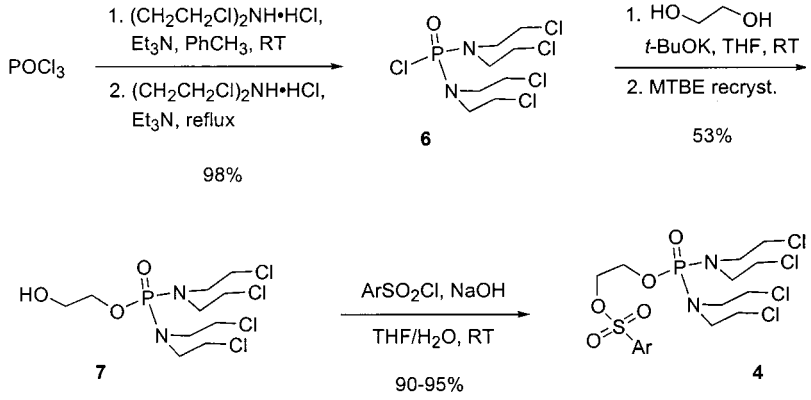
Scheme 3



7 in good yields (47−63%). Unfortunately, some of the product was lost to the aqueous layer during workup due to the partitioning effect of the excess ethylene glycol in the mixed solvent system, and all attempts to recover **7** from the aqueous wash by removal of ethylene glycol were largely unsuccessful. Stoichiometric variations of the reaction conditions showed that fewer equivalents of the diol or greater amounts of base led to decreases in yield due to the formation of greater amounts of inseparable dimeric by-products. However, these by-products were easily removed after one recrystallization from methyl *tert*-butyl ether to provide **7** as a white powder in excellent purity (greater than 98% by HPLC analysis) and with minimal loss of material. Starting with 5.2 kg of the chloride **6**, a 2.9 kg lot of 2-hydroxyethyl *N*,*N*,*N*′,*N*′-tetrakis(2-chloroethyl)phosphorodiamidate (**7**) was prepared by this process in 53% isolated yield.

Due to the unavailability of commercial quantities of the diamidic monochloride **6**,[4] a process was developed for the large-scale preparation of this compound from phosphorus oxychloride and 2 equiv of bis(2-chloroethyl)amine. Several research groups have shown that phosphorus oxychloride may be coupled with 1 equiv of bis(2-chloroethyl)amine to provide bis(2-chloroethyl)phosphoroamidic dichloride, which may be coupled with other amines to provide analogues of **6** in a separate step.[5,6] While many of these preparations involved refluxing a suspension of the mustard chloride in excess phosphorus oxychloride, we found that phosphorus oxychloride could be treated with 1 equiv of bis(2-chloroethyl)amine hydrochloride in toluene in the presence of 2 equiv of triethylamine at room temperature to provide the intermediate dichloride adduct in quantitative yield (Scheme 3). This in situ intermediate was then treated with a second equivalent of the amine and an additional 2 equiv of triethylamine, and the mixture was heated at reflux to provide tetrakis(2-chloroethyl)phosphorodiamidic chloride (**6**) in near-quantitative yields. Filtration of the cooled mixture to remove the insoluble triethylamine hydrochloride salts was usually all that was necessary to produce multikilogram quantities of **6** in excellent purity (greater than 98% by proton NMR analysis), although occasionally a slurry of the mixture with activated charcoal followed by filtration through Celite was useful to decolorize the product. Using 1.0 L of phosphorus oxychloride, a 4.0 kg lot of **6** was prepared by this two-step process in 98% isolated yield.

It is interesting to note that the first amine addition was accompanied by a slow exothermic heating to 30 °C over a 2−3 h period, while the second amine addition required the (endothermic) heating of the mixture to reflux to accomplish reaction completion. When 2 equiv of the amine were added to phosphorus oxychloride at room temperature in toluene, complete conversion to the amidic dichloride intermediate was observed with only a small amount of the diamidic monochloride formed. When 2 equiv of the amine were added to phosphorus oxychloride in toluene and heated at reflux, the product yield was reduced in addition to the formation of significant amounts of unidentified by-products. It should also be mentioned that the chloride **6** was typically not crystallized,[7] but used as an oil for the coupling reaction with ethylene glycol, as described above (Scheme 3). When run in tandem, this process was used for the preparation of multikilogram quantities of the mustard alcohol **7** from phosphorus oxychloride with an overall isolated yield of 52% and with a purity of greater than 98% (by proton NMR and HPLC analyses).

The mustard alcohol **7** was treated with sodium hydroxide and several sulfonyl chlorides in a water/tetrahydrofuran solvent mixture to provide a series of mustard sulfonate derivatives **4** (Scheme 3), which in turn were examined as coupling partners with glutathione **2**. The results of these studies will be presented in a separate article.

Conclusions

An efficient process for the preparation of 2-hydroxyethyl *N*,*N*,*N*′,*N*′-tetrakis(2-chloroethyl)phosphorodiamidate (**7**) from phosphorus oxychloride has been achieved in substantially pure form by a two-step preparation of the intermediate mustard chloride in one pot, followed by the base-catalyzed

(4) This work was performed in 1997. At that time, the availability of **4** from Aldrich (the only vendor of the reagent) was $42.90/5 g at a maximum of 200 g deliverable.

(5) (a) Friedman, O. M.; Seligman, A. M. *J. Am. Chem. Soc*. **1954**, *76*, 655. (b) Radu, V.; Nicoleta, V.; Ion, N. Romanian Patent 84,601 B, 1985; *Chem. Abstr*. **1985**, *103*, 105152. (c) Borch, R. F.; Canute, G. W. *J. Med. Chem*. **1991**, *34*, 3044. (d) McGuigan, C.; Narashiman, P. *Synthesis* **1993**, 311. (e) Wan, H.; Modro, T. A. *Synthesis* **1996**, 1227.

(6) For methods involving the bis-amination of phosphorous trichloride, followed by oxidation to analogues of **4**, see: (a) Mulcahy, R. T.; Gipp, J. J.; Schmidt, J. P.; Joswig, C.; Borch, R. F. *J. Med. Chem*. **1994**, *37*, 1610. (b) Ghosh, A. K.; Farquhar, D. *Tetrahedron Lett*. **1997**, *38*, 8795.

(7) Compound **6** can be isolated as a solid, if necessary, but in this process the crude oil was suitable for use without purification.

reaction with excess ethylene glycol. Following a purification by recrystallization, this process has been carried out to provide 2.9 kg of this key drug substance intermediate in 52% overall isolated yield. The synthetic transformations that were performed to provide multikilogram quantities of the glutathione drug substance **1** will be discussed in a forthcoming publication.

Experimental Section

Reagents and solvents were used as received from vendors, and no attempts were made to purify or dry these components further. Thin-layer chromatography was performed using 1 in. × 3 in. Analtech GF 350 silica gel plates with fluorescent indicator. Visualization of TLC plates was made by observation in iodine vapors. The proton and carbon magnetic resonance spectra were obtained on a Bruker AC 300 MHz nuclear magnetic resonance spectrometer, using tetramethylsilane as an internal reference. Melting points were obtained using an Electrothermal melting point apparatus and are uncorrected. Infrared spectra were obtained as KBr pellets and obtained on a Perkin-Elmer Spectrum 1000 FT-infrared spectrophotometer. CI mass spectroscopic analyses were performed on a Shimadzu QP-5000 GC/mass spectrometer (methane) by direct injection. Thermal analyses were run on a Mettler Toledo DSC821e differential scanning calorimeter. HPLC analysis was performed using a Zorbax RX-SIL 5 μm column (4.6 mm × 250 mm) and a methanol/chloroform (99:1) isocratic mobile phase (flow rate = 1 mL/min) with ELSD detection (drift tube, 45 °C, gas flow 1.6).

Preparation of *N*,*N*,*N*′,*N*′-Tetrakis(2-chloroethyl)phosphorodiamidic Chloride (6). A 72-L, three-neck, round-bottom flask in an electric heating mantle was equipped with an overhead mechanical stirrer, a water-cooled reflux condenser and a 5-L pressure-equalizing addition funnel capped with a nitrogen inlet/outlet bubbler. The flask was charged with phosphorus oxychloride (1.04 mL, 11.2 mol), bis(2-chloroethyl)amine hydrochloride (2.0 kg, 11.2 mol), and toluene (25 L). The stirrer was set to agitate at a moderate rate (to provide a clear solution), and triethylamine (3.28 L, 23.5 mol) was added to the reaction mixture over 10 min. The reaction mixture was then stirred for 26 h at room temperature. To this mixture was charged bis(2-chloroethyl)-amine hydrochloride (2.0 kg, 11.2 mol) and triethylamine (3.3 L, 23.5 mol) over 10 min. The tan suspension was heated to toluene reflux (110 °C) and stirred for 20 h. The mixture was then cooled to room temperature and filtered to remove the triethylamine hydrochloride salt. The solvent was removed under reduced pressure (30 mmHg, final bath temperature at 50 °C) on a rotary evaporator to produce *N*,*N*,*N*′,*N*′-tetrakis(2-chloroethyl)phosphorodiamidic chloride (**6**) as a reddish-brown oil (4.0 kg, 98% yield): TLC R_f = 0.71 (ethyl acetate/hexanes = 1:1). The proton NMR spectrum ($CDCl_3$) was consistent with the proton NMR spectrum of a commercially available batch of this material (Aldrich, lot no. 11608 TZ).

Preparation of 2-Hydroxyethyl N,N,N′,N′-Tetrakis(2-chloroethyl)-phosphorodiamidate (7). A 72-L, three-neck, round-bottom flask was equipped with an overhead mechanical stirrer, a thermometer, and a nitrogen inlet/outlet bubbler. The flask was charged with a solution of *N*,*N*,*N*′,*N*′-tetrakis-(2-chloroethyl)phosphorodiamidic chloride (**6**, 5.18 kg, 14.2 mol) in tetrahydrofuran (17.5 L) and ethylene glycol (7.8 L, 112.9 mol). The stirrer was set to agitate at a moderate rate (to provide a clear red solution), and the mixture was cooled to 2 °C using an ice/water bath. To this cooled solution was added potassium *tert*-butoxide (1.9 kg, 16.9 mol) in portions over 30 min, maintaining the temperature below 8 °C. After the addition was completed, the ice and water were removed, and the reaction mixture was allowed to reach ambient temperature and stirred for a total of 20 h. Hydrochloric acid (36 L, 1.2 M) was introduced into the flask over 10 min. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (15 L). The combined organic layers were washed with saturated aqueous sodium chloride solution (2 × 6 L), and the solvents were removed under reduced pressure (30 mmHg vacuum, final bath temperature at 50 °C) on a rotary evaporator to provide the crude product **7** as a dark reddish-brown oil. This crude organic product was then dissolved with warm (40 °C) methyl *tert*-butyl ether (6 L) and allowed to cool to room temperature, with stirring, for a total of 16 h. The resulting slurry was stirred for 30 min at 0 °C, after which the precipitate was collected by vacuum filtration and washed with cold methyl *tert*-butyl ether (2 L). The solid product was dried overnight (30 mmHg, 25 °C) to produce 2-hydroxyethyl *N*,*N*,*N*′,*N*′-tetrakis-(2-chloroethyl)phosphorodiamidate (**7**) as an off-white powder (2.90 kg, 53% yield): mp 79−81 °C; TLC R_f = 0.50 (methanol/ethyl acetate = 1:20); ^{1}H NMR ($CDCl_3$) δ 4.10−4.20 (m, 2H), 3.80−3.90 (m, 2H), 3.60−3.70 (m, 8H), 3.40−3.50 (m, 8H) ppm; ^{13}C NMR (CD_3OD) δ 68.7, 62.3 (d), 50.6, 43.3 ppm; IR (KBr) 3372, 2957, 1446 cm^{-1}; MS (CI, methane) *m*/*z* 391.07 [$(C_{10}H_{21}Cl_4N_2O_3P + H)^+$]. Anal. Calcd for $C_{10}H_{21}Cl_4N_2O_3P$: C, 30.79; H, 5.43; N, 7.18. Found: C, 30.89; H, 5.65; N, 7.10. A thermal analysis (DSC) showed two exothermic decomposition ranges of 164−179 and 222−231 °C. HPLC analysis chemical purity (t_R = 23.7 min) showed one major peak, with a purity of 99.8%.

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