Tetrahedron Letters, Vol.31, No.6, pp 887-890, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

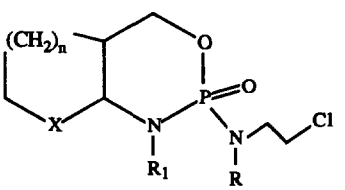
SYNTHESIS AND CONFIGURATIONAL ASSIGNMENT OF BICYCLIC "PREACTIVATED"[1] ANALOGUES OF CYCLOPHOSPHAMIDE

B. Lilo, M. Moreau and D. Bouchu*
E.S.C.I.L., Université Claude Bernard , Laboratoire de Synthèse Organique Appliquée
43, bd du 11 Novembre 1918, 69622 Villeurbanne, France.

Summary : *The synthesis of a new class of bicyclic "preactivated" analogues of Cyclophosphamide, 3-[bis(2-chloroethyl)amino]-2-aza-3-phospha-4,10-dioxabicyclo(4.4.0)decane 3-oxide is described. The configurational assignment of the stereoisomers was made by ^{31}P and ^{1}H NMR.*

Studies on the metabolism of Cyclophosphamide (CPA)[2], one of the most widely used anticancer agents[3], have shown that 4-hydroxycyclophosphamide (4-hydroxy-CPA) is a major metabolite in the process which leads to the liberation of the ultimate cytostatic agent "phosphoramide mustard" in tumor cells[4]. Unfortunately, hydroxycyclophosphamide itself is very unstable[5] and many attempts have been made to synthesize more stable derivatives[6]. One of the most successful was accomplished by the Asta Gruppe which introduced Mafosfamide (4-sulfoethylthio-cyclophosphamide)[7] as a stable derivative of 4-hydroxy-CPA (this was clearly less toxic than CPA with respect to the bone marrow, the immune system and the urinary tract)[8]. However the application of this stabilized "activated" cyclophosphamide is limited by severe local toxicity[9] due to its rapid decomposition.

We have undertaken the synthesis of bicyclic "preactivated" analogues of cyclophosphamide of the type :

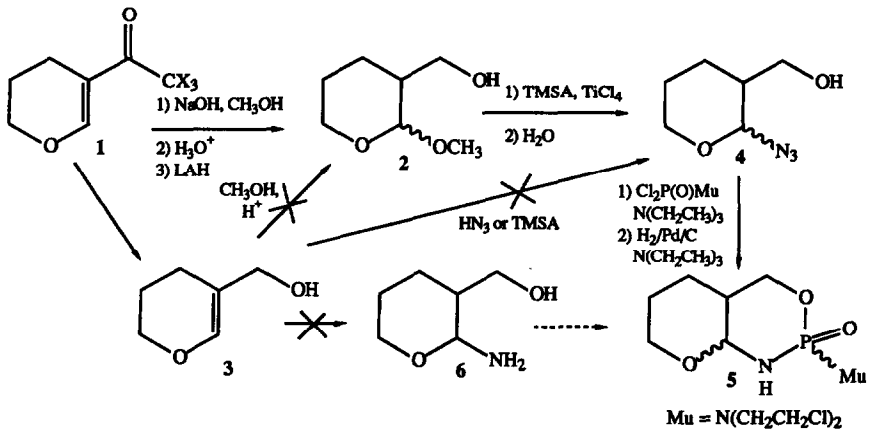


X = O, S
R = H, CH_2CH_2Cl
R_1 = H, CH_2CH_2Cl
n = 1, 2

Compounds of this kind are "preactivated" analogues of CPA since the hydrolysis of the aminal function can lead to an intermediate which has the potentiality to decompose to "phosphoramide mustard". Because of the bicyclic structure of these compounds we can expect a different behaviour as compared to the known monocyclic "preactivated" analogues of CPA. The cis or trans fusion of the rings, the configuration at the phosphorus atom, the size of the ring adjacent to the oxazaphosphorinane cycle, the anomeric effect on the hydrolysis are many factors which may affect the pharmacokinetics and the biological availability of the drug. Furthermore, the presence of an hydroxyl or a thiol group, which could react intramolecularely with either the half-aminal function of the intermediate formed after hydrolysis or with the α,β-unsaturated system of the substituted acrolein after a β-elimination, is another important factor which might influence the biological properties of these analogues.

We describe here the synthesis of three diastereoisomers of 3-[bis(2-chloroethyl)amino]-2-aza-3-phospha-4,10-dioxabicyclo(4.4.0)decane 3-oxide **5**. The scheme below outlines the pathway we have used to obtain the target molecules.

The preparation of the aminoalcohol precursor, 2-amino-3-hydroxymethyltetrahydropyran **6** proved to be not straightforward. After several attempts were made to obtain this relatively unstable compound[10] directly as the



starting material, we decided to use the azido group as a masked amine function. The 3,4-dihydro-5-hydroxymethyl-*2H*-pyran **3**, obtained from 3,4-dihydro-*2H*-pyran-5-carboxylic acid[11] by lithium aluminium hydride reduction, gave neither the methoxyalcohol **2** nor the azidoalcohol **4** by either acid catalysed addition of methanol or hydrazoic acid, respectively[12].

Alternatively, 3,4-dihydro-5-trichloroacetyl-*2H*-pyran[11] **1** was converted to 2-methoxy-3-hydroxymethyl-tetrahydropyran **2** (cis : 80%, trans : 20%)[13] by reaction with methanolic sodium hydroxide (yield 70%). The reaction proceeds probably by a Michael type addition of methanol followed by the second step of the haloform reaction. The reaction of **2** with azidotrimethylsilane (1 eq.) at room temperature (4 h) in the presence of a catalytic amount of titanium tetrachloride followed by the workup (addition of sodium bicarbonate to pH = 4, stirring 30 mn, neutralisation at pH = 7) led to the 2-azido-3-hydroxymethyltetrahydropyran **4** with a 65% overall yield (cis : 60%, trans : 40%). This reaction is an adaptation of the acid catalysed substitution of methoxy groups by azide in linear acetals or ketals[14,15]. It was not possible to separate the cis and trans isomers at this stage.

The condensation of the cis-trans mixture of **4** with the bis(2-chloroethyl)aminophosphoryl dichloride in the presence of one equivalent of triethylamine, isolation of the phosphorylated azide, and the subsequent reduction of the azido group by hydrogenation over palladium on charcoal in the presence of another equivalent of triethylamine, led to a mixture of four stereoisomers of **5** with a 60% yield[16]. Three of these isomers were separated by high performance liquid chromatography[17] as oils.

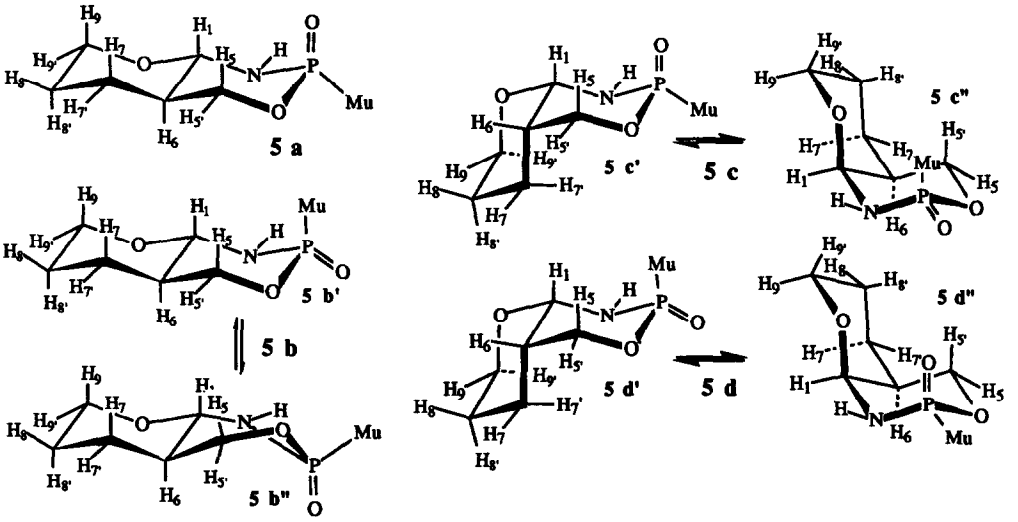
The configurational and conformational analysis were derived from the first order analysis of the ^1H NMR spectra obtained at high field (300 MHz) and confirmed by ^{31}P chemical shifts. Selected NMR parameters are listed in the table below.

SELECTED NMR PARAMETERS OF ISOMERS **5a, 5c, 5d** (δ* in ppm and J in Hz)											
Isomer	δ ^{31}P	δ H1	δ H5	δ H5'	δ H6	J H1-P	J H5-P	J H5'-P	J H1-H6	J H5-H6	J H5'-H6
5a	*12.8*	4.43	4.08	4.0	1.85	< 2	3.3	24.0	8.6	11.5	4.0
5c	*7.8*	4.91	4.25	4.33	2.24	9.4	11	13.5	3.3	4.2	7.7
5d**	*8.6*	4.6	3.95	4.87	2.2	25.4	22.1	3.0	< 2	4.5	11

*$CDCl_3$ ** J_{H1-H5} = 1.4 Hz

The cis-trans relationship of the rings was firmly established by comparison of the $^3J_{H1-H6}$ values : 8,6 Hz for **5a**, 3.3 Hz for **5c** and < 2 Hz for **5d**.

The structure of the compound **5a** was assigned on the basis of the large value of $J_{H5'-P}$ (24.0 Hz) and the small values of J_{H5-P} and J_{H1-P} (3.3 and < 2 Hz respectively) combined with J_{H5-H6} = 11.5 Hz and $J_{H5'-H6}$ = 4.0 Hz. These $^3J_{H-P}$ coupling constants are consistent with an oxazaphosphorinane ring existing exclusively in a chair conformation[18, 19]. The known preference of the bis-(2-chloroethyl)amino group for the equatorial orientation in bicyclic oxazaphosphorinane 2-one[18, 19] further supports the structural assignment given to **5a** with the P=O bond being axially oriented (scheme).



The configurational assignment of the cis isomers is not as easily deduced as in the trans case. Indeed, the cis fusion of the bicyclic system allows conformational mobility and the compounds **5c** and **5d** can experience chair-chair equilibrium in such a way that the bis-(2-chloroethyl)amino group can be in an equatorial prefered orientation (**5c'**, **5d"**). We have here to take into account the other exocyclic substituents (relative to the oxazaphosphorinane ring), namely alkoxy and alkyl parts of the tetrahydropyran ring. A decisive feature in the structural assignment of one compound was the "W" ^4J coupling constant (1.4 Hz) between H_1 and H_5 (or $H_{5'}$). In this case we assign **5d"** conformation, where the preference of the bis-(2-chloroethyl)amino group is preserved. Additionnal proof is provided by the $J_{H5'-P}$ (3.0 Hz), J_{H5-P} (25.4 Hz) and J_{H1-P} (22.1 Hz) values combined with J_{H5-H6} = 11.5 Hz and $J_{H5'-H6}$ = 4.0 Hz indicating of a conformationaly biased compound. **5c** experiences conformational mobility between **5c'** and **5c"** (the $^3J_{H-P}$ and $^3J_{H-H}$ are population-weighted averaged values) due to the fact that their is a balance between the prefered orientations of the substituents around the oxazaphosphorinane ring. Further conclusive arguments could be found in the ^1H and ^{31}P chemical shifts values. For example, we notice only the $\delta H_{5'}$ = 4.87 ppm value in the case of **5d"**, which denotes the well known deshielding effect of the P=O bond, and the expected order of ^{31}P chemical shifts ($\delta\,^{31}P$ = 8.6 ppm for **5d** > $\delta\,^{31}P$ = 7.8 ppm for **5c**)[18-20] in accordance with the prefered conformation of **5d** and the equilibrium between two chair conformations for **5c**.

References and notes

1) Both terms, "activated" or "preactivated", refer to compounds which do not need liver biological oxidation and are active *in vitro*.

2) For a review : a) D. L. Hill, *A Review of Cyclophosphamide*, 1975, E. Thomas, Springfield; b) O. M. Friedman, A. Myles and M. Colvin, *Adv. Cancer Chemother.*,1979, **1**, 143 ; c) W. J. Stec, *Organophosphorus Chem.*, 1982, **13**, 145 ; d) G. Zon, *Prog. Med. Chem.*, 1982, **19**, 205.

3) M. Colvin and J. Hilton, *Cancer Treat. Rep.*, 1981, **65**, 89.

4) a) H. J. Hohorst, U. Draeger, G. Peter and G. Voelcker , *Cancer Treat. Rep.*, 1976, **60**, 309 ; b) N. Brock and H. J. Hohorst, *Z. Krebsforsch.*, 1977, **88**, 185 ; c) R. F. Borch, T. R. Hoye and T. A. Swanson, *J. Med. Chem.*, 1984, **27**, 490 ; d) G. Zon, S. Ludeman, J. A. Brandt, V. L. Boyd, G. Okkau, W. Egan and K-I. Shao, *J. Med. Chem.*, 1984, **27**, 466 ; f) P. Hilgard and N. Brock, *Invest. New Drugs*, 1984, **2**, 131 ; g) R. F. Borch and J. A. Millard, *J. Med. Chem.*, 1987, **30**, 427.

5) A. Takamizawa, S. Matsumoto, T. Iwata, K. Katagari, Y. Tochino and K. Yamaguchi, *J. Am. Chem. Soc.*, 1973, **95**, 985.

6) a) T. A. Connors, P. J. Cox, P. B. Farber, A. B. Foster and M. Jarman, *Biochem. Pharmacol.*, 1974, **23**, 115 ; b) A. Takamizawa, S. Matsumoto, T. Iwata, Y. Tochino, K. Katagari, K. Yamaguchi and O. Shiratori, *J. Med. Chem.*, 1975, **18**, 376; c) G. Peter, T. Wagner and H.-J. Hohorst, *Cancer Treat. Rep.*, 1976, **60**, 429 ; d) G. Peter and H.-J. Hohorst, *Cancer Chemother. Pharmacol.*, 1979, **3**, 181 ; e) U. Niemeyer, G. Scheffler and G. Nonnenmacher, *Arzneim.-Forsch./Drug Res.*, 1982, **32**, 478.

7) a) U. Niemeyer, J. Engel, G. Scheffler, K. Molke, D. Sauerbier, W. Weigert, *Invest. New Drugs*, 1984, **2**, 133 ; b) U. Niemeyer, J. Engel, G. Scheffler, J. Pohl, G. Hägele and B. Krebs, *Phosphorus and Sulfur*, 1987, **30**, 585 ; c) C.-H. Kwon, R. F. Borch, J. Engel and U. Niemeyer, *J. Med. Chem.*, 1987, **30**, 395.

8) J. Pohl, P. Hilgard, W. Jahn and H.-J. Zechel, *Invest. New Drugs*, 1984, **2**, 201.

9) J. D. Roberts, M. P. Hacker, R. A. Newman, J. J. McCormack and I. H. Krakoff, *Invest. New Drugs*, 1984, **2**, 215.

10) Half-aminal in tetrahydropyran series are usually very unstable. a) C. Glacet, *Bull. Soc. Chim. France*, 1954, 575 ; b) H. S. Isbell and H. L. Frush, *J. Org. Chem.*, 1958, **23**, 1309.

11) M. Hojo, R. Masuda, S. Sakaguchi and M. Takagawa, *Synthesis*, 1986, 1016.

12) The main product was in fact 3-(3-hydroxypropyl)butenal resulting from acid catalysed dehydration.

13) Percentages obtained by integration of the ^1H NMR signals of H_2 proton. This compound can also be prepared by methoxymercuration-demercuration of **3**.

14) a) S. Kirchmeyer, A. Mertens and G. A. Olah, *Synthesis*, 1983, 500 ; b) R. H. Moriarty, K-C Hou and R. S. Miller, *Synthesis*, 1984, 683.

15) It is noteworthy that this reaction cleanly gave the exocyclic alkoxy substitution's product contrary to cyanotrimethylsilane which gives, in this case, also the ring opened compound. Full experimental details will be published on the use of TMSA in the synthesis of heterocyclic α-alkoxy azides.

16) It is possible to obtain **5** from **4** by reduction of the azido group in the presence of $Cl_2P(O)Mu$ and two equivalents of triethylamine, but in this case the yield is much lower (20%).

17) Lichrosorb Si 60, chloroform/methanol (97 : 3). **5a** was eluted first, then **5d** and **5c**. These compounds are stable for at least six months at 10°C. We detected the fourth stereoisomer **5b** only by ^{31}P NMR (δ = 8.1 ppm). It isomerizes quickly into **5d**.

18) H. Halimi, *Thesis Lyon*, 1981.

19) J.-C Yang, D. O. Shah, N. U. M. Rao, W. A. Freeman, G. Sosnovsky and D. G. Gorenstein, *Tetrahedron*, 1988, **44**, 6305.

20) W. G. Bentrude, W. N. Setzer, A. E. Sopchik, G. S. Bajwa, D. D. Burright and J. P. Hutchinson, *J. Am. Chem. Soc.*, 1986, **108**, 6669.

(Received in France 30 October 1989)