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Synthesis of potential metabolites of (*S*)-(—)-bromofosfamide

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Received September 22, 2003, accepted December 10, 2003

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Pharmazie 59: 668–672 (2004)

(*S*)-(−)-Bromofosfamide, a newly obtained anticancer agent, recently became a subject of phase I clinical trials in Poland. With the aim to study its metabolism in humans using phosphorus nuclear magnetic resonance a group of potential metabolites of this agent was synthesized.

1. Introduction

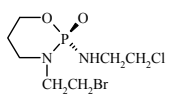
(*S*)-(−)-Bromofosfamide [(*S*)-(−)-3-(2-bromoethyl)-*N*-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine-2-amine 2-oxide, SBF] is an enantiomerically pure bromo analogue of ifosfamide (IF), an akylating anticancer drug introduced into clinical practice in the 1980s and particularly useful in the treatment of soft tissue sarcomas and a variety of pediatric tumours (O'Byrne and Steward 1999; Advani 1998).

SBF was selected from a group of newly obtained oxazaphosphorines possessing bromine atom(s) and synthesized in both racemic and enantiomeric forms. Studies on the synthesis of SBF (Misiura et al. 2001), its crystal and molecular structure (Karolak-Wojcichowska et al. 1999), HPLC chromatographic properties (Bielajewska et al. 1999), microsomal *in vitro* metabolism (Hladon et al. 1997), and stereoselective pharmacokinetics (Sloderbach et al. 1997; Kobylinska et al. 2001a) along with activity against many experimental tumours in mice (Misiura et al. 2001) were a continuation of our early studies on ifosfamide analogues possessing modified 2-chloroethylamino moieties responsible for bis-alkylation (cross-linking) of target DNA (Misiura et al. 1988). Recently SBF became a subject of phase I clinical trials in several hospitals in Poland. Its pharmacokinetics and toxicity in lung cancer patients have been established (Kobylinska et al. 2001b).

It is assumed that metabolism of SBF in humans is similar to that evaluated for ifosfamide (Boddy and Yule 2000; Kerbusch et al. 2001) (Scheme 1).

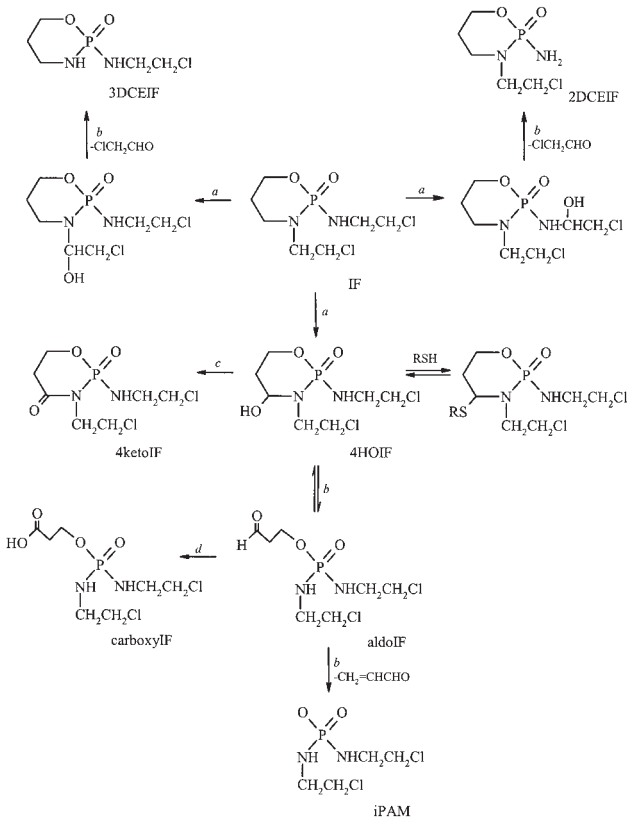
Ifosfamide is a pro-drug activated *in vivo* by cytochrome P450 mediated hydroxylation on the C-4 atom of the tetrahydro-2*H*-1,3,2-oxazaphosphorine ring. The 4-hydroxyifosfamide (4HOIF) obtained is in tautomeric equilibrium with aldoifosfamide (aldoIF), which spontaneously releases isophosphoroamide mustard (iPAM), a final DNA bis-alkylating metabolite. At the same time 4HOIF and aldoIF are oxidized into 4-ketoifosfamide (4ketoIF) and carboxyifosfamide (carboxyIF), respectively. Both metabolites are inactive and excreted in urine. Hydroxylation of C-1 atoms of 2-chloroethyl chains leads to unstable hydroxy intermediates, which spontaneously collapse into 2- and 3-dechloroethylated metabolites 2DCEIF and 3DCEIF and chloracetaldehyde.

In our laboratory a new method to study ifosfamide metabolism in humans was established employing phosphorus

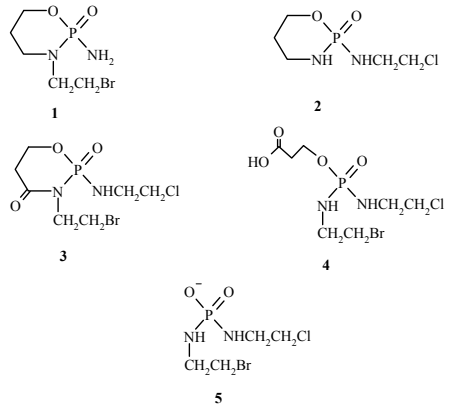


SBF

Scheme 1



(a) cytochrome P45O (b) spontaneous
(c) mechanism unknown (d) aldehyde dehydrogenase



nuclear magnetic resonance (^{31}P NMR) (Misiura et al. 1983) which was very recently optimized and used for the analysis of the metabolism of this drug in children suffering from cancer (Misiura et al. 2003). With the aim to examine the metabolism (*S*)-(−)-bromofosfamide in humans using ^{31}P NMR we started with the synthesis of potential metabolites of this experimental drug.

2. Investigations, results and discussion

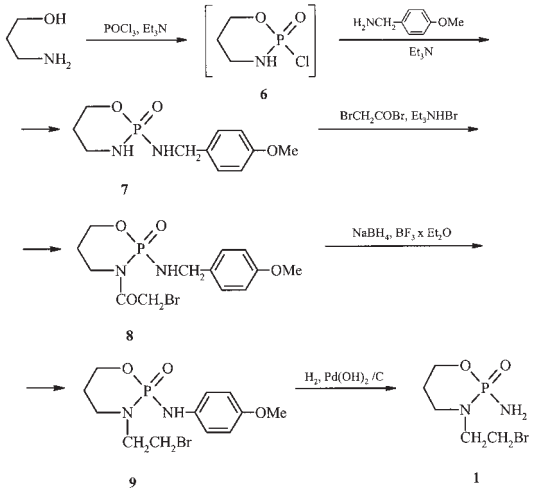
By analogy to the well-established metabolism of ifosfamide (Boddy and Yule 2000; Kerbusch et al. 2001), it was presumed that major metabolites of (*S*)-(−)-bromofosfamide would consist of compounds **1**–**5.**

Since compounds **1**–**5** were needed as standards for ^{31}P NMR and stereochemical analysis in the presence of chiral shift reagents designed to performed in a same way as previously done for ifosfamide (Misiura et al. 1983), the chiral metabolites **1**–**4** were obtained as racemate.

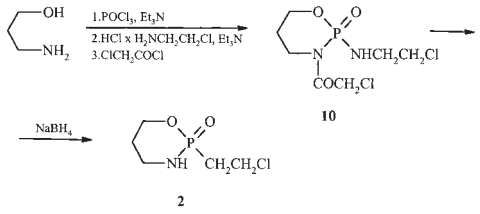
The synthesis of the potential metabolite **1**, which is a bromo analogue of 2DCEIF, is depicted in Scheme 2.

Starting from 3-aminopropanol the chloroamidophosphate **6** was obtained which without isolation reacted with *p*-methoxybenzyl amine in the presence of triethylamine to give the bisamide **7**. Compound **7** was acylated with bromoacetyl bromide in the presence of triethylamine hydrobromide to yield compound **8**. The reduction of the carbonyl group in **8** to a methylene one was performed using sodium borohydride in the presence of boron trifluoride etherate (Misiura et al. 2001). The protected bisamide **9**

Scheme 2



Scheme 3



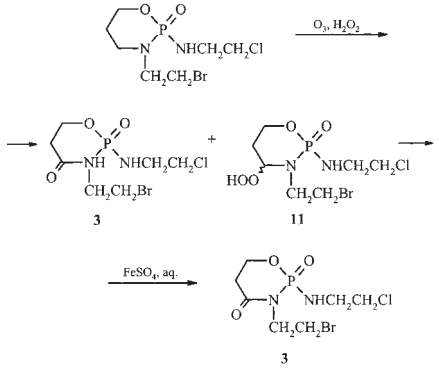
obtained was subjected to hydrogenolysis. Only palladium hydroxide on carbon proved to be effective to give **1**. Other palladium catalysts commonly used for debenzylation resulted in large amounts of side-products probably coming out from the reduction of the C–Br bond. Hydrogen bromide formed in this reaction could catalyze subsequent hydrolysis of P–N bonds. The total yield of **1** was 8%.

Synthesis of compound **2**, which is 3DCEIF, was performed earlier by Takamizawa (Takamizawa et al. 1977). However, repeating this method proved that the yield of **2** is low (<10%) and the desired product, even after careful silica gel purification and crystallization, was contaminated with triethylamine hydrochloride. So a new method for the synthesis of **2** was elaborated which is depicted in Scheme 3.

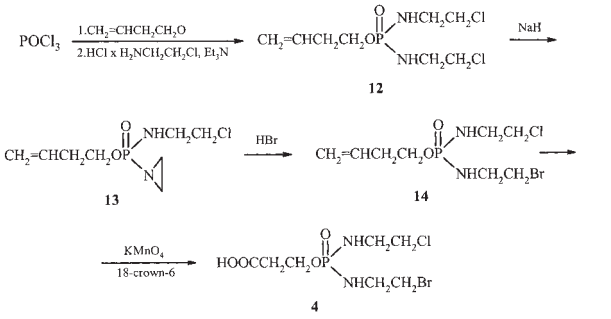
Starting from 3-aminopropanol, in a reaction with phosphorus oxychloride in the presence of triethylamine, the chloroamidophosphate **6** was obtained which reacted without isolation with 2-chlorethylamine hydrochloride and then with chloroacetyl chloride to give compound **10**. All these three steps were performed in a one-pot mode. This process is also a key step in the industrial production of ifosfamide (Stec et al. 1990). Compound **10** was easy to purify by chromatography on silica gel and the product obtained was free from any impurities. The chloroacetyl group was removed from **10** by reduction with sodium borohydride. The most probable mechanism of this reaction is the reduction of the carbonyl group into a hydroxyl group and subsequent spontaneous elimination of chloracetaldehyde similarly to the ifosfamide metabolism. The total yield of **2** was 37%.

First attempts to synthesize compound **3** were performed using Fenton reagent ($FeSO_4/H_2O_2$) for the oxidation of bromofosfamide as previously done for cyclophosphamide (Misiura et al. 1981). The yields of **3** were very low (1–3%). It was also found that bromofosfamide is resistant against oxidation with *t*-butyl peroxide, 3-chloroperbenzoic acid, and selenium dioxide. Treatment of bromofosfamide with an aqueous solution of potassium permanganate under neutral and acidic conditions yielded **3** in low yields

Scheme 4



Scheme 5



of 10–15% (^{31}P NMR assay). However, ozonolysis of bromofosfamide (Misiura et al. 2002) in a presence of hydrogen peroxide gave a mixture of **3** and **11** (ca. 1 : 1 ratio, ^{31}P NMR and TLC assays) in quantitative yield (Scheme 4).

To avoid separation of **3** and **11** the latter compound was *in situ* reduced to **3** by treatment with iron(II) sulfate. The crude product obtained was crystallized from a mixture of chloroform and *n*-hexane to give **3** in 38% yield.

The synthesis of compound **4**, which is a bromo analogue of carboxyIF, is depicted in Scheme 5.

By condensing phosphorus oxychloride with buten-3-ol-1 and subsequently with 2-chloroethylamine, compound **12** was obtained. One chloride atom in **12** was exchanged into bromine by sodium hydride promoted cyclization to the aziridinyl compound **13** and ring opening in the presence of hydrogen bromide. The bisamide **14** was oxidized by means of potassium permanganate/18-crown-6 ether (Mukaiyama et al. 1983) to give crude compound **4** in low yield. Attempts to purify **4** using silica gel chromatography failed probably due to chemical instability of **4**.

Synthesis of compound **5**, which is a bromo analogue of iPAM, was performed by a method similar to that earlier used for the synthesis of isophosphoroamide mustard (Misiura et al. 1983) (Scheme 6).

Dichloro bisamide **15**, monoaziridate **16**, and its chlorobromo bisamide **17** were obtained by methods similar to the synthesis of compound **4**. The key intermediate **17** was subjected to hydrogenolysis in a presence of palladium catalyst and when the removal of the benzyl group was completed (TLC assay) the reaction mixture was concentrated to give **5** in a total yield of 9%.

Since it is important to prove that the metabolism of (*S*)-(−)-bromofosfamide is stereospecific and no racemization process take place we used racemic bromofosfamide and obtained chiral metabolites **1**–**4** to establish conditions for analysis of the enantiomeric composition by means of ^{31}P

Scheme 6

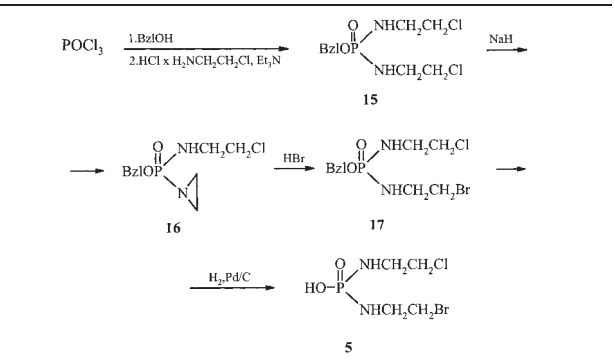


Table: Conditions for determination of enantiomeric purity of compounds 1–3 using ^{31}P NMR

Compd.	Chiral shift reagent (CSR)	Molar ratio CSR/Comp.	Solvent	^{31}P NMR, δ
1	Eu(tfc)$_3$	0.4 : 1	$CDCl_3$	−29.2, −31.8
2	Eu(tfc)$_3$	0.5 : 1	C_6D_6	−33.7, −36.5
3	Pr(tfc)$_3$	0.5 : 1	C_6D_6	11.2, 9.8

NMR done in the presence of a chiral shift reagent (Misiura et al. 1983; Pankiewicz et al. 1979). For compounds **1**–**3**, under the appropriate conditions, two well-separated signals were observed (Table and Fig.).

The expected metabolites of (*S*)-(−)-bromofosfamide were synthesized similar to that of known ifosfamide metabolites, however, in some cases the procedures had to be changed due to lower stability of the C–Br bond compared to C–Cl one. Compounds **1**–**5** will be used as standards in a study of the metabolism of (*S*)-(−)-bromofosfamide in humans. Stereochemical aspects of this metabolism will be also considered using chiral shift reagents or a new method recently introduced for the assignment of enantiomeric purity of oxazaphosphorine compounds by means of solid-state NMR (Potrzebowski et al. 2002).

3. Experimental

Tetrahydrofuran and triethylamine were dried by distillation from calcium hydride. Ozone was obtained from oxygen using a Fischer Ozone Generator 501. Chiral shift reagents Eu(tfc)$_3$ and Pr(tfc)$_3$ were from Merck. Racemic bromofosfamide (m.p. 68–69 °C) was obtained in the same way as enantiomer (Misiura et al. 2001) starting from racemic α-methylbenzyl amine. Unless otherwise stated, the progress of each reaction was monitored by silica gel TLC. ^{1}H and ^{31}P NMR spectra were recorded on a Bruker AC-200. Mass spectrometry analyses were performed using a Finnigan MAT 95 apparatus. The elemental analyses of compounds **1**–**5** gave results in an acceptable range.

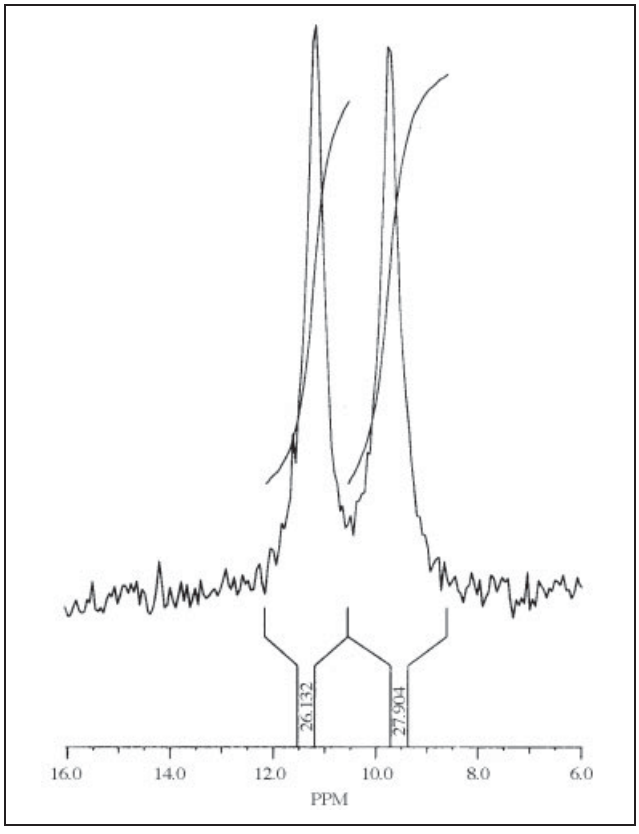


Fig.: ^{31}P NMR spectra of compound **3** in the presence of Pr(tfc)$_3$

3.1. N-(p-Methoxybenzyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (7)

Into stirred, cooled (temp. −15−−10 °C) solution of phosphorus oxychloride (7.65 g, 50 mmol) in chloroform stabilized with amylene (200 mL) a solution of 3-aminopropanol 3.75 g, 50 mmol) and dry triethylamine (7.55 mL, 55 mmol) in dry chloroform (50 mL) was added dropwise. After 30 min the temperature of the reaction mixture was raised to 0–5 °C and another portion of triethylamine (7.55 mL, 55 mmol) was added dropwise. The reaction mixture was stirred until its temperature reached 10 °C. Then, a solution of *p*-methoxybenzyl amine (6.9 g, 50 mmol) and triethylamine (7.55 mL, 55 mmol) in chloroform (50 mL) was added dropwise. The reaction mixture was left for 2 h at room temperature. The suspension obtained was filtered and the filtrate was washed with a brine (100 mL) and water (50 mL). Chloroform solution was dried ($MgSO_4$), passed through silica gel (20 g), and concentrated. The solid obtained was re-crystallized from a mixture of chloroform and *n*-hexane to give product **7**: 6.11 g (62%): m.p 113–115 °C; TLC (chloroform/ethanol, 9 : 1) R_f 0.43; ^{1}H NMR ($CDCl_3$) δ: 1.67–1.94 (m, 2 H), 2.90 (br.s, 2 H), 2.98–3.47 (m, 2 H), 3.78 (s, 3 H), 4.03–4.08 (m, 2 H), 4.14–4.49 (m, 2 H), 6.86–7.25 (m, 4 H).

3.2. 3-Bromoacetyl-N-(p-methoxybenzyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (8)

To the solution of **7** (2.3 g, 9 mmol) in chloroform stabilized with amylene (90 mL), triethylamine bromohydride (4.86 g, 27 mmol) and bromoacetyl bromide (0.82 mL, 9.9 mmol) were added and reaction mixture was stirred for 2 h. Then it was washed with H_2O (3 × 30 mL), dried ($MgSO_4$) and concentrated. The crude product obtained was purified on silica gel using a mixture of chloroform and ethanol 27 : 1 (v/v) as eluent. Appropriate fractions were concentrated and the residue was crystallized from a mixture of chloroform and ethyl ether to give **8**: 2.11 g (54%); m.p 101–102 °C; TLC ($CHCl_3$/EtOH, 9 : 1) R_f 0.69; ^{1}H NMR ($CDCl_3$) δ: 1.80–2.20 (m, 2 H), 3.23 (br.s, 1 H), 3.74 (s, 3 H), 3.96–4.51 (m, 8 H), 6.81–7.20 (m, 4 H).

3.3. 3-(2-Bromoethy1)-N-(p-methoxybenzyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (9)

To the solution of **8** (1.32 g, 35 mmol) in dry THF (150 mL) sodium borohydrate (0.45 g) and then BF_3xEt_2O (3.0 mL) were added. The reaction mixture was stirred for 1.5 h and then it was poured into water (150 mL). The solution obtained was concentrated up to ca. half of its initial volume and then extracted with chloroform (3 × 60 mL). The combined extracts were dried ($MgSO_4$) and evaporated to dryness. The oil obtained was crystallized from a mixture of ethanol and ethyl ether to provide **9** (0.8l g, 64%), which was used in the next reaction without further purification.

3.4. 3-(2-Bromoethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (1)

To a solution of **9** (2.9 g, 8 mmol) in ethanol (100 mL) palladium hydroxide an active carbon (0.2 g) was added. The reaction mixture was stirred under a hydrogen atmosphere. The progress of debenzylation was monitored by TLC. After 6 h the catalyst was filtered off and the filtrate was evaporated. The residue obtained was chromatographed on silica gel using a mixture of chloroform and ethanol in a ratio of 9 : 1 (v/v) as eluent. Appropriate fractions were evaporated and the solid obtained was re-crystallized from a mixture of chloroform and ethyl ether to give **1**: 0.70 (36%); m.p 108–109 °C; TLC ($CHCl_3$/EtOH, 9 : 1) R_f 0.25; ^{1}H NMR ($CDCl_3$) δ: 1.88–1.99 (m, 2 H), 2.94 (br.s, 2 H), 3.18–3.69 (m, 6 H), 4.15–4.48 (m, 2 H); ^{31}P NMR ($CDCl_3$) δ 12.9; MS, EI 242, 244 ($M^{+\bullet}$, 1Br).

3.5. 3-Chloroacetyltetrahydro-2H-l,3,2-oxazaphosphorin-2-amine-2-oxide (10)

A solution of $POCl_3$ (15.3 g, 100 mmol) in chloroform stabilized with amylene (150 mL) was cooled to −15 °C and a solution of 3-aminopropanol (7.5 g, 100 mmol) and triethylamine (15,1 mL, 110 mmol) in chloroform (150 mL) was added dropwise under stirring. Stirring of the reaction mixture was continued for 15 min at −5 °C and then it was allowed to warm up to room temperature. Triethylamine (15.1 mL, 110 mmol) and 2-chloroethylamine hydrochloride (12.8 g, 110 mmol) were added and stirring was continued for 1 h. Then chloroacetyl chloride (13.6 g, 110 mmol) was added and the reaction mixture was kept at 40 °C for 1 h. The solution was washed with 5% $NaHCO_3$ solution (250 mL), dried ($MgSO_4$) and evaporated. The crude compound **10** was purified by silica gel chromatography using a mixture of chloroform and ethanol (19 : 1 v/v). The appropriate fractions were evaporated and the solid obtained was re-crystallized to give **10**: 14.0 g (50%); m.p 54–57 °C; TLC (chloroform/ethanol, 9 : 1) R_f 0.46; ^{1}H NMR ($CDCl_3$) δ: 1.78–2.23 (m, 2H), 3.04–3.78 (m, 7H), 4.24–4.63 (m, 2H), 4.72 (s, 2H).

3.6. N-(2-Chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (2)

A solution of **10** (8.25 g, 30 mmol) in isopropanol (60 mL) was added dropwise into a stirred suspension of sodium borohydride (0.40 g) in isopropanol (30 mL). The reaction mixture was stirred for another 40 min and then it was filtered. The filtrate was concentrated. The solid obtained was dissolved in a mixture of chloroform and ethanol (4 : 1 v/v) and passed through silica gel (l0 g). The silica gel was additionally washed with the same mixture of chloroform and ethanol (3 × 40 mL). The solutions were combined and evaporated to dryness. The solid obtained was re-crystallized from a chloroform, acetone, and *n*-hexane mixture to give **2**: 4.35 g (73%); m.p 110–111 °C; TLC ($CHCl_3$-EtOH, 9 : 1) R_f 0.42; ^{1}H NMR ($CDCl_3$) δ: 1.76–1.86 (m, 2H), 2.76 (br.s, 1 H), 3.13–3.50 (m, 5H), 3.61 (t, J = 5.7 Hz, 2 H), 4.17–4.49 (m, 2 H); ^{31}P NMR ($CDC1_3$) δ 11.8; MS, EI 198, 200 (M^+, 1Cl).

3.7. 3-(2-Bromoethy1)-N-(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2,4-dioxide (3)

To a solution of bromofosfamide (3.05 g, 10 mmol) in a mixture of acetone and water (2 : 1 (v/v), 60 mL) 30% hydrogen peroxide (6 mL) was added. This solution was cooled to ca. 0 °C and then ozone was bubbled through it (flow rate 0.5 g/h) for 6 h. The reaction mixture was concentrated to remove acetone; the aqueous suspension was extracted with chloroform (3 × 20 mL). The chloroform extract was washed with 4% $FeSO_4$ solution (60 mL), dried ($MgSO_4$) and concentrated. The crude product **3** was purified by silica gel chromatography using a mixture of chloroform and acetone 9 : 1 (v/v) as eluent. Appropriate fractions were concentrated. The oily product obtained was crystallized from a mixture of chloroform and *n*-hexane (1 : 1 v/v). Compound **3**: 1.22 g (38%); mp. 113–114 °C; TLC ($CHCl_3$-EtOH, 19 : 1) R_f 0.40; ^{1}H NMR ($CDCl_3$) δ: 1.62 (s, lH), 2.64–2.95 (m, 2 H), 3.30 (dt, $J_{H-H} = J_{H-P} = 6.0$ Hz, 2 H), 3.44–3.65 (m, 4 H), 3.68–4.10 (m, 2 H), 4.14–4.61 (m, 2 H); ^{31}P NMR ($CDCl_3$) δ 7.8; MS, EI, 318, 320, 322 ($M^{+\bullet}$, 1Br1Cl).

3.8. O-Buten-3-yl N,N′-bis(2-chloroethyl)phosphorodiamidate (12)

To the cooled 0–5 °C solution of phosphorus oxychloride (3.83 g, 25 mmol) in chloroform stabilized with amylene (50 mL), a solution of buten-3-ol-l (1.80 g, 25 mmol) and dry triethylamine (3.48 mL, 27.5 mmol) in chloroform stabilized with amylene (50 mL) was added dropwise. Stirring was continued for 30 min at room temperature. Then 2-chloroethylamine hydrochloride (5.79 g, 50 mmol) was added. Triethylamine (13.87 mL, 100 mmol) was added dropwise in such a speed to keep the temperature of the reaction mixture below 25 °C. Stirring was continued for 2 h and then reaction mixture was washed with water (3 × 50 mL). The chloroform solution was dried ($MgSO_4$) and evaporated. The residue obtained was purified on silica gel using a mixture of chloroform and ethanol in a ratio 19 : 1 (v/v). Appropriate fractions were evaporated to give **12** as a colorless oil: 3.58 g (52%); TLC ($CHCl_3$/acetone, 1 : 1) R_f 0.48; ^{1}H NMR ($CDCl_3$) δ: 2.37–2.47 (m, 2 H), 2.97–3.04 (m, 2H), 3.20–3.35 (m, 4 H), 3.61 (t, J = 5.6 Hz, 4 H), 4.05 (dt, $J_{H-H} = J_{H-P} = J = 6.6$ Hz, 2 H), 5.07–5.20 (m, 2 H), 5.70–5.87 (m, 1 H); ^{31}P NMR ($CDCl_3$) δ 14.7.

3.9. O-Buten-3-yl aziridinyl-N-(2-chloroethyl)-phosphorodiamidate (13)

To the stirred solution of **2** (5.5 g, 20 mmol) in dry THF (150 mL) sodium hydride was added in small portions. The progress of cyclization was monitored by TLC using a mixture of *n*-butyl acetate and isopropanol in a ratio of 1 : 1 as eluent. Gradual disappearance of substrate (R_f = 0.72) and formation of the monoaziridate **13** (R_f = 0.66) and the diaziridate (R_f = 0.49) were observed. Adding of NaH was stopped when the intensity of each spot was approximately the same. The mixture was concentrated and separated using silica gel chromatography in a mixture of n-butyl acetate and isopropanol 3 : 1. The fractions containing **13** (TLC assay) were concentrated to give the title compound as a colorless liquid: 1.78 g (37%), ^{31}P NMR ($CDCl_3$) δ 23.6.

3.10. O-Buten-3-yl N-(2-bromoethyl)-N′-(2-chloroethyl)phosphorodiamidate (14)

To the stirred solution of **13** (2.39 g, 10 mmol) in THF (50 mL), 40% HBr (1 mL) was added dropwise. Then the reaction mixture was concentrated and the residue was purified on silica gel using a mixture of chloroform and ethanol in a ratio of 19 : 1 as an eluent. Appropriate fractions were concentrated to give **14** as an colorless oil: 3.03 g (95%); TLC ($CHCl_3$/EtOH 19 : 1) R_f 0.40; ^{1}H NMR ($CDCl_3$) δ: 2.40–2.44 (m, 2 H), 3.03 (br.s, 2 H), 3.24–3.36 (m, 4 H), 3.47 (t, J = 5.9 Hz, 2 H), 3.60 (t, J = 5.7 Hz, 2 H), 4.02–4.06 (m, 2 H), 5.10–5.31 (m, 2 H), 5.76–5.83 (m, 1 H); MS, + FAB 319, 321, 323 [$(M + H)^+$, 1Br1Cl].

3.11. O-(3-Carboxypropyl) N-(2-bromoethyl)-N′-(2-chloroethyl)phosphorodiamidate (4)

To a stirred solution of **14** (1.34 g, 4.1 mmol) in a mixture of benzene (8 mL) and water (2 mL), 18-crown-6 ether (0.10 g) was added and then

$KMnO_4$ (2.09 g) was added in a few portions. The suspension obtained was stirred for 24 h and then it was concentrated. The residue obtained was re-suspended in chloroform (20 mL) and applied on silica gel (l0 g). Silica gel was eluted with chloroform (100 mL), a mixture of chloroform and ethanol in a ratio of 19 : 1 (100 mL) and ethanol (100 mL). The fractions possessing a R_f value of 0.2 ($CHC1_3$/EtOH, 3 : 1) were concentrated to give **4** as a sticky crystals: 120 mg (8% yield); ^{31}P NMR (d_6-DMSO) δ 17.2; MS, −FAB 335, 337, 339 [(M-H)$^-$, 1Br1Cl].

3.12. O-Benzyl N,N′-bis(2-chloroethyl)phosphorodiamidate (**15**)

To the stirred and cooled −40−−30 °C solution of phosphorus oxychloride (15.3 g, 100 mmol) in chloroform stabilized with amylene (400 mL), a solution of benzyl alcohol (10.8 g, 100 mmol) and triethylamine (13.87 mL, 100 mmol) in chloroform stabilized with amylene (30 mL) was added dropwise. Stirring of the reaction mixture was continued at −30 °C for 30 min and then the temperature was raised to −10 °C. 2-Chloroethylamine hydrochloride (23.2 g, 200 mmol) was added to the reaction mixture and then triethylamine (55.48 mL, 400 mmol) was added dropwise maintaining the temperature in a range of −10−−5 °C. Stirring of the obtained suspension was continued for 1 h after it had reached room temperature. Then it was filtered and the chloroform solution obtained was washed with brine (100 mL) and water (2 × 50 mL) and was dried afterwards ($MgSO_4$). The organic solution was concentrated and the obtained crude product was purified on silica gel using a mixture of chloroform and ethanol 27 : 1 (v/v) as eluent. Appropriate fractions were concentrated and dried under vaccum to give product **15**: 19.6 g (61%) of a as an oil: TLC (chloroform, ethanol 19 : 1) R_f 0.36; ^{1}H NMR ($CDCl_3$) δ: 3.01–3.15 (m, 2 H), 3.17–3.31 (m, 4 H), 3.55 (t, J = 5.6 Hz, 4 H), 5.03 (d, J = 8.5 Hz, 2 H), 7.33–7.41) (m, 5 H); ^{31}P NMR ($CDCl_3$) δ 15.2.

3.13. O-Benzyl aziridinyl-N-(2-chloroethyl)phosphorodiamidate (**16**)

To a stirred solution of **15** (6.22 g, 20 mmol) in dry THF (150 mL) sodium hydride was added in portions. The progress of cyclization was observed by TLC using a mixture of ethyl acetate and ethanol in a ratio of 9 : 1. A gradual disappearance the spot corresponding to the substrate ($R_f = 0.72$) and formation of the monoaziridate **16** ($R_f = 0.66$) and the diaziridate ($R_f = 0.50$) was observed. Adding of NaH was stopped when the intensities of all three spots were approximately the same. Then reaction mixture was filtered, concentrated and purified by silica gel chromatography using a mixture of ethyl acetate and ethanol in a ratio 27 : 1 as an eluent. Pure **16** was obtained as a colorless oil: 1.64 g (30%); ^{31}P NMR ($CDCl_3$) δ 23.8.

3.14. O-Benzyl N-(2-bromoethyl)-N′-(2-chloroethyl)phosphorodiamidate (**17**)

To the stirred solution of **16** (1.06 g, 3.9 mmol) in THF a 40% HBr (0.65 mL) was added dropwise. The solution obtained was concentrated to give **17** as a colorless oil: 1.38 g, TLC (CH_3COOEt/EtOH, 9 : 1) R_f 0.75, which was used in the next reaction without further purification.

3.15. N-(2-Bromoethy1)-N′-(2-chloroethyl)phosphorodiamidic acid (5)

To a solution of benzyl ester **17** (4.2 g, 118 mmol) in methanol (120 mL) a palladium on activated carbon (ca. 120 mg) was added and the suspension obtained was stirred and maintained in a hydrogen atmosphere for 2 h. The catalyst was filtered off and the filtrate was concentrated up to ca. 30 mL. Crystals were filtered washed with cold methanol (10 mL) and acetone (20 mL) and dried under vacuum to give product **5**: 1.51 g (48%); m.p 106–107 °C; TLC ($CHCl_3$/EtOH, 7 : 3) R_f 0.58; ^{1}H NMR (d_6-DMSO) δ: 2.93–3.12 (m, 2 H), 3.47 (t, J = 7.0 Hz, 4 H), 3.54 (t, J = 7.0 Hz, 4 H), 10.8 (br.s, 1 H); ^{31}P NMR(d_6-DMSO) δ 12.9; MS, -FAB 263, 265, 267 [(M-H)$^-$, 1Br1Cl].

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