Synthesis of Some Amino Acid Linked Nitrogen Mustard Derivatives

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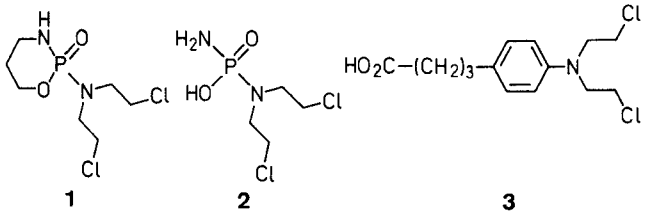
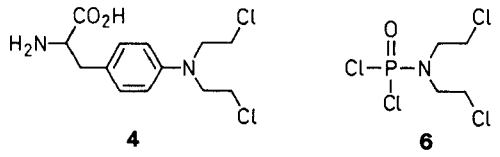
Dichlorophosphoramide is readily prepared by the reaction of bis(β-chloroethyl)amine hydrochloride with phosphoryl chloride at elevated temperature. The crude product is pure without need for troublesome vacuum distillation. However, this reagent reacts only poorly with ethanol, propanol, and the methyl ester of valine. Thus, an alternative route was sought to amino acid linked derivatives of nitrogen mustards. This involved the synthesis of the appropriate alkyl phosphorodichloridate, and its reaction with bis(β-chloroethyl)amine, followed by condensation with an amino acid carboxyl ester. Reactions proceed in high yield, under mild conditions. In all but the case of glycine derivatives, the presence of multiple chiral centres in the final product leads to the generation of diastereoisomers, which can be observed by spectroscopic and analytical (HPLC) methods.

Cyclophosphamide (**1**), an alkylating agent prepared over 30 years ago by Arnold,[1] has become very widely used in cancer chemotherapy.[2] Its unique mode of action involves hydroxylation of the heterocycle, followed by breakdown to generate phosphoramide mustard **2**, which is a potent DNA alkylator.[3] Other agents containing the masked bis(β-chloroethyl)amine group are also in clinical use, or pre-clinical evaluation. These include the phenylbutyric acid compound chlorambucil (**3**),[4] and the phenylanine derivative melphalan (**4**).[5] We wondered whether phosphoramide derivatives bearing an (esterified) amino acid group on the phosphorus centre would display useful antineoplastic properties. The reasons for studying these particular derivatives were threefold. Firstly, the attachment of an amino acid to a drug is a potential vehicle for enhanced cellular uptake, which has been used previously in a number of therapeutic areas.[6] Secondly, if hydrolysis of the P–N bond to the amino acid were to ensue, the released amine would be an amino acid, which would be of limited toxicity. The release of the acyclic component acrolein, is one of the major sources of toxicity with cyclophosphamide.[7] Lastly, we have noted that the attachment of an amino acid to the phosphate moiety of certain anti-HIV nucleotides may lead to an enhancement of chemotherapeutic properties.[8] Thus the target compounds were of general structure **5**.



1 **2** **3**



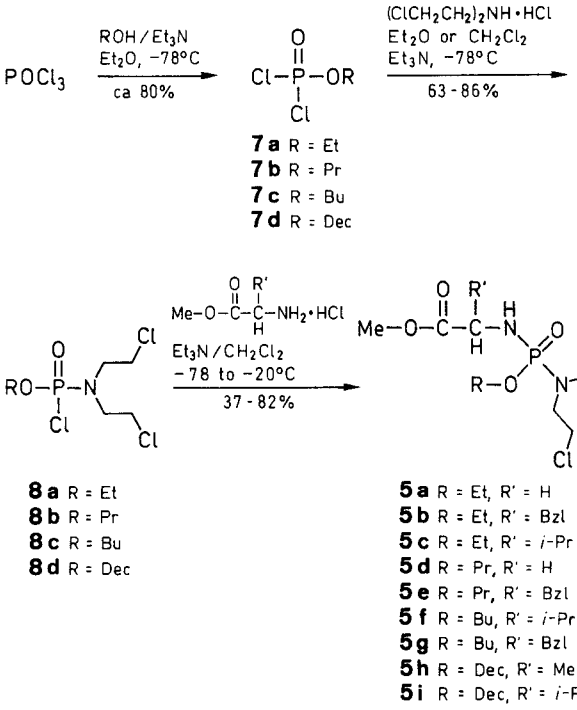
4 **6**

By analogy to the preparation of cyclophosphamide,[1] it seemed likely that such compounds could be generated by the reaction of dichlorophosphoramide **6** sequentially with an alcohol, and an esterified amino acid. Thus, phosphoryl chloride was allowed to react with bis(β-chloroethyl)amine at elevated temperature, in the absence of solvent or base. In the literature report[9] the resulting dichlorophosphoramide is vacuum distilled. In fact, we find that the crude product is pure. For example, ^{31}P NMR reveals a single peak of chemical shift [$\delta_P = +15.8$] similar to that reported for the model compound dipropylamidophosphorodichloridate [$\delta_P = +15.3$].[10] ^{13}C NMR data also confirms the structure of the product; two doublets are noted (split by phosphorus coupling) at chemical shift values comparable to those reported for the bis(β-chloroethyl)amine moiety of cyclophosphamide.[11] Proton NMR and microanalysis data further confirm the structure and purity of the product. We regard this observation as an important discovery, because the use of dichlorophosphoramide is widespread, and vacuuum distillation of this (solid) material is troublesome; we found that the crude yield is quantitative, and the crude product is pure by spectroscopic and analytical methods.

Dichlorophosphoramide **6** was allowed to react with ethanol in diethyl ether containing triethylamine, but only a small degree of reaction (ca. 18%) had occurred after 16 hours as judged by ^{31}P NMR. Similarly, the reaction of dichlorophosphoramide with propanol had only proceeded to a small extent (ca. 13%) after 136 hours. Whilst it was possible to extend these times, or to increase the reaction temperature, it appeared that this route would be of limited utility, especially for longer chain alcohols, particularly given the apparent large rate difference between ethanol and propanol.

Thus, an alternative approach was studied. This involved the reaction of dichlorophosphoramide **6** with a carboxyl-protected amino acid. However, again, the reaction of the dichloridate with L-valine methyl ester hydrochloride was extremely slow in dichloromethane in the presence of triethylamine. The failure of these two alternative routes indicated the low reactivity of dichlorophosphoramide, and/or the instability of the resulting phosphorochloridate.

Thus, a further route was studied. This involved the prior synthesis of the alkyl phosphorodichloridate, and its subsequent reaction, first with bis(β-chloroethyl)amine and then with the appropriate amino acid ester. The reaction of ethanol with phosphoryl chloride in diethyl ether in the presence of triethylamine gave ethyl phosphorodichloridate (**7a**), as a crude product in 80% yield. This was pure by ^{31}P NMR [$\delta_P = +5.0$] and by ^{13}C NMR, with data confirming the structure. This reagent was



allowed to react with bis(β-chloroethyl)amine hydrochloride, under similar conditions to the first stage, to give the phosphorochloridate **8a** in 86% yield. This gave ^{31}P, ^{13}C, and ^{1}H NMR data, and FAB mass spectrometry data entirely consistent with the proposed structure. Compound **8a** was allowed to react with glycine methyl ester hydrochloride in dichloromethane in the presence of triethylamine, at low temperature. Repeated extraction of the crude product with ether gave **5a** in 82% yield. The compound was entirely pure as judged by a range of spectroscopic and analytical methods. The ^{31}P NMR spectrum revealed a single peak at a chemical shift in the region expected for a structure of this type ($\delta_P = +12.6$).[10] In the ^{13}C NMR, all resonances displayed phosphorus coupling, apart from those of the methoxy and chloromethylene groups. Full ^{13}C NMR data for **5a**, and for the subsequent target compounds **5b–i** are given in Table 1. Mass spectral and analytical data also confirmed the structure and purity of **5a**. Similarly prepared were the phenylalanine **5b** and valine **5c** analogues. However, several differences were noted here. In particular, the crude products were not entirely pure, as judged by ^{31}P NMR, and purification by column chromatography was necessary, in contrast to the preparation of the glycine compound **5a**. In the absence of a UV chromophore, the eluting samples were detected using iodine adsorbed on silica. Furthermore the ^{31}P NMR of **5b–c** were more complicated than those obtained for the glycine compound **5a**. Thus, two very closely spaced resonances were noted ($\Delta\delta$ ca. 0.1–0.2 ppm), and in the case of **5c** these data indicated an unequal generation of the two diastereoisomers (2:1). In addition, some of the signals in the ^{13}C NMR appeared as multiplets due to a combination of diastereomeric splitting and phosphorus coupling.

Besides variation in the amino acid moiety, it was also of interest to probe variation in the alkyl group. Thus, propyl phosphorodichloridate **(7b)** was prepared as above, and reacted with bis(β-chloroethyl)amine to give the phosphorochloridate **8b**. This was allowed to react with glycine methyl ester and with phenylalanine methyl ester to give **5d** and **5e**, respectively. Again, column chromatography was needed in each case, and the products displayed splitting in the NMR spectra consistent with the generation of a diastereoisomeric mixture. Using the same procedure, the valine and phenylalanine derivatives **5f** and **5g** were prepared from butyl phosphorodichloridate **(7c)** via the chloridate **8c**. Lastly, decyl phosphorodichloridate **(7d)** was used to access the chloridate **8d** and thus the target alanine **5h** and valine **5i** compounds.

In conclusion, the reaction of alkyl phosphorodichloridates with bis(β-chloroethyl)amine gives the corresponding phosphorochloridates in high yield, and these react with amino acid methyl esters to give novel amino acid

Table. ^{13}C NMR data for **5a–i** recorded at 100 MHz in $CDCl_3$: phosphorus-carbon coupling constants (Hz) are given in parentheses. Many peaks are split due to diastereoisomers; in these cases the mean δ and coupling constant are given.

Signal	5a	5b	5c	5d	5e	5f	5g	5h[c]	5i[e]
NCH_2CH_2Cl	49.0 (4.7)	49.1 (4.6)	49.2	49.2 (4.5)	49.1 (4.6)	49.2	49.1 (4.7)	49.3	49.2
NCH_2CH_2Cl	42.3	42.5	42.6	42.5	42.5	42.6	42.5	42.5	42.6
NHCH*	42.2 (2.0)	55.3	59.4	42.3 (2.2)	55.2	59.3	55.2	49.6	59.3
C=O	171.4 (8.0)	173.3 (4.7)	173.8 (3.6)	171.5 (8.5)	173.4 (4.8)	173.8 (3.0)	173.4 (5.0)	174.6 (7.3)	173.8
OMe	52.0	52.3 (3.2)	52.1	52.3 (6.6)	52.3 (6.2)	52.1	52.2	52.4 (2.5)	52.0
Val–CH	–	–	32.3 (6.0)	–	–	[a]	–	–	32.5 (5.9)
Val–Me (1)	–	–	19.0	–	–	[b]	–	–	18.9
Val–Me (2)	–	–	17.7	–	–	17.6	–	–	17.6
Phe	–	127–136	–	–	127–136	–	127–136	–	–
Phe–CH_2	–	40.6 (6.0)	–	–	40.6 (6.2)	–	40.6 (6.0)	–	–
Ala–Me	–	–	–	–	–	–	–	21.2 (4.7)	–
P–O–C	61.6 (5.1)	61.8 (NR)	61.9 (5.2)	67.3 (5.4)	67.3 (5.2)	65.6 (5.5)	65.5 (5.4)	65.7 (4.3)	65.9 (5.4)
P–O–CC	16.0 (7.1)	16.2 (3.8)	16.3 (6.0)	23.7 (7.2)	23.7 (7.4)	[a]	32.4 (7.3)	25.6	25.6
P–O–CCC	–	–	–	10.1	10.1	[b]	18.8	[d]	30.4
P–O–CCCC	–	–	–	–	–	13.7	13.7	–	–

[a] Multiplet, $\delta = 32.1–32.5$.
[b] Multiplet, $\delta = 18.8–18.9$.
[c] Also contains signals at $\delta = 31.8$ (C3), 22.6 (C2) and 14.0 (C1).
[d] Overlap with methylene signals $\delta = 29.1–30.4$.
[e] Also contains signals at $\delta = 31.8$ (C3), 30.3 (C7), 29.5 (C6), 29.3 (C5), 29.1 (C4), 22.6 (C2) and 14.1 (C1).

linked nitrogen mustard derivatives, as diastereomeric mixtures, in high yield.

^{31}P NMR spectra were recorded on a Varian XL-200 spectrometer operating at 82 MHz or a Varian VXR400 instrument operating at 164 MHz and shifts are reported in units of δ relative to 85% phosphoric acid as external standard, positive shifts are downfield. ^{13}C NMR spectra were recorded on a Varian XL-200 spectrometer operating at 50 MHz or a Varian VXR400 instrument operating at 100 MHz and shifts are in units of δ relative to TMS. Both ^{31}P and ^{13}P NMR spectra were proton noise decoupled and all signals were singlets unless otherwise stated. ^1H NMR spectra were recorded on a Varian XL-200 spectrometer operating at 200 MHz or a Varian VXR400 instrument operating at 400 MHz and are reported in units of δ relative to TMS. All NMR spectra were recorded in $CDCl_3$ unless otherwise stated, and all coupling constants are reported in Hz. EI MS were recorded on a VG7070H spectrometer fitted with a Finnigan Incos II data system, and fast atom bombardment (FAB) mass spectra were recorded on a VG Zab 1F spectrometer, courtesy of Dr. M. Mruzek (University College London). Microanalyses were performed at University College London courtesy of the Group of Mr. A.T.T. Stones. For all new compounds satisfactory Microanalyses were obtained: C ± 0.28, H ± 0.28, except **5h** C ± 0.71, H ± 0.54. All experiments involving water sensitive reagents were carried out under scrupulously dry conditions. Where needed, anhydrous solvents and reagents were obtained in the following ways: Et_3N, Et_2O, hexane and CH_2Cl_2 were refluxed over CaH_2 for several hours and distilled. All but Et_3N were further dried over activated 4Å molecular sieves. All alcohols except decanol were distilled onto activated 4Å molecular sieves. Decanol was then dried in vacuo at room temperature for several hours. All alkyl moieties are of the n-form unless otherwise stated.

Bis(β-chloroethyl)aminophosphoryl Dichloride (6):
Dry bis(β-chloroethyl)amine hydrochloride (10.0 g, 56.3 mmol) was mixed with phosphoryl chloride (26 mL, 0.27 mol) and the mixture heated under reflux at 120–140°C for 75 h. Excess phosphoryl chloride was evaporated under reduced pressure, to yield the crude product as off-white crystals (14.5 g, 100%), mp 50–51°C.

^{31}P NMR: δ = 15.8.

^{13}C NMR: δ = 49.3 (d, CH_2N, J = 4.1), 40.8 (d, CH_2Cl, J = 2.7).

^1H NMR: δ = 3.55–3.75 (m).

MS (FAB): m/z = 542 (M_2HNa^+, 2 × ^{37}Cl, 4%), 540 (M_2HNa^+, ^{37}Cl, 9), 538 (M_2HNa^+, 6), 284 (MNa^+, 2 × ^{37}Cl, 44), 282 (MNa^+, ^{37}Cl, 100), 280 (MNa^+, 76), 262 (MH^+, 2 × ^{37}Cl, 14), 260 (MH^+, ^{37}Cl, 37), 258 (MH^+, 33), 224 ($M^+ - Cl$, ^{37}Cl, 4), 222 ($M^+ - Cl$, 4).

Ethyl Phosphorodichloridate (7a):
Dry Et_3N (7.48 mL, 53.6 mmol) and EtOH (3.15 mL, 53.6 mmol) in Et_2O (100 mL) were added dropwise with vigorous stirring to phosphoryl chloride (5.0 mL, 53.6 mmol) in Et_2O (100 mL) at −78°C under an atmosphere of nitrogen. The mixture was allowed to warm to ambient temperature, stirred for 20 h, then filtered, and the solvent removed under reduced pressure to give the crude product (6.99 g, 80%).

^{31}P NMR: δ = 5.0.

^{13}C NMR: δ = 68.5 (d, CH_2, J = 9.0), 15.4 (d, Me, J = 2.6).
Other alkyl phosphorodichloridates were similarly prepared.

Ethyl *N,N*-Bis(β-chloroethyl)amidophosphorochloridate (8a):
Dry Et_3N (3.42 mL, 24.6 mmol) in Et_2O (50 mL) was added dropwise with vigorous stirring to a mixture of ethyl phosphorodichloridate (2.0 g, 12.3 mmol) and $(ClCH_2CH_2)_2NH \cdot HCl$ (2.19 g, 12.3 mmol) in Et_2O (50 mL) at −78°C. The mixture was allowed to warm to ambient temperature, stirred for 42 h, then filtered, and the solvent removed under reduced pressure. Hexane (150 mL) was added to the residue, and the mixture was filtered and the solvent removed under reduced pressure, to give the crude product (2.84 g, 86%).

^{31}P NMR: δ = 13.4.

^{13}C NMR: δ = 65.0 (d, CH_2OP, J = 6.0), 49.9 (d, CH_2N, J = 4.3), 41.5 (d, CH_2Cl, J = 2.5), 15.8 (d, Me, J = 8.0).

^1H NMR: δ = 4.3 (2H, m, CH_2OP), 3.6 (4H, m, CH_2N), 3.5 (4H, m, CH_2Cl), 1.4 (3H, dt, Me).

MS (FAB): m/z = 270 (MH^+, ^{37}Cl, 3%), 268 (MH^+, 4), 144 (14), 142 (26), 102 (100).

Ethyl *N,N*-Bis(β-chloroethyl)amido(methoxyglycinyl)phosphate (5a):
Dry Et_3N (0.21 mL, 1.5 mmol) in CH_2Cl_2 (6 mL) was added dropwise with vigorous stirring to a mixture of $(ClCH_2CH_2)_2NP(O)(OEt)Cl$ **(8a)** (0.2 g, 0.74 mmol) and glycine methyl ester hydrochloride (0.094 g, 0.74 mmol) in CH_2Cl_2 (12 mL) at −78°C under an atmosphere of nitrogen. The mixture was allowed to warm to ambient temperature, with stirring for 42 h. The solvent was removed under reduced pressure, and the residue extracted with Et_2O (2 × 150 mL). The extracts were filtered, combined and evaporated under reduced pressure to yield the product as a colourless oil (0.2 g, 82%).

^{31}P NMR: δ = 12.6.

^1H NMR: δ = 4.1 (2H, m, CH_2OP), 3.7 (3H, s, MeO), 3.65 (4H, m, CH_2N), 3.4–3.5 (6H, m, CH_2Cl, CH_2NH), 3.1 (1H, m, NH), 1.3 (3H, t, Me).

MS (EI): m/z = 323 (MH^+, ^{37}Cl, 0.03%, 321.0544 (MH^+, 0.02, calc. for $C_9H_{20}Cl_2N_2O_4P$ 321.0538), 273 (5), 271 (16), 180 (100), 152 (90).

Ethyl *N,N*-Bis(β-chloroethyl)amido(methoxyphenylalaninyl)phosphate (5b):
This was prepared as described for **5a** above, using phenylalanine methyl ester hydrochloride (0.16 g, 0.74 mmol), except that the isolation procedure was altered. Thus, the mixture was evaporated under reduced pressure, and the residue dissolved in chloroform (20 mL) and washed with aqueous sodium $NaHCO_3$ bicarbonate (20 mL) and then water (3 × 10 mL). The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to give a pale yellow oil which was purified by column chromatography on silica. Elution with chloroform, then pooling and evaporation of appropriate fractions gave the product as a white solid (0.24 g, 77%).

^{31}P NMR: δ = 14.1, 14.0 (1:1).

^1H NMR: δ = 7.2 (5H, m, Ph), 4.1 (1H, m, CHNH), 4.0 (2H, m, CH_2OP), 3.7 (3H, 2 × s, MeO), 3.5 (4H, m, CH_2N), 3.2 (4H, m, CH_2Cl), 3.0 (3H, m, NH, $PhCH_2$), 1.2 (3H, 2 × t, Me).

MS (FAB): m/z 413 (MH^+, ^{37}Cl, 9%), 411 (MH^+, 15), 353 (3), 351 (7), 270 (58), 242 (29).

Ethyl *N,N*-Bis(β-chloroethyl)amido(methoxyvalinyl)phosphate (5c):
This was prepared as described for **5b** above, using valine methyl ester hydrochloride (0.45 g, 2.68 mmol), except that two consecutive chromatographic columns were required, with elution by 2.5% MeOH in $CHCl_3$, followed by neat $CHCl_3$. Pooling and evaporation of appropriate fractions gave the product as a colourless oil (0.63 g, 65%).

^{31}P NMR: δ = 4.1 (2H, m, CH_2OP), 3.75 (3H, s, MeO), 3.7 (5H, m, Ch_2N, CHNH), 3.4 (4H, m, CH_2Cl), 3.2 (1H, 2 × t, NH), 2.1 (1H, m, Me_2CH), 1.3 (3H, 2 × t, CH_3CH_2), 0.95 (6H, m, Me_2C).

MS (FAB): m/z 365 (MH^+, ^{37}CI, 6%), 363 (MH^+, 12), 305 (6), 303 (12), 222 (100), 194 (35).

Propyl *N,N*-Bis(β-chloroethyl)amidophosphorochloridate (8b):
This ws prepared as described for $(ClCH_2CH_2)_2NP(O)(OEt)Cl$ **(8a)** above, using propyl phosphorodichloridate (1.98 g, 11.2 mmol), the product being isolated as a colourless oil (2.00 g, 63%).

^{31}P NMR: δ = 13.60.

^{13}C NMR: δ = 70.3 (d, CH_2OP, J = 6.7), 49.9 (d, CH_2N, J = 4.4), 41.6 (d, CH_2Cl, J = 2.4), 23.3 (d, CH_3CH_2, J = 8.3), 10.0 (Me).

^1H NMR: δ = 4.2 (2H, m, CH_2OP), 3.7 (4H, m, CH_2N), 3.5 (4H, m, CH_2Cl), 1.8 (2H, m, CH_2), 1.0 (3H, t, Me).

MS (FAB): m/z 308 (MNa^+, 2 × ^{37}Cl, 4%), 306 (MNa^+, ^{37}Cl, 18), 304 (MNa^+, 17), 144 (3), 142 (9), 65 (12), 63 (47).

Propyl *N,N*-Bis(β-chloroethyl)amido(methoxyglycinyl)phosphate (5d):
This was prepared as described for **5a** above, using $(ClCH_2CH_2)_2$-NP(O)(OPr)Cl **(8b)** (0.2 g, 0.71 mmol), except that the crude product was further purified by chromatography on silica, with elution by $CHCl_3$. Pooling and evaporation of appropriate fractions gave the product as a colourless oil (0.14 g, 57%).

^{31}P NMR: δ = 12.5.

^{1}H NMR: δ = 3.9 (2 H, m, CH_2OP), 3.7 (3 H, s, MeO), 3.6 (6 H, m, CH_2N, $C\underline{H}_2NH$), 3.4 (4 H, m, CH_2Cl), 3.2 (1 H, m, NH), 1.6 (2 H, m, CH_2), 0.9 (3 H, t, Me).

MS (FAB): *m/z* = 339 (MH^+, 2 × ^{37}Cl, 3.5%), 338 (2), 337 (NH^+, ^{37}Cl, 51), 336 (8), 335 (NH^+, 83), 194 (100), 152 (56).

Propyl *N,N*-Bis(β-chloroethyl)amido(methoxyphenylalaninyl)phosphate (5e):
This was prepared as described for **5d** above, using phenylalanine methyl ester hydrochloride (0.23 g, 1.06 mmol), except that hexane extraction (4 × 50 mL) was carried out on the concentrated Et_2O extracts and the chromatographic column was eluted with 20% $CHCl_3$ in light petroleum (bp 60–80 °C). Pooling and evaporation of appropriate fractions gave the product as a white solid (0.06 g, 37%).

^{31}P NMR: δ = 11.8, 11.7 (1:1).

^{1}H NMR: δ = 7.25 (5 H, m, Ph), 4.1 (1 H, m, $C\underline{H}NH$), 3.8 (2 H, m, CH_2OP), 3.7 (3 H, 2 × s, MeO), 3.5 (4 H, m, CH_2N), 3.3 (4 H, m, CH_2Cl), 3.1 (3 H, m, NH, $PhC\underline{H}_2$), 1.6 (2 H, m, CH_2), 0.9 (3 H, 2 × t, Me).

MS (EI): *m/z* 425.1147 (MH^+, 0.12%, calc. for $C_{17}H_{28}Cl_2N_2O_4P$ 425.1164), 367 (11), 365 (16), 335 (33), 333 (49), 284 (26), 91 (100).

Butyl *N,N*-Bis(β-chloroethyl)amidophosphorochloridate (8c):
This was prepared as described for $(ClCH_2CH_2)_2NP(O)(OEt)Cl$ **(8a)** above, using butyl phosphorodichloridate (0.70 g, 3.64 mmol), the product being isolated as a pale yellow oil (0.93 g, 86%).

^{31}P NMR: δ = 13.60.

^{13}C NMR: δ = 68.6 (d, CH_2OP, J = 3.9), 49.9 (d, CH_2N, J = 4.4), 41.6 (d, CH_2Cl, J = 2.1), 31.8 (d, $\underline{C}H_2CH_2OP$, J = 8.0 Hz), 18.7 ($CH_3\underline{C}H_2$), 13.5 (Me).

^{1}H NMR: δ = 4.2 (2 H, m, CH_2OP), 3.5 (8 H, m, CH_2N, CH_2Cl), 1.7 (2 H, m, CH_2), 1.4 (2 H, m, CH_2), 0.9 (3 H, t, Me)

Butyl *N,N*-Bis(β-chloroethyl)amino(methoxyvalinyl)phosphate (5f):
This was prepared as described for **5b** above, using valine methyl ester hydrochloride (0.28 g, 1.69 mmol) to give the product as a white solid (0.45 g, 69%).

^{31}P NMR: δ = 12.5, 12.3 (1:1).

^{1}H NMR: δ = 4.0 (2 H, m, CH_2OP), 3.73 (3 H, s, MeO), 3.7 (5 H, m, CH_2N, $C\underline{H}NH$), 3.4 (4 H, m, CH_2Cl), 3.1 (1 H, m, NH), 2.0 (1 H, m, $Me_2C\underline{H}$), 1.6 (2 H, m, $C\underline{H}_2CH_2OP$), 1.4 (2 H, m, $CH_3C\underline{H}_2$), 0.9 (9 H, m, Me).

MS (FAB): *m/z* = 393 (MH^+, ^{37}Cl, 15%), 391 (MH^+, 24), 333 (5), 331 (10), 250 (100).

Butyl *N,N*-Bis(β-chloroethyl)amido(methoxyphenylalaninyl)phosphate (5g):
This was prepared as described for **5b** above, using phenylalanine methyl ester hydrochloride (0.22 g, 1.01 mmol) to give the product as a white solid (0.25 g, 56%).

^{31}P NMR: δ = 11.7.

^{1}H NMR: δ = 7.25 (5 H, m, Ph), 4.2 (1 H, m, $C\underline{H}NH$), 3.9 (2 H, m, CH_2OP), 3.7 (3 H, 2 × s, MeO), 3.5 (4 H, m, CH_2N), 3.3 (4 H, m, CH_2Cl), 3.0 (3 H, m, NH, $PhC\underline{H}_2$), 1.6 (2 H, m, $C\underline{H}_2CH_2OP$), 1.3 (2 H, m, $CH_3C\underline{H}_2$), 0.9 (3 H, 2 × t, Me).

MS (FAB): *m/z* 441 (MH^+, ^{37}Cl, 16%), 439 (MH^+, 30), 298 (59), 270 (6), 120 (100), 91 (43).

Decyl *N,N*-Bis(β-chloroethyl)amidophosphorochloridate (8d):
This was prepared as described for $(ClCH_2CH_2)NP(O)(OEt)Cl$ **(8a)** above, using decyl phosphorodichloridate (2.50 g, 9.09 mmol), except that dichloromethane was used as the solvent, stirring was continued for 96 h at ambient temperature, and the crude product was extracted with Et_2O (2 × 100 mL). The product was isolated as a pale yellow oil (2.87 g, 83%).

^{31}P NMR: δ = 13.4.

^{13}C NMR: δ = 68.9 (d, CH_2OP, J = 6.5), 49.9 (d, CH_2N, J = 4.3), 41.5 (d, CH_2Cl, J = 2.5), 31.9 (CH_2), 29.0–30.2 (m, CH_2), 25.4 (CH_2), 22.6 (CH_2), 14.1 (Me).

^{1}H NMR: δ = 4.2 (2 H, m, CH_2OP), 3.6 (4 H, m, CH_2N), 3.5 (4 H, m, CH_2Cl), 1.7 (2 H, m, CH_2), 1.2 (14 H, m, CH_2), 0.9 (3 H, t, Me).

Decyl *N,N*-Bis(β-chloroethyl)amido(methoxyalaninyl)phosphate (5h):
This was prepared as described for **5a** above, using alanine methyl ester hydrochloride (0.18 g, 1.31 mmol), except that the product was further purified by column chromatography on silica. Elution with 30% $CHCl_3$ in light petroleum (bp 60–80 °C) followed by pooling and evaporation of appropriate fractions gave the product as a colourless oil (0.35 g, 60%).

^{31}P NMR: δ = 11.8, 11.4 (1:1).

^{1}H NMR: δ = 3.9 (2 H, m, CH_2OP), 3.7 (3 H, s, MeO), 3.6 (5 H, m, CH_2N, $C\underline{H}NH$), 3.4 (4 H, m, CH_2Cl), 3.2 (1 H, m, NH), 1.6 (2 H, m, CH_2), 1.4 (3 H, m, $CHC\underline{H}_3$), 1.2 (14 H, bs, CH_2), 0.8 (3 H, t, Me).

MS (FAB): *m/z* 451 (MH^+, 2 × ^{37}Cl, 4%), 449 (MH^+, ^{37}Cl, 27), 447 (MH^+, 36), 306 (59), 249 (9), 247 (16), 166 (100).

Decyl *N,N*-Bis(β-chloroethyl)amido(methoxyvalinyl)phosphate (5i):
This was prepared as described for **5h** above, using valine methyl ester hydrochloride (0.20 g, 1.19 mmol) to give the product as a colourless oil (0.28 g, 48%).

^{31}P NMR: δ = 15.0, 14.7 (2:1).

^{1}H NMR: δ = 3.9 (2 H, m, CH_2OP), 3.7 (3 H, s, MeO), 3.6 (5 H, m, 2 × CH_2N, $C\underline{H}NH$), 3.4 (4 H, m, CH_2Cl), 3.1 (1 H, m, NH), 2.0 (1 H, m, $Me_2C\underline{H}$), 1.6 (2 H, m, CH_2), 1.2 (14 H, m, CH_2), 0.9 (3 H, m, $CH\underline{Me}$), 0.85 (6 H, m, $CH\underline{Me}$).

MS (FAB): *m/z* 477 (MH^+, ^{37}Cl, 8%), 475 (MH^+, 17), 334 (70), 277 (11), 275 (23), 194 (78).

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