Angelo Paci, Dominique Guillaume*† and Henri-Philippe Husson

Laboratoire de Chimie thérapeutique, UMR 8638 du CNRS,
Faculté des Sciences Pharmaceutiques et Biologiques, 4 Avenue de l'Observatoire, 75006 Paris cedex 06, France
Received April 9, 2001

Three analogs of the cytostatic drug ifosfamide incorporating 1-methyl-2-chloroethyl side chains were designed and prepared as an attempt to obtain drugs of lower toxicity.

*J. Heterocyclic Chem.*, **38**, 1131 (2001).

Ifosfamide (**1a**, 2-(2-chloroethylamino)-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide) is a structural isomer of cyclophosphamide (**1b**, 2-[bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide) differing only in the position of the alkylating groups (Figure).

Both compounds are efficient antitumor agents employed as a racemic mixture in the chemotherapy of several tumors [1]. They also share a similar enzyme-catalyzed activation process necessary for the therapeutic activity [2]. This process begins by the formation of 4-hydroxylated derivatives (**2**) and ultimately ends with the release of an isophosphoramide mustard (**3**) that is the true alkylating species (Figure). Concomitantly, a competitive deactivation pathway occurs by oxidation of the chloroethyl groups and leads to the liberation of chloroacetaldehyde (**4**, Figure). This latter is suspected of being responsible of the observed neurotoxicity, urotoxicity and cardiotoxicity of the oxazaphosphorines [3].

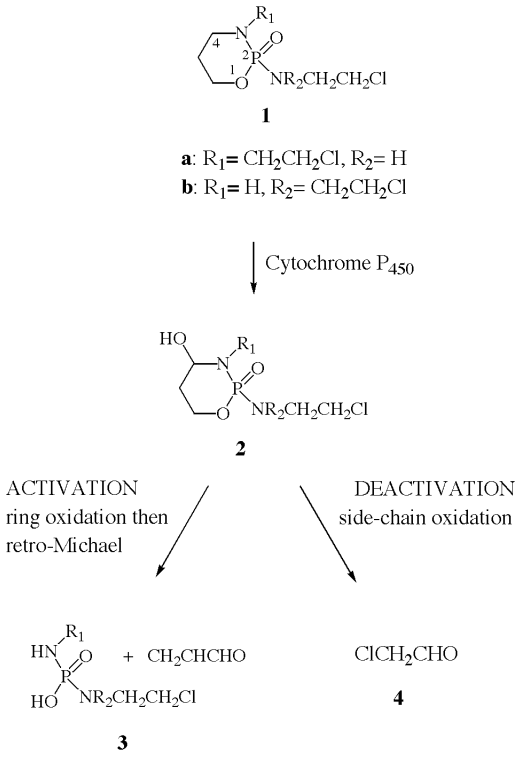



Figure. Schematic representation of the oxidative activation and deactivation pathways of **1**.

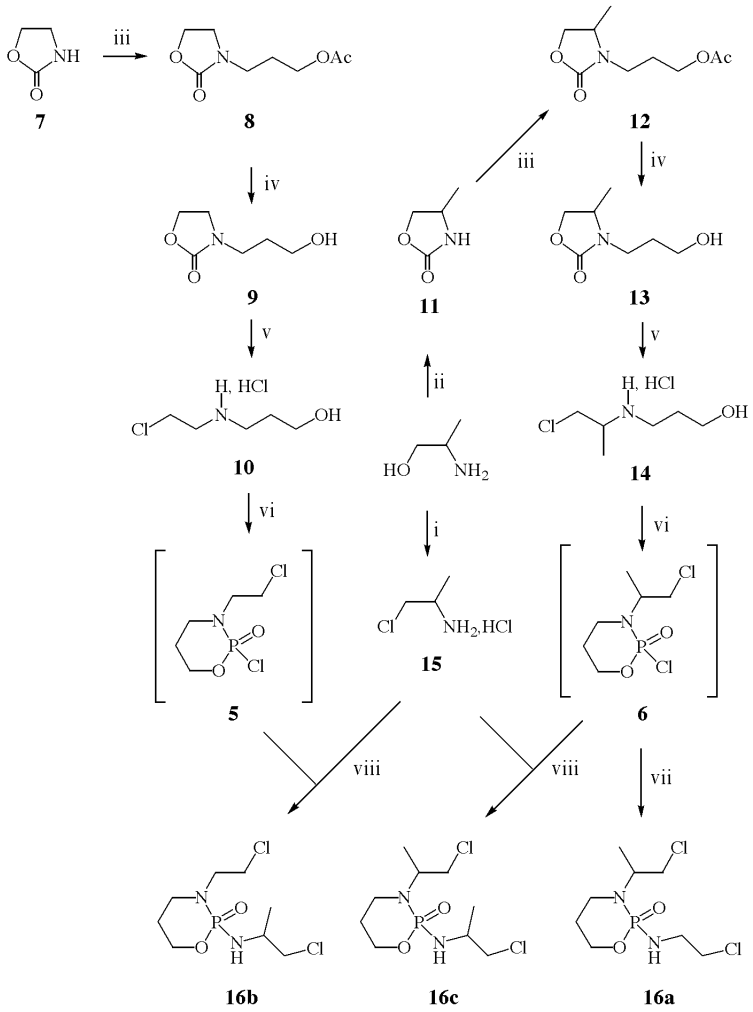
Furthermore, it has also been observed that the deactivation route has a faster kinetic in the case of **1a** compared to **1b**, hence inducing higher blood levels of **4**. The release of **4** in the blood stream is a serious drawback particularly in the case of high-dose treatment and generally prevents the use of ifosfamide that is however intrinsically a more efficient alkylating drug than cyclophosphamide.

Though the reasons for this difference in term of side chain metabolism are still unclear, we postulated that it could be due to an easier access by the oxidative enzymatic core to the 1-position of the 2-chloroethyl side chains of **1a**. Indeed, the two chloroethyl chains of **1b** substituting the same nitrogen atom, the bulky bis(2-chloroethyl)amino group is probably less accessible to the enzyme. Consequently we reasoned that substitution of this sensitive 1-position by a methyl group would induce some hindrance that could reduce or even eliminate the possibility of oxidation with minimum perturbation of the alkylating properties. We did not envisage a substitution of the 2-position or a 1,2-disubstitution since, in the cyclophosphamide series, these kinds of derivatives have been shown to be poorly therapeutically efficient [4]. Thus, we designed three compounds (**16a-c**) in which a single or both 2-chloroethyl residues of **1a** have been replaced by a 1-methyl-2-chloroethyl chain. We herein wish to report the synthesis of these three compounds.

Synthesis of 2-alkylamino-3-substitued-2*H*-1,3,2-oxazaphosphorine 2-oxide can generally be easily accomplished according two routes. Either a 2-chloro-3-substituted-2*H*-1,3,2-oxazaphosphorine 2-oxide, prepared by condensing an aminoalcohol with $POCl_3$, reacts with an amine [5,6] or $POCl_3$ is first stoichiometrically condensed with an amine and the resulting species is cyclized using an appropriate aminoalcohol [7].

The synthesis of **16a-c** was performed following an identical strategy that follows the first route (Scheme). In both cases a suitable chloro-amino-alcohol was condensed with $POCl_3$ to afford the corresponding 2-chloro-3-substituted-2*H*-1,3,2-oxazaphosphorine 2-oxide that was subsequently condensed under basic conditions with the selected chloroalkylamine hydrochloride to afford the desired ifosfamide analog.

Scheme




Reagents, conditions and yields: i: $SOCl_2$, 75%; ii: DIC, Ph-Me, reflux, 74%; iii: NaH, DMF, $Br(CH_2)_3OAc$, 120 °C, 80%; iv: $N_2H_4$, 95%; v: HCl gas, 70%; vi: $POCl_3$, $CH_2Cl_2$, $Et_3N$; vii: $NH_2(CH_2)_2Cl$, HCl, $Et_3N$, reflux, 60%; viii: $Et_3N$, reflux, 35% (**16c**) 52% (**16b**).

Preparation of the 2-Chloro-3-substituted-2*H*-1,3,2-oxazaphosphorine 2-oxide Moities **5** and **6**.

We first decided to prepare **5** whose N-3 atom is substituted with a chloroethyl side chain. Reaction of 2-oxazolidinone **7** with 3-bromopropylacetate in anhydrous DMF at 120 °C and in the presence of NaH afforded *N*-substituted 2-oxazolidinone **8** in 80% yield. Deacetylation of this latter, performed in almost quantitative yield by use of aqueous hydrazine was followed by the opening of the oxazolidinone (HCl gas) furnishing **10** in 70% yield. Cyclization of **10** into **5** (65% yield) was accomplished in refluxing dichloromethane by use of $POCl_3$ in the presence of 3 equivalents of $Et_3N$ (Scheme).

Synthesis of **6** whose N-3 atom is substituted with a 1-methyl-2-chloroethyl side chain was performed by applying a strictly similar protocole to oxazolidinone **11** that was prepared in 74% yield by refluxing racemic 2-aminopropanol and *N*-*N*'-carbonyldiimidazole (DIC) in toluene [8]. Compound **6** and its synthetic intermediates were obtained in yields almost identical to those obtained for the preparation of **5**.

Preparation of Diastereomers **16**.

Although compounds **5** and **6** are relatively stable, we observed that derivatives **16** were obtained in much better yields when the synthetic procedure was carried out

rapidly and without isolation of the 2-chloro-oxazaphosphorine 2-oxide **5** and **6**. Consequently, and as reported in the phosphotriamide series [9], we currently synthesized compounds **16** using a one pot procedure.

Synthesis of **16a** was realized in 60% yield by directly adding to a refluxing solution of **6** in 2-chloroethylamine and 2 equivalents of $Et_3N$.

Synthesis of **16b,c** required the preparation of 1-methyl-2-chloroethylamine hydrochoride **15**. This was achieved in 75% yield by treatment of (±)-2-aminopropanol with $SOCl_2$ [10]. Condensation of **15** with **5** or **6**, in the conditions depicted for the preparation of **16a** led to the isolation of **16b** (52%) and **16c** (35%), respectively.

## Conclusion.

We have been able to prepare three ifosfamide analogs designed to prevent the enzymatic oxidation of the side chains and hence the release of chloroacetaldehyde, a highly toxic metabolite. Although, chirality of the phosphorus atom is not a critical factor for the therapeutic use, chirality on the side chains has still to be evaluated. In case of importance, our synthetic strategy can easily be applied, on a large scale, in asymmetric series using commercially available optically pure 2-aminopropanol. Biological data and importance of the side-chain chirality will be the subject of a forthcoming communication.

## EXPERIMENTAL

IR spectra were recorded on a Perkin Elmer FTIR-1600 spectrometer. NMR spectra were obtained with a Bruker AC-300 instrument using $CD_3OD$ as a solvent unless otherwise indicated. $^1H$ and $^{13}C$ chemical shifts are reported from residual non-deuteriated solvent traces. $^{31}P$-NMR spectra were calibrated using phosphoric acid as external reference. Coupling constants (*J*) are given in Hz. Chromatographic separations were carried out on a silicagel column (Merck; 230-400 mesh). TLC were developped on Merck $60F_{254}$ plates and revealed by heating after spraying a 5% ethanolic solution of phosphomolybdic acid. Ionization of the samples for mass analysis was performed by electronic impact or chemical ionization using $NH_3$ as vector. Some synthetic intermediates are potentially carcinogenic and consequently should be handled with care.

Synthesis of 3-(2-Oxo-1,3-oxazolidin-3-yl)-propylacetate (**8**) and 3-(4-Methyl-2-oxo-1,3-oxazolidin-3-yl)-propylacetate (**12**).

A solution of oxazolidinone (**7** or **11** [6]) (190 and 220 mg respectively, 2.2 mmol) in 4 mL of DMF was cooled at 0 °C. Sodium hydride (1.25 equivalent) was carefully added and the solution allowed to warm up to room temperature then heated to 50 °C for 1 hour. Then 3-bromopropylacetate (1.5 equivalent) was added and the solution was refluxed for 2 hours. The organic solution was cooled at room temperature then slowly poured in 1.5 mL of a saturated aqueous ammonium chloride. The crude mixture was concentrated under reduced pressure and compounds **8** or **12** were flash chromatographed using dichloromethane/methanol (19:1) as an eluent.

Compound **8** was obtained as an oil; yield 82%; IR (neat) 2930, 1732, 1430, 1244 $cm^{-1}$; $^1H$-NMR ($CDCl_3$): δ 1.95 (m, 2H), 2.12 (s, 3H), 3.46 (t, *J*= 7, 2H), 3.71 (m, 2H), 4.18 (t, *J*=6, 2H), 4.40 (m, 2H) ; $^{13}C$-NMR ($CDCl_3$): δ 20.8, 27.3, 42.1, 45.6, 62.9, 63.6, 160.8, 172.6 ; MS *m/z* (rel. int., %) 205 ($C_8H_{13}NO_4+NH_4^+$, 100), 188 ($C_8H_{13}NO_4+H^+$, 23).

Compound **12** was obtined as an oil; yield 80%; IR (neat) 2930, 1738, 1732 $cm^{-1}$ ; $^1H$-NMR: δ 1.23 (d, *J*= 6, 3H), 1.94 (m, 2H), 2.01 (s, 3H), 3.14 (m, 1H), 3.45 (m, 1H), 3.82 (m, 2H), 4.05 (t, *J*=6, 2H), 4.35 (t, *J*= 6.5, 1H); $^{13}C$-NMR: δ 17.9, 20.8, 26.3, 38.6, 51.0, 61.6, 68.7, 157.9, 170.8 ; MS *m/z* (rel. int., %) 219 ($C_9H_{15}NO_4+NH_4^+$, 100), 202 ($C_9H_{13}NO_4+H^+$, 46).

Synthesis of 3-(2-Oxo-1,3-oxazolidin-3-yl)-propan-1-ol (**9**) and 3-(4-Methyl-2-oxo-1,3-oxazolidin-3-yl)-propan-1-ol (**13**).

To a solution of **8** or **12** (346 and 372 mg respectively, 1.85 mmol) in 5 mL of methanol was added at room temperature 1.1 equivalent of hydrazine hydrate. The solution was heated at 50 °C for 4 hours and 0.2 equivalent of hydrazine hydrate were re-added. The solution was allowed to return to room temperature and stirred for 2 hours. The solution was concentrated under reduced pressure and the residue flash chromatographed using dichloromethane/methanol (98/1) as an eluent. Compounds **9** and **13** were obtained in 95% yield and used for the next step without further characterization.

Synthesis of 3-(2-Chloroethylamino)-propan-1-ol (**10**) and 3-(2-Chloro-1-methylethylamino)-propan-1-ol (**14**).

A solution of oxazolidinone **9** or **13** (1 and 1.1 g respectively, 6.89 mmol) in 30 mL of THF was placed under a stream of HCl at room temperature for 30 minutes. The solution was refluxed for 4 hours then stirred at room temperature overnight. The solution was concentrated under reduced pressure and the residue flash chromatographed using dichloromethane/methanol (9/2) as an eluent.

Compound **10** was obtained as a powder; yield 73%; IR (neat) 3380, 1634, 1435 $cm^{-1}$; $^1H$-NMR: δ 2.02 (m, 2H), 3.25 (t, *J*=7, 2H), 3.51 (t, *J*=7, 2H), 3.73 (t, *J*=7, 2H), 4.02 (t, *J*=7, 2H); $^{13}C$-NMR 29.5, 40.4, 47.7, 50.3, 60.8; MS *m/z* (rel. int., %) 138 ($C_5H_{12}NOCl+H^+$, 100), 140 ($C_5H_{11}NOCl+H^+$, 31).

Compound **14** was obtained as a powder; yield 70%; IR (neat) 3400, 1634, 1432 $cm^{-1}$; $^1H$-NMR: δ 1.53 (d, *J*= 6.5, 3H), 2.02 (m, 2H), 3.25 (m, 2H), 3.75 (m, 3H), 3.84 (m, 2H), 4.06 (t, *J*=7, 2H); $^{13}C$-NMR: δ 14.3, 29.5, 44.9, 45.9, 55.8, 60.5; MS *m/z* (rel. int., %) 152 ($C_6H_{14}NOCl+H^+$, 100), 154 ($C_8H_{13}NO_4+H^+$, 39).

Synthesis of 2-(2-Chloroethylamino)-3-(1-methyl-2-chloroethyl)-tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide (**16a**), 2-(1-Methyl-2-chloroethylamino)-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide (**16b**), and 2-(1-Methyl-2-chloroethylamino)-3-(1-methyl-2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide (**16c**).

To a solution of **10** or **14** (226 and 244 mg respectively, 1.3 mmol) in dichloromethane (100 mL), $Et_3N$ (1 equivalent) was added. Then, phosphorus oxychloride (1 equivalent) was slowly and carefully added and the solution was cooled in case of excessive heating. Triethylamine (2 equivalents) was further added. The solution was stirred for 2 hours then the hydrochloride salt of the suitable chloroethylamine was added together with two more equivalents of $Et_3N$. The solution was refluxed for 6 hours. The solvent was then evaporated and the crude

product was chromatographed using dichloromethane/methanol (98:2) as an eluent. Compounds **16a**, **16b** and **16c** were obtained in pure form in 60%, 52%, and 35% yield, respectively.

Compound **16a** was obtained as a thick oil; IR (neat) 3216, 1455, 1265 $cm^{-1}$ ; $^1$H-NMR (mixture of diastereomers): δ 1.32 (6H), 1.95 (m, 4H), 3.15-3.31 (m, 8H), 3.55 (m, 4H), 3.65 (m, 4H), 3.76 (m, 1H), 3.92 (m, 1H), 4.21 (m, 2H), 4.35 (m, 2H); $^{31}$P-NMR (mixture of diastereomers): δ 13.6, 14.4 ; MS *m/z* (rel. int., %) 292 ($C_8H_{17}N_2O_2PCl_2+NH_4^+$, 100), 294 ($C_8H_{17}N_2O_2PCl_2+NH_4^+$, 38), 275 ($C_8H_{17}N_2O_2PCl_2+H^+$, 42), 277 ($C_8H_{17}N_2O_2PCl_2+H^+$, 12).

*Anal*. Calcd for $C_8H_{17}N_2O_2PCl_2 \cdot 3H_2O$: C, 29.19; H, 7.04; N, 8.51. Found: C, 29.23; H, 7.14; N, 8.67.

Compound **16b** was obtained as a thick oil; IR (neat) 3210, 1455, 1265 $cm^{-1}$; $^1$H-NMR (mixture of diastereomers): δ 1.32 (6H), 1.95 (m, 2H), 2.15 (m, 2H), 3.10-3.35 (m, 6H), 3.40 (m, 2H), 3.55-3.75 (m, 10H), 4.25 (m, 2H), 4.35 (m, 2H); $^{31}$P-NMR (mixture of diastereomers): δ 13.6, 14.4 ; MS *m/z* (rel. int., %) 292 ($C_8H_{17}N_2O_2PCl_2+NH_4^+$, 100), 294 ($C_8H_{17}N_2O_2PCl_2+NH_4^+$, 40), 296 ($C_8H_{17}N_2O_2PCl_2+NH_4^+$, 4), 275 ($C_8H_{17}N_2O_2PCl_2+H^+$, 23), 277 ($C_8H_{17}N_2O_2PCl_2+H^+$, 12), 279 ($C_8H_{17}N_2O_2PCl_2$, 8).

*Anal*. Calcd for $C_8H_{17}N_2O_2PCl_2 \cdot 2H_2O$: C, 30.88; H, 6.80; N, 9.00. Found: C, 30.61; H, 6.97; N, 9.01.

Compound **16c** was obtained as a thick oil; IR (neat) 3216, 1455, 1265 $cm^{-1}$; $^1$H-NMR: complex mixture of diastereomers; $^{31}$P-NMR (mixture of diastereomers) 10.6, 11.4, 13.6 ; MS *m/z* (rel. int., %) 306 ($C_9H_{19}N_2O_2PCl_2+NH_4^+$, 100), 308 ($C_9H_{19}N_2O_2PCl_2+NH_4^+$, 52), 310 ($C_9H_{19}N_2O_2PCl_2+NH_4^+$, 8), 289 ($C_9H_{19}N_2O_2PCl_2+H^+$, 23), 291 ($C_9H_{19}N_2O_2PCl_2+H^+$, 9), 293 ($C_9H_{19}N_2O_2PCl_2$, 2).

*Anal*. Calcd for $C_9H_{19}N_2O_2PCl_2 \cdot 4H_2O$: C, 29.93; H, 7.53; N, 7.76. Found: C, 30.11; H, 7.39; N, 7.91.

## REFERENCES AND NOTES

[†*] Permanent address: Laboratoire de Chimie Thérapeutique, 1 rue des Louvels, 80037 Amiens cedex 1, France. dominique.guillaume@sa.u-picardie.fr.

[1] D. L. Hill, A Review of Cycloposphamide, C. C. Thomas: Springfield, Il, 1975; H. Burket and H. C. Voigt, Proceedings of the International Holoxan Symposium, Düsseldorf, March 21-23, 1977.

[2] N. E. Sladek, *Pharmacol. Ther.*, **37**, 301 (1988); G. P. Kaijser, J. H. Beijnen, A. Bult and W. J. M. Underberg, *Anticancer Res.*, **14**, 517 (1994).

[3] M. P. Goren, R. K. Wright, C. B. Pratt and F. E. Pell, *Lancet II*, 1219 (1986); J. Pohl, J. Stekar and P. Hilgard, *Arzneim. Forsch.*, **39**, 704 (1989); C. Joqueviel, M. Malet-Martino and R. Martino, *Cell. Mol. Biol.*, **43**, 773 (1997).

[4] H. Arnold, F. Bourseaux and N. Brock, *Arzneim.-Forsch.*, **11**, 143 (1961).

[5] K. Misuiura, A. Okruszek, K. Pankiewcz, W. J. Stec, Z. Czownicki and B. Utracka, *J. Med. Chem.*, **26**, 674 (1983).

[6] B. Kutscher, U. Niemeyer, J. Engel, A. Kleeman, P. Hilgard, J. Pohl and G. Scheffler, *Arzneim.-Forsch.*, **45**, 323 (1995).

[7] S. M. Ludeman, D. L. Bartlett and G. Zon, *J. Org. Chem.*, **44**, 1163 (1979).

[8] J. R. Piper, C. R. Stringfellow Jr, R. D. Elliott and T. P. Johnston, *J. Med. Chem.*, **14**, 345 (1971).

[9] D. Guillaume and G. R. Marshall, *Synthetic Commun.*, **28**, 2903 (1998).

[10] K. Kashiwabara, I. Kinoshita, T. Ito and J. Fujita, *Bull. Chem. Soc. Jpn.*, **54**, 725 (1981).