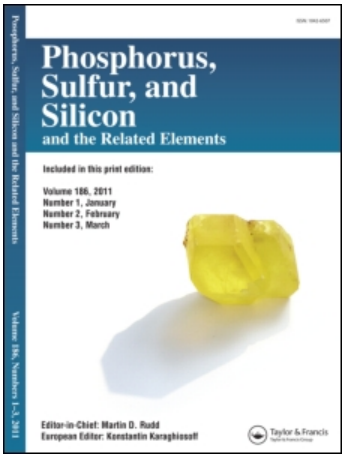


Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Hydrolytic Pathway of Glufosfamide, a New Phosphorylated Anticancer Agent

Marie-Caroline Aoust de Rouvèze[a]; Veronique Gilard[a]; Robert Martino[a]; Myriam Malet-Martino[a]; Ulf Niemeyer[b]

[a] Biomedical NMR Group, IMRCP Laboratory, Université Paul Sabatier, Toulouse, France [b] ASTA Medica AG, Franckfurt am Main, Germany

Online publication date: 27 October 2010

To cite this Article de Rouvèze, Marie-Caroline Aoust , Gilard, Veronique , Martino, Robert , Malet-Martino, Myriam and Niemeyer, Ulf(2002) 'Hydrolytic Pathway of Glufosfamide, a New Phosphorylated Anticancer Agent', Phosphorus, Sulfur, and Silicon and the Related Elements, 177: 6, 1735 — 1738

To link to this Article: DOI: 10.1080/10426500212229

URL: http://dx.doi.org/10.1080/10426500212229

Phosphorus, Sulfur and Silicon, 2002, Vol. 177:1735–1738

1042-6507/02 $12.00 + .00
DOI: 10.1080/10426500290093333

HYDROLYTIC PATHWAY OF GLUFOSFAMIDE, A NEW PHOSPHORYLATED ANTICANCER AGENT

Marie-Caroline Aoust de Rouvèze,[a] *Veronique Gilard,*[a] *Robert Martino,*[a] *Myriam Malet-Martino,*[a] *and Ulf Niemeyer*[b]
Biomedical NMR Group, IMRCP Laboratory, Université Paul Sabatier, 118, route de Narbonne, 31062 Toulouse, France[a] *and ASTA Medica AG, Franckfurt am Main, Germany*[b]

^{31}P NMR was used to demonstrate that glufosfamide is hydrolyzed into isophosphoramide mustard (IPM) with a half-life of about 25 h at 37°C and pH 7.4 in buffer as well as in human plasma. Through a cascade of reactions, IPM gives rise to three final products, dihydroxyIPM, phosphate ion, and phosphorylethanolamine, which is by far the major compound.

Keywords: Glufosfamide; hydrolysis; isophosphoramide mustard; ^{31}P NMR

INTRODUCTION

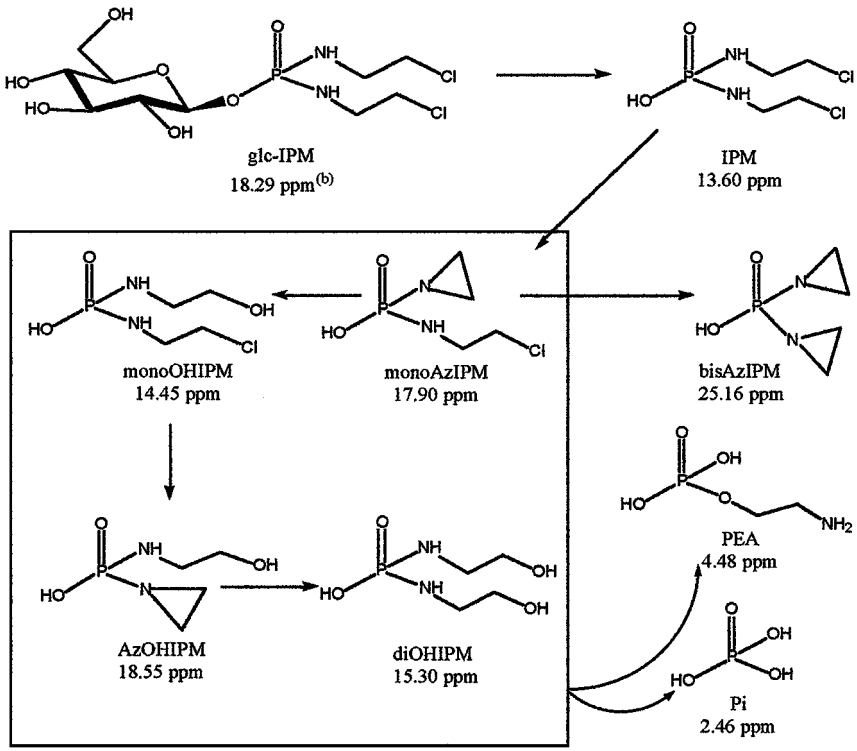
The anticancer drug glufosfamide (β-D-glucosylisophosphoramide mustard, glc-IPM) entered a clinical phase II trial in 1999. In this drug, IPM is coupled to the C1 of D-glucose in a β-glycosidic bond. Inside the cells, glc-IPM is mainly cleaved by glucosidases into D-glucose and IPM, but nonenzymatic hydrolysis could also contribute to its decay.[1] In this study, we investigated the time course of glc-IPM hydrolysis in buffered solutions at pH 7.4 and in human urine and plasma. Since the first stage of hydrolysis gives IPM, the chemical stability and the fate of IPM was also determined in the same conditions.

RESULTS AND DISCUSSION

^{31}P NMR monitoring of the glc-IPM hydrolysis in cacodylate buffer at pH 7.4 shows that this compound ($\delta = 18.29$ ppm) gives IPM

Address correspondence to R. Martino. E-mail: martino@chimie.ups-tlse.fn

($\delta = 13.60$ ppm) as the first intermediate, which is transformed into the monoaziridinyl derivative of IPM (monoAzIPM) ($\delta = 17.90$ ppm) (Figure 1 and Scheme 1). This is in accordance with the fact that IPM gives also monoAzIPM at the same pH (data not shown). The rates of degradation of glc-IPM were ≈12–25-fold slower than those of IPM, depending on the temperature and medium (Table I). Up to now, the chemical stability of glc-IPM in any aqueous medium has not been reported. This study shows that its half-life ($t_{1/2}$) at 37°C was ≈1 day at pH 7.4 in buffer and plasma, slightly shorter (≈0.8 day) at pH 6 in urine, and slightly longer (≈1.2 day) at pH 8 in deproteinized plasma. The $t_{1/2}$ of IPM (Table I) were in agreement with the literature data (350 min at pH 7.0 and 25°C and in the range 49–84 min at pH 7.0–7.4 at 37°C).[2,3]



SCHEME 1 Hydrolytic pathway of glc-IPM at pH 7.4.[a]

[a]All the compounds are represented in neutral form. Structures were characterized from one- and two-dimensional ^{1}H, ^{13}C, and ^{31}P NMR experiments.

[b]δ are given at pH 7.4 in cacodylate buffer and 37°C. They are expressed in ppm relative to 85% H_3PO_4 as external standard.

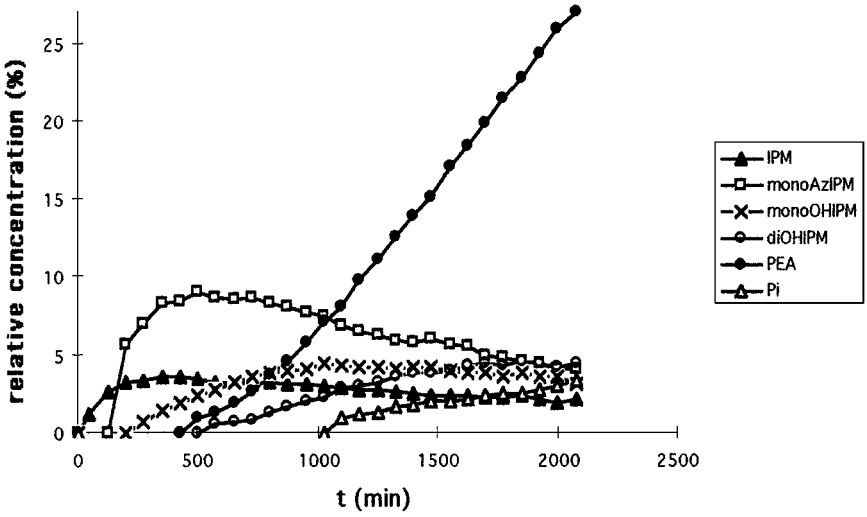


FIGURE 1 ^{31}P NMR time course of the main hydrolytic products of glc-IPM at pH 7.4 (cacodylate buffer) and 37°C.

On the basis of the formation in time and the temporal patterns of the degradation compounds of glc-IPM at pH 7.4, its hydrolytic pathway can be summarized as illustrated in Scheme 1. IPM, monoAzIPM, and monohydroxyIPM (monoOHIPM) are intermediates. DihydroxyIPM (diOHIPM), phosphorylethanolamine (PEA), and phosphate ion (Pi) are the final products, PEA being the major one (Figure 1).

Identical hydrolytic profiles are observed for glc-IPM and IPM in urine and plasma; however, in plasma, additional ^{31}P signals were

TABLE I ^{31}P NMR-Derived Kinetic Data for Hydrolysis of glc-IPM and IPM

Conditions	Glc-IPM			IPM	
	T	(n)[a]	$t_{1/2}$[a]	(n)[a]	$t_{1/2}$[a]
Cacodylate buffer 7.4	25°C	(3)	127 ± 5 h	(3)	373 ± 33 min
	37°C	(4)	25 ± 3 h	(5)	65 ± 12 min
Urine pH 6.0[b]	37°C	(5)	20 ± 3 h	(6)	98 ± 14 min
Plasma pH 7.4[b]	37°C	(4)	24.5 ± 1 h	(5)	107 ± 16 min
Deproteinized plasma pH 8.3[b]	37°C	(1)	30 h		

[a](n): number of experiments; $t_{1/2}$: half-life.

[b]Initial pH can increase or decrease by 1–1.5 pH units, depending on the temperature, concentration of the solution, and duration of the experiments.

detected in the resonance area of IPM at 13.93 ppm (broad), 14.57 ppm, and 13.25 ppm. These δ suggest that these compounds correspond very probably to adducts of a nucleophile present in plasma with IPM via its aziridinyl derivatives.

In conclusion, this study demonstrated that glc-IPM is nonenzymatically hydrolyzed into IPM and determined the successive steps of its hydrolysis.

REFERENCES

[1] H. Seker, B. Bertram, A. Burkle, B. Kaina, J. Pohl, H. Koepsell, and M. Wiebler, *Br. J. Cancer*, **82,** 629 (2000).

[2] H. A. A. M. Dirven, L. Megens, M. J. Houdshoorn, M. A. Digemanse, B. van Ommen, and P. J. van Bladeren, *Chem. Res. Toxicol.*, **8**, 979 (1995).

[3] J. H. Boal, M. Williamson, V. L. Boyd, S. M. Ludeman, and W. Egan, *J. Med. Chem.*, **32**, 1768 (1989).