A New Method for the Preparation of Ifosfamide and Cyclophosphamide

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Dedicated to Professor Dr. med. Dr. h. c. Norbert Brock on the occasion of his 85th birthday

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The reaction of 2-chloro-3-(2-chloroethyl)-tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-oxide **1** and 2-chloro-tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-oxide **2** with 2-(trimethylsilyloxy)ethylamine **3** and bis-[2-(trimethylsiloxy)ethyl]amine **4**, respectively, yielded the trimethylsilylated compounds **5** and **6**, analogous to ifosfamide and cyclophosphamide. The reaction of **5** and **6** with 2-chloro-1,3,5-trimethyl-1,3,5-triaza-2-phosphorin-4,6-dione **7** led to the diphosphorus compounds **8** and **9** which could be transformed to ifosfamide **11** and cyclophosphamide **12** by treatment with sulfuryl chloride. This synthesis shows that the alkylating agents 2-chloroethylammonium chloride and bis-(2-chloroethyl)ammonium chloride can be avoided and the chlorine atom can be introduced in the final reaction step of the synthesis of **11** and **12**.

Introduction

Ifosfamide **11** and cyclophosphamide **12** are two clinically widely used alkylating agents in the treatment of many types of human cancers [1, 2]. 2-Chloroethylammonium chloride and bis-(2-chloroethyl)ammonium chloride (nitrogen mustard HCl-salt) are important educts for the synthesis of **11** [3, 4] and **12** [5, 6], respectively. In order to avoid the use of these alkylating agents we developed a new synthesis in which chlorine is introduced in the final reaction step.

Results and Discussion

The substitution of chlorine in **1** and **2** by 2-(trimethylsiloxy)ethylamine **3** and bis-[2-(trimethylsiloxy)ethyl]amine **4**, respectively, yielded the products **5** and **6** in nearly quantitative yield. Hydrolysis of these silylated compounds led to the corresponding hydroxy compound only in the case of **5**. Its direct chlorination with thionyl chloride gave **11** in low yield. Having in mind that 2-alkoxy-1,3,5-trimethyl-1,3,5-triaza-2-phosphorin-4,6-diones react with sulfuryl chloride with formation of 2-chloro-1,3,5-trimethyl-1,3,5-triaza-2-phosphorin-4,6-dion-2-oxide **10** and the corresponding alkyl chloride [7], the silylated compounds **5** and **6**, consequently, were allowed to react with **7** to form the $\sigma^4\lambda^5/\sigma^3\lambda^3$-diphosphorus species **8** and **9**. Treatment of **8** and **9** with sulfuryl chloride led to ifosfamide **11** and cyclophosphamide **12**, respectively, but the compounds were not stable in the reaction mixture. A ^{31}P NMR spectrum, recorded after half an hour of reaction time, showed a yield of *ca.* 75%. On recrystallisation of the product mixtures from diethyl ether only 2-chloro-1,3,5-trimethyl-1,3,5-triaza-2-phosphorin-4,6-dion-2-oxide **10** crystallized in a pure state. Ifosfamide **11** and cyclophosphamide **12**, could be separated by column chromatography of the ethereal mother liquor; the yield after recrystallisation amounted to 10 - 15%.

The previously known methods for the synthesis of ifosfamide and cyclophosphamide do not require chromatographic separation of the products. Our work has demonstrated an alternative synthesis of compounds **11** and **12**, avoiding the use of the alkylating agents, 2-chloroethylammonium chloride and bis(2-chloroethyl)ammonium chloride, as starting compounds.

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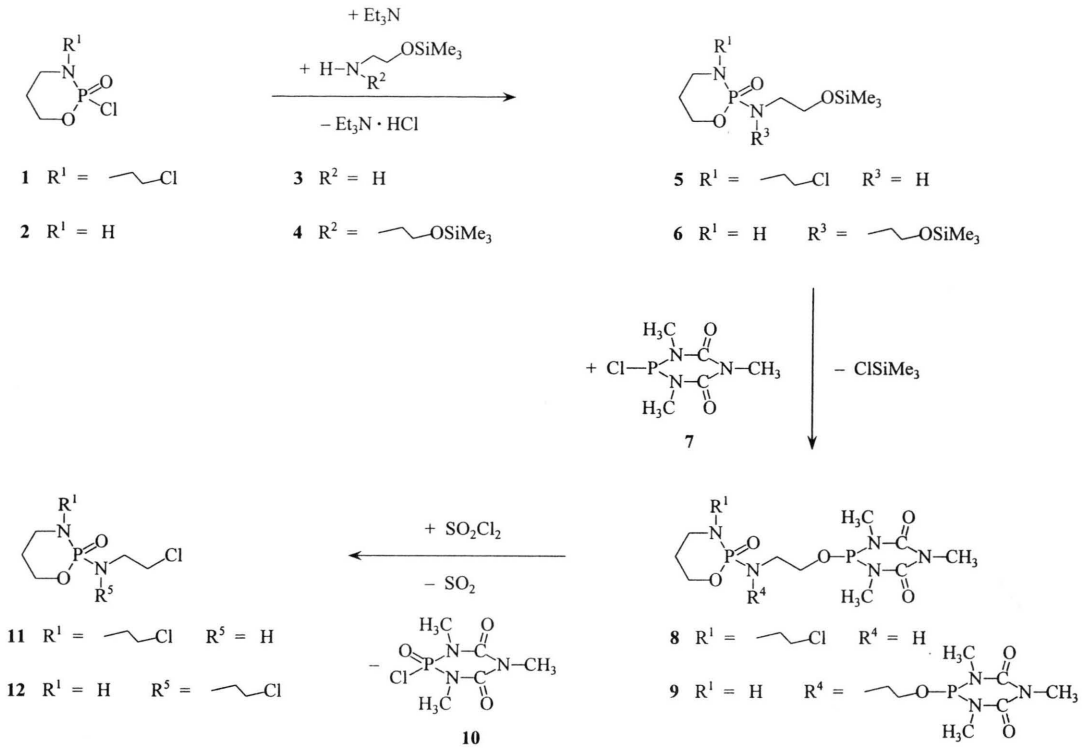

Experimental

All experiments were carried out with exclusion of air and moisture; solvents were purified and dried according to the usual methods [8]. "In vacuo" (i. v.) refers to a pressure of 0.1 mm Hg at 25°C. - NMR spectra were recorded on a Bruker AC 200 spectrometer at 200.1 MHz (^{1}H), 50.3 MHz (^{13}C), and 81.0 MHz (^{31}P). Chemical shifts δ are given relative to $Si(CH_3)_4$ (TMS) (^{1}H, ^{13}C); 85% H_3PO_4 (^{31}P). High-field shifts were given negative, low-field shifts positive signs. - MS: Finnigan MAT 8430. - Elemental analyses: Analytisches Laboratorium des Instituts für Anorganische und Analytische Chemie der Technischen Universität, Braunschweig. - Precursor compounds: 2-chloro-3-(2-chloroethyl)-tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-oxide (**1**) [9], 2-chloro-tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-oxide (**2**) [10], 2-(trimethylsiloxy)ethylamine (**3**) [11], bis-[2-(trimethylsiloxy)ethyl]amine (**4**) [11], 2-chloro-1,3,5-trimethyl-1,3,5-triaza-2-phosphorin-4,6-dione (**7**) [12].

*3-(2-Chloroethyl)-2-[2-(trimethylsiloxy)ethylamino]-tetrahydro-2H-1,3,2 -oxazaphosphorin-2-oxide (**5**)*

A solution of **1** (2.00 g, 15 mmol) and of triethylamine (1.52 g, 15 mmol) in 30 ml of dichloromethane was cooled to -15°C (ethanol/liqu. nitrogen) and a solution of 2-(trimethylsiloxy)ethylamine **3** (3.27 g, 15 mmol) in 20 ml of dichloromethane was added dropwise during 15 min. After stirring the reaction mixture for 5 h at -15°C the solvent and all volatile components were removed i. v. The residue was extracted with 50 ml of diethyl ether, and triethylammonium chloride was filtered off. After removing the diethyl ether i. v. **5** was left as a colourless oil; yield: 4.48 g (94.9%); b.p.: >200°C (1 mm Hg).

^{1}H NMR ($CDCl_3$, 200.1 MHz): δ = 0.79 (s, 9 H, $Si(CH_3)_3$), 1.83-1.95 (m, 2 H, $CH_2CH_2CH_2$), 2.90-3.65 (m, 11 H, $3xNCH_2$, $2xNCH_2CH_2$, N*H*), 4.13-4.37 (m, 2 H, $POCH_2$). - ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ = -0.63 (s, $SiCH_3$), 26.41 (d, ^3J(PC) = 4.6 Hz, $PNCH_2CH_2CH_2$), 42.01 (d, ^2J(PC) = 4.0 Hz, $PNCH_2CH_2CH_2$), 43.22; 62.56 (s; d, 6.4 Hz, $PNHCH_2CH_2OSi$), 47.73 (s, CH_2Cl), 50.15 (d, ^2J(PC) = 2.7 Hz, $PNCH_2CH_2Cl$), 67.01 (d, ^2J(PC) = 6.8 Hz, $POCH_2CH_2CH_2$). - ^{31}P NMR ($CDCl_3$, 81.0 MHz): δ = 13.23 (s) - EI-MS: *m/z* (%) = 314 (4) [M^+], 299 (24) [M^+- CH_3], 211 (100) [M^+- $CH_2OSi(CH_3)_3$], 182 (28) [M^+- $NHCH_2CH_2OSi(CH_3)_3$].

$C_{10}H_{24}ClN_2O_3PSi$ (314.82)

Calcd C 38.15 H 7.68 N 8.90%,
Found C 36.88 H 7.45 N 9.28%.

The experimental C-value deviates from the calculated value, presumably because of the formation of SiC.

*2-Bis-[2-(trimethylsiloxy)ethylamino]-tetrahydro-2H-1,3,2-oxazaphosphorin-2-oxide (**6**)*

A solution of **2** (5.80 g, 37mmol) and of triethylamine (3.77 g, 37 mmol) in 50 ml of dichloromethane was cooled to 0°C. A solution of bis-[2-(trimethylsiloxy)ethyl]amine **4** (9.30 g, 37 mmol) in 50 ml of dichloromethane was added dropwise during 1 h. After stirring the reaction mixture for 18 h at r.t., the solvent and all volatile components were removed i. v. The residue was extracted with 100 ml of diethyl ether, and triethylammonium chloride was filtered off. After removing the diethyl ether i.v. **6** was left as a colourless oil; yield: 12.08 g (88.7%); b.p.: >200°C (1 mm Hg).

^{1}H NMR ($CDCl_3$, 200.1 MHz): δ = 0.05 (s, 18 H, $Si(CH_3)_3$), 1.62-1.94 (m, 2 H, $CH_2CH_2CH_2$), 2.87-2.96 (br., 1 H, N*H*), 3.02-3.45 (m, 6 H, 3xNCH_2), 3.60 (t, 4 H, ^3J(HH) = 6.2 Hz, NCH_2CH_2OSi), 4.05-4.46 (m, 2 H, $POCH_2$). - ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ = -0.89 (s, $SiCH_3$), 25.86 (d, ^3J(PC) = 6.4 Hz, $PNCH_2CH_2CH_2$), 40.90 (d, ^2J(PC) = 2.8 Hz, $PNCH_2CH_2CH_2$), 48.17 (d, ^2J(PC) = 4.1 Hz, $PN(CH_2CH_2OSi)_2$), 60.91 (d, ^3J(PC) = 1.1 Hz, $PN(CH_2CH_2OSi)_2$), 67.08 (d, ^2J(PC) = 6.7 Hz, $POCH_2CH_2CH_2$). - ^{31}P NMR ($CDCl_3$, 81.0 MHz): δ = 13.84 (s). - EI-MS: *m/z* (%) = 368 (1) [M^+], 353 (16) [M^+- CH_3], 265 (100) [M^+- $CH_2OSi(CH_3)_3$], 194 (13) [M^+- $CH_2CH_2OSi(CH_3)_3$ -$NHCH_2CH_2CH_2$].

$C_{13}H_{33}N_2O_4PSi_2$ (368.56)
Calcd C 42.37 H 9.02 N 7.60%,
Found C 41.39 H 9.03 N 7.71%.

The experimental C-value deviates from the calculated value, presumably because of the formation of SiC.

*3-(2-Chloroethyl)-2-[2-(1,3,5-trimethyl-1,3,5,2-triazaphosphorin-4,6-dion-2-yloxy)ethylamino]-tetrahydro-2H-1,3,2-oxazaphosphorin-2-oxide (**8**)*

To a solution of 3.15 g (10 mmol) of **5** in 50 ml of dichloromethane 2.10 g (10 mmol) of **7** was added. After stirring the reaction mixture for 1 d at r.t., the solvent and all volatile components were removed i.v. The residue was washed with 50 ml of n-hexane and dried i.v.; yield: 3.60 g (86.5%); m.p.: 54°C.

^{1}H NMR ($CDCl_3$, 200.1 MHz): δ = 1.86-1.91 (m, 2 H, $CH_2CH_2CH_2$), 2.99-3.62 (m, 11 H, 3xNCH_2, 2xNCH_2CH_2, N*H*), 3.08 (d, 6 H, ^3J(PH) = 11.7 Hz, $P(NCH_3)_2$), 3.24 (s, 3 H, $[C(:O)]_2NCH_3$), 4.08-4.33 (m, 2 H, $P(:O)OCH_2$). - ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ = 26.32 (d, ^3J(PC) = 4.4 Hz, $PNCH_2CH_2CH_2$), 30.20 (s, $[C(:O)]_2NCH_3$), 33.43 (d, ^2J(PC) = 36.3 Hz, $P(NCH_3)_2$), 41.60; 41.87; 63.98 (3d, ^2J(PC) = 4.2 Hz; 4.6 Hz; 4.8 Hz, $PNCH_2CH_2CH_2$, $PNHCH_2CH_2OP$), 47.40 (s, CH_2Cl), 49.92 (d, ^2J(PC) = 2.6 Hz, $PNCH_2CH_2Cl$), 67.07 (d, ^2J(PC) = 6.8 Hz, $POCH_2CH_2CH_2$), 153.27 (d, ^2J(PC) = 9.9 Hz, $P(NCH_3C(:O))_2$). - ^{31}P NMR ($CDCl_3$, 81.0 MHz): δ = 90.93 (s), 13.06 (s). - EI-MS: *m/z* (%) = 415 (2) [M^+], 380 (10) [M^+- Cl], 366 (5) [M^+- CH_2Cl], 225 (100) [M^+- $OP\{NCH_3C(:O)\}_2NCH_3$], 211 (42) [M^+- $CH_2OP\{NCH_3C(:O)\}_2NCH_3$].

$C_{12}H_{24}ClN_5O_5P_2$ (415.75)
Calcd C 34.67 H 5.82 N 16.85%,
Found C 34.05 H 5.99 N 16.04%.

*2-Bis-[2-(1,3,5-trimethyl-1,3,5,2-triazaphosphorin-4,6-dion-2-yloxy)ethylamino]-tetrahydro-2H-1,3,2-oxazaphosphorin-2-oxide (**9**)*

To a solution of 3.70 g (10 mmol) of **6** in 50 ml of dichloromethane 4.20 g (20 mmol) of **7** was added. After stirring the reaction mixture for 1 d at r.t., the solvent and all volatile components were removed i.v. The residue was washed with 50 ml of n-hexane and dried i.v.; yield: 4.35 g (76.3%); m.p.: 58°C.

^{1}H NMR ($CDCl_3$, 200.1 MHz): δ = 1.72-1.88 (m, 2 H, $CH_2CH_2CH_2$), 2.61 (br., 1 H, N*H*), 2.98-3.33; 3.52-3.69 (2 m, 10 H, 3xNCH_2, 2xNCH_2CH_2), 3.09 (d, 12 H, ^3J(PH) = 11.6 Hz, $P(NCH_3)_2$), 3.27 (s, 6 H, $[C(:O)]_2NCH_3$), 4.07-4.45 (m, 2 H, $P(:O)OCH_2$). - ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ = 25.72 (d, ^3J(PC) = 5.5 Hz, $PNCH_2CH_2CH_2$), 30.26 (s, $[C(:O)]_2NCH_3$), 33.51 (d, ^2J(PC) = 36.3 Hz, $P(NCH_3)_2$), 41.57 (d, ^2J(PC) = 2.9 Hz, $PNCH_2CH_2CH_2$), 47.40 (d, ^2J(PC) = 3.8 Hz, $PN(CH_2CH_2O\text{-})_2$), 63.14 (s, $PN(CH_2CH_2O\text{-})_2$), 67.41 (d, ^2J(PC) = 6.8 Hz, $POCH_2CH_2CH_2$), 153.35 (d, ^2J(PC) = 11.3 Hz, $P(NCH_3C(:O))_2$. - ^{31}P NMR ($CDCl_3$, 81.0 MHz): δ = 91.67 (s), 13.35 (s). - EI-MS: *m/z* (%) = 570 (0.4) [M^+], 380 (100) [M^+- $OP\{NCH_3C(O)\}_2NCH_3$], 366 (5) [M^+- $CH_2OP\{NCH_3C(:O)\}_2NCH_3$], 174 (73) [$OP\{NCH_3C(:O)\}_2NCH_3$].

$C_{17}H_{33}N_8O_8P_3$ (570.42)
Calcd C 35.80 H 5.83 N 19.64%,
Found C 35.95 H 6.28 N 19.33%.

*3-(2-Chloroethyl)-2-[(2-chloroethyl)amino]-tetrahydro-2H-1,3,2-oxazaphosphorin-2-oxide (Ifosfamide) (**11**) and 2-bis-[(2-chloroethyl)amino]-tetrahydro-2H-1,3,2-oxazaphosphorin-2-oxide (Cyclophosphamide) (**12**)*

To solutions of 2.49 g (6 mmol) of **8** and of 3.42 g (6 mmol) of **9**, respectively, in 50 ml of dichloromethane sulfuryl chloride (0.81 g, 6 mmol) was added at 0°C. After stirring the solutions for 1 h at 0°C, the solvent and all volatile components were removed i.v. The residues were dissolved in 30 ml of diethyl ether each and kept overnight at -20°C. Pure 2-chloro-1,3,5-trimethyl-1,3,5-triaza-2-phosphorin-4,6-dion-2-oxide **10** crystallized from both

solutions, while ifosfamide **11**, cyclophosphamide **12** and other by-products remained in solution. After filtration, both **11** and **12** were separated by column chromatography on silica gel (CH_2Cl_2/n-hexane, 5:2) of the ethereal mother liquor and recrystallized from 5 ml of diethyl ether each at -20°C. The identity of **10**, of ifosfamide **11** and of cyclophosphamide **12** was established by ^{1}H- and ^{31}P NMR-spectroscopy [4, 6, 12]; yield: **11**: 0.24 g (14.8%); **12**: 0.17 g (10.9%).

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