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New Strategy for the Diastereoselective Synthesis of Bicyclic "Pre-activated" Analogues of Cyclophosphamide[1]

P. Maynard-Faure, C. Gonser,[†] V. Vaime[†] and D. Bouchu*[#]

Université Claude Bernard LYON I, CPE LYON, Laboratoire de Chimiométrie et Synthèse Organique
43, bd du 11 novembre 1918, 69622 Villeurbanne, France

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Abstract: The diastereoselective synthesis of "pre-activated" analogues of cyclophosphamide in the 3-[bis(2-chloroethyl)amino]-2-aza-4,9-dioxa-3-phosphabicyclo(4.3.0)nonane 3-oxide series is described, using either the phosphorylation of an azidoalcohol followed by a reductive cyclisation or the phosphorylation of an acetal alcohol followed by an unprecedent direct Lewis acid catalyzed intramolecular substitution. © 1998 Elsevier Science Ltd. All rights reserved.

Cyclophosphamide, an extensively used anticancer and immunosuppressive agent, is itself a prodrug that is activated by the hepatic cytochrome P450 system.[2] The resulting 4-hydroxycyclophosphamide is in equilibrium with the opened form aldophosphamide which leads to the cytotoxic phosphoramide mustard and acrolein by β-elimination. Acrolein, toxic to cultured tumor cells,[3] does not play a significant role in the anticancer activity of cyclophosphamide but is responsible for various side effects[4] which are dose limiting. For this reason, many attemps have been made to trap acrolein or avoid its production during clinical treatment.[5] We have previously described[6] the synthesis of analogues in the 3-[bis(2-chloroethyl)amino]-2-aza-4,10-dioxa-3-phosphabicyclo(4.4.0)decane 3-oxide series, designed to perform an intramolecular trapping of the α,β-unsaturated carbonyl function formed after hydrolysis of the aminal function and β-elimination of the phosphoramide mustard. Encouraging preliminary tests[7] on P388 leukemia in mice prompted us to continue developing more convenient synthetic pathways.

We describe herein the diastereoselective synthesis of epimeric cyclophosphamide analogues in the 3-[bis(2-chloroethyl)amino]-2-aza-4,9-dioxa-3-phosphabicyclo(4.3.0)nonane 3-oxide series. Taking into account the synthetic methods used (*vide infra*), we expected a limited number of diastereomers for this skeleton with the juxtaposition of a six membered oxazaphosphorinane ring and a tetrahydrofuran ring. The first synthetic method described is an optimization of previously reported route developed in this group. The second approach is entirely new.

The treatment of the 2,3-dihydro-4-trichloroacetylfuran[8] **1** by methanol in the presence of potassium carbonate according to conditions employed by Tietze et al.[9] leads to the expected methyl (2-methoxytetrahydro-3-furan)carboxylate **2**.[10] This acetal ester **2** is reduced in a conventional way by lithium

* Fax (33) (0)4 72 44 83 19; E-mail: denis.bouchu@univ-lyon1.fr

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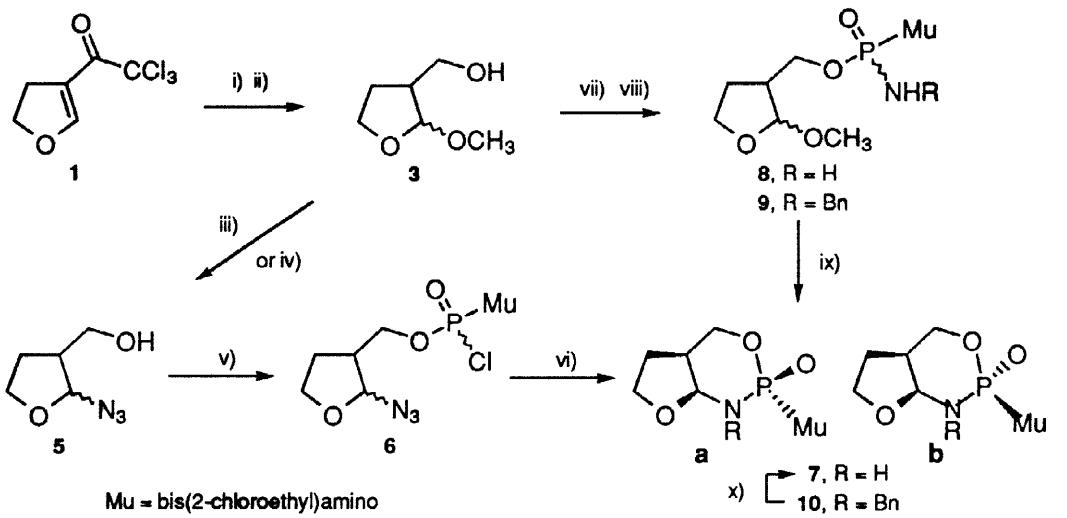
aluminium hydride into (2-methoxytetrahydro-3-furanyl)methanol[11] **3** with a yield of 68%. This pivotal alcohol **3** is isolated in the form of two diastereomers *cis* (12 to 25 %) and *trans* (88 to 75 %),[12] inseparable by chromatography on silica gel. The mixture can be transformed into the (2-azidotetrahydro-3-furanyl)methanol[13] **5** in a one pot procedure: titanium tetrachloride catalyzed reaction with azidotrimethylsilane (1.1 eq.) at room temperature (4 h) followed by workup (hydrolysis of the trimethylsilyl ether at pH 4 for 30 mn) (60% yield). A better procedure involves the successive protection of the hydroxyl group (TMS or TBDMS), trimethylsilyl triflate catalyzed nucleophilic substitution of the exocyclic acetal methoxy group with azidotrimethysilane (at 0°C for **4a** and -60°C for **4b**) followed by deprotection with tetrabutylammonium fluoride in tetrahydrofuran (80% yield). The condensation of **5** with bis(2-chloroethyl)aminophosphoryl dichloride (1.5 eq.) in the presence of ethyldiisopropylamine (1 eq.) and DMAP (0.2 eq.) resulted in the isolation of the phosphorylated intermediate **6** (δ ^{31}P = 15.97 ppm). Reductive cyclisation of **6** (H_2/Pd/C) in the presence of ethyl-diisopropylamine (1eq.) led to the epimeric 3-[bis(2-chloroethyl)amino]-2-aza-4,9-dioxa-3-phosphabicyclo-(4.3.0)nonane 3-oxides **7a** (76%, δ ^{31}P = 8.02 ppm) and **7b** (24%, δ ^{31}P = 11.14 ppm) with 50% overall yield.



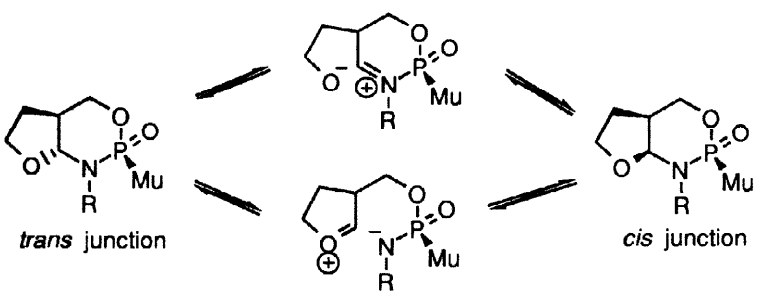
Scheme 1

i) CH_3OH, K_2CO_3; ii) $LiAlH_4$; iii) (a) TMSA, $TiCl_4$, $CHCl_3$; (b) H_2O; iv) (a) $TMSN_3$ or TBDMSCl, imidazole, DMF; (b) $TMSN_3$, TMSOTf, CH_2Cl_2, (c) TBAF, THF; v) $Cl_2P(O)Mu$, $(iPr)_2NEt$, DMAP; vi) H_2, Pd/C, $(iPr)_2NEt$; vii) $Cl_2P(O)Mu$, $(iPr)_2NEt$, DMAP or (a) nBuLi, THF; (b) $Cl_2P(O)Mu$; viii) NH_3 or $BnNH_2$; ix) TMSOTf, $CHCl_3$; x) H_2, Pd/C.

Alternatively the phosphorylation of the alcohol **3**, under the above conditions or by reaction of its lithium alcoholate with bis(2-chloroethyl)aminophosphoryl dichloride, followed by aminolysis (ammonia or benzylamine respectively) led to the phosphorodiamidates **8** (δ ^{31}P = 16.24 and 16.40 ppm) and **9** (δ ^{31}P = 15.72 and 15.55 ppm). These analogues of aldophosphamide are neither separable by thin layer, flash chromatography on silica gel, nor by HPLC.

It is well reported that it is possible to substitute the exocyclic alkoxy group of a hemicyclic acetal by various nucleophiles with a Lewis acid catalyst.[6,14] Although we did not find, to the best of our knowledge, phosphoramidates among these nucleophiles, Vorbrüggen glycosylation[15] showed that it is possible to substitute an exocyclic acyloxy or alkoxy group in anomeric position by a silylated base in these conditions. Recent work of Lipshutz et al.[16] concerning the stereoselective intramolecular N-glycosylation starting from a methyl glycoside prompted us to try the direct cyclization starting from the phosphorodiamidates **8** and **9**. Actually, when the reaction was attempted as in the work cited above, this reaction in dichloromethane failed. Replacement of the latter by chloroform as the solvent led cleanly *via* the catalyzed TMSOTf cyclisation[17] to the bicyclic compounds **7** (**7a**: 67%, **7b**: 33%) and **10** (**10a**: 23%, δ ^{31}P = 11.35 ppm and **10b**: 77%, δ ^{31}P = 13.40 ppm) with 41% and 70% yield respectively. The subsequent debenzylation of **10** led to **7**. In each case only two of the four possible diastereomers were formed and isolated after HPLC.

It is noteworthy that the two synthetic procedures lead to a mixture with a *cis* ring junction[18] regardeless of the configuration of the starting material. Several mechanisms can be visualized to give this result. One can invoke either an epimerisation at the level of the hemi-aminal before the cyclisation in the case of the reductive cyclisation, or the stereoselective attack of the oxocarbenium intermediate ion in the case of the Lewis acid catalyzed cyclisation. But it seems also reasonable to consider yet another mechanism, which can operate after cyclisation (scheme below), reminescent of that proposed by Alanine et al.[19] for the epimerisation of spirocyclic aminals. Indeed we have already noted the stereochemical instability of this stereocenter in the 3-[bis(2-chloroethyl)amino]-2-aza-4,10-dioxa-3-phosphabicyclo(4.4.0)decane 3-oxide series[6] and in other series.[20]



Scheme 2

Furthermore, we noted a slower epimerisation at the phosphorus atom (monitoring by ^{31}P NMR in $CDCl_3$). This phenomenon, for which we cannot yet propose a clear mechanism, was already mentioned by others[21] for "preactivated" analogues of cyclophosphamide.[22]

In conclusion, we have demonstrated that new convenient synthetic pathways toward "preactivated" analogues of cyclophosphamide are available, using a reductive cyclisation or a direct intramolecular substitution on an acetal function. Further work is in progress in our laboratory to find new applications of these strategies.

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10. The reaction probably takes place in two successive steps (1,4 addition of the methanolate, then substitution of the trichloromethyl group) both having very similar kinetics since one can sometimes isolate a trace amount of methyl (2,3-dihydro-4-furan)carboxylate. This results from the substitution of the trichloromethyl group, at this stage the insufficiently activated double bond cannot undergo further 1,4 addition.
11. This alcohol was previously prepared starting from γ-butyrolactone: Vader J.; Koopmans R.; De Groot A.; Van Veldhuizen A. and Van der Kerk S. *Tetrahedron*, **1988**, 44, 2663-2674.
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13. IR ν_{-N_3} = 2105 cm^{-1}; ^{1}H NMR : *cis* ($\delta_{H\text{-}2'}$ = 5.55 ppm, $J_{2,3}$ = 5.37 Hz) and *trans* ($\delta_{H\text{-}2'}$ = 5.42 ppm, $J_{2,3}$ = 1.68 Hz).
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17. *Typical procedure for the cyclisation*: In a Schlenk reaction vessel placed under argon, the solution of 0.5 g (3.78 mmol) of **9** in 5 mL of anhydrous chloroform containing 4 Å molecular sieves is agitated at -78°C. TMSOTf (58 µL, 0.3 mmol) is added over a period of 5 mn. The reaction mixture is allowed to return to room temperature and further agitated during three hours. 4 mL of buffer solution (pH 7) is then added. After the workup the stereomers **10a** and **10b** (70% yield) are separated by HPLC on Macherey-Nagel silica gel column with chloroform/methanol (97/3) as eluant. It is noted that the same cyclisation reaction is observed when the phosphorodiamidates are placed in "old" chloroform during two weeks.
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22. Apart from the fact that there is a lack of appreciable stereochemical discrimination between the enantiomers of cyclophosphamide during initial hepatic microsomal activation,[23] these two conjugate epimerisations could explain why no significant difference in activity was noted between the enantiomers of cyclophosphamide.[23,24] In light of our results we could indeed expect that the enzymatic activation (hydroxylation of the carbon α to the nitrogen atom of the oxazaphosphorinane ring) would create a stereochemical instability of the resulting molecule.[25]
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25. The work of Tsui F.-P; Brandt J. A. and Zon G.[23] rules out the possibilty of racemisation of cyclophosphamide during *in vitro* liver microsomal metabolism but not of the 4-hydroxycyclophosphamide formed thereafter.