered and allowed to stand overnight at room temperature. The reaction mixture was filtered, concentrated, and purified by flash chromatography (2 cm x 15 cm column, $CH_3OH$ - $CHCl_3$, 4:96). Alpha-$^{13}$C-**8** was recovered as an oil ($R_f$ 0.30, 602 mg, 2.2 mmol, 78%). $^1H$ NMR (ppm): 6.03 - 5.58 (m, 1 H, $CH_2$=C<u>H</u>), 5.28 - 5.00 (m, 2H, $CH_2$=), 4.04 (doubled, doubled triplet, $J_{HH}$ = 6.8 Hz and 1.2 Hz, $^3J_{HP}$ = 6.8 Hz, 2H, $CH_2O$), 3.77 - 3.30 (m, 6H, $NCH_2$ and 2 $CH_2Cl$), 3.51 (doubled, doubled triplet, $^1J_{CH}$ = 140.6 Hz, $^3J_{HP}$ = 11.5 Hz, $^3J_{HH}$ = 6.8 Hz, 2H, $N^{13}CH_2$), 2.85 (broad s, 2H, $NH_2$), and 2.4 (apparent q, $J$ = 6.6 Hz, 2H, $CH_2CH_2O$). $^{13}C$ NMR (ppm): 49.31 (d, $^2J_{CP}$ = 4.9 Hz). $^{31}P$ NMR (ppm): 15.62 (d, $^2J_{CP}$ = 4.9 Hz).

**ALPHA-$^{13}$C-4-HYDROPEROXYCYCLOPHOSPHAMIDE (ALPHA-$^{13}$C-7).** The title compound was prepared from alpha-$^{13}$C-**8** (594 mg, 2.2 mmol) using the same procedure described above for the synthesis of 4-$^{13}$C-**7**. Alpha-$^{13}$C-**7** was obtained as a white microcrystalline solid in 43% yield (274 mg, 0.93 mmol) with cis stereochemistry (4,20,21). $^1H$ NMR (ppm): 5.15 (doubled q, $J_{HH}$ = 2 Hz, $^3J_{HP}$ = 26 Hz, 1 H, H-COOH), 5.10 - 3.90 (m, 3H, OOH and $CH_2O$), 3.80 - 3.20 (m, 6H, $NCH_2$ and 2 $CH_2Cl$), 3.32 (doubled, doubled triplet, $^1J_{CH}$ = 135 Hz, $^3J_{HH}$ = 6.8 Hz, $^3J_{HP}$ = 12.2 Hz, 2H,

$N^{13}CH_2$), and 2.20 - 1.91 (m, 3H, NH and $\underline{CH}_2CH_2O$). $^{13}C$ NMR (ppm): 48.72 (d, $^2J_{CP}$ = 4.9 Hz). $^{31}P$ NMR (ppm): 9.86 (d, $^2J_{CP}$ = 4.9 Hz).

**ACKNOWLEDGMENT**

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