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Short communication

The synthesis of novel melphalan derivatives as potential antineoplastic agents

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Summary — Five derivatives of melphalan ((*S*)-4-[bis(2-chloroethyl)amino]phenylalanine), an alkylating agent presently employed as an antineoplastic in humans, were designed and synthesised as potential prodrugs. Their antitumour activity was tested against P388 leukaemia in mouse after acute intraperitoneal or oral administration and compared with that of the parent compound.

melphalan / antineoplastic / leukaemia

Introduction

Melphalan **1** (fig 1) is a well-known alkylating agent presently employed as an antineoplastic agent in humans, and is often the drug of choice in the treatment of metastatic melanoma, ovarian and breast cancer. Belonging to the nitrogen mustard family of compounds, this amino-acid is renowned for its toxicity which greatly hinders its benefits. The nitrogen mustards are cell-cycle non-specific cytotoxic anticancer drugs and hence can kill cells at all stages. Melphalan (*H*-Mel-OH) is currently administered per os (2 mg tds) and absorption is irresolute and deficient; with a half-life of 90 min in the plasma, 20–50% is recovered in the stools and up to 15% is eliminated in the urine [1–3]. In an attempt to improve the oral bioavailability and overcome some of the toxicity limitations of melphalan, we sought to synthesise and test the novel glycerol derivatives **2–4**.

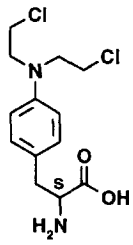

Fig 1. Structure of melphalan **1**.

In accordance with its chemical structure, melphalan may enter cells by amino-acid transporters. Hence, impairing amino-acid recognition is a possible means to less toxic melphalan derivatives and/or prodrugs [4, 5]. Indeed, various modifications of the amino group [6, 7] or the carboxylic acid function have been envisaged [8–15]. In particular, Deverre, Loiseau and coworkers [14, 15] devised a lymphotrophic, above portal [9] system, prodrug of melphalan whereby the carboxylic acid function of the amino-acid is esterified to the β-position of 1,3-dipalmitoyl glycerol. The prodrug was pharmacologically assayed by its macrofilaricidal activity [15]. Our recent investigations on the hypolipidemic agent nicotinic acid [16, 17] determined that covalently bonding this product to the α-position of long-chain diacyl glycerols, although not favouring lymphatic absorption, provided derivatives with greatly improved pharmacokinetics; a steady release of the active, parent drug into the plasma was achieved, which in the case of nicotinic acid significantly diminished the dose-dependent side effect of flushing. Here at Servier, our priority was to determine if the new α-bound diacyl glycerol derivatives of melphalan **2–4** were indeed compounds with possible therapeutic advantages over the parent amino-acid, specifically compositions with relatively diminished toxicity and necessitating an overall, lower molar dose of the alkylating component upon oral

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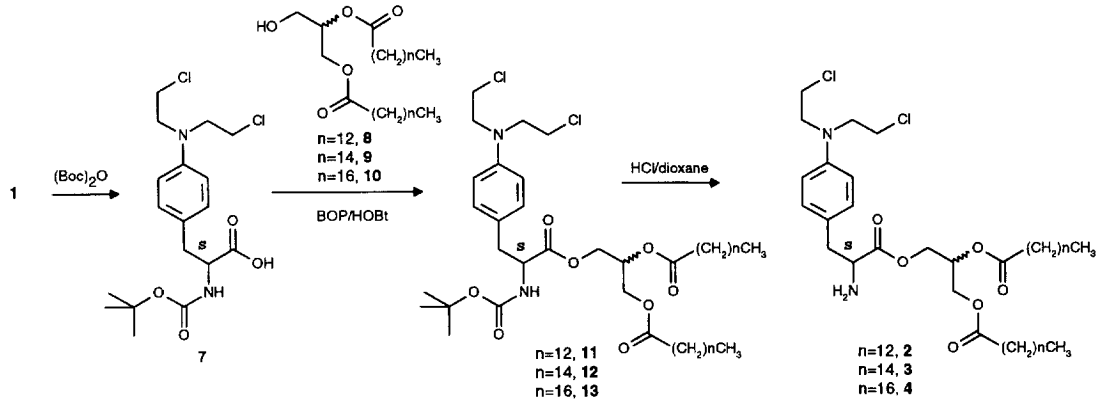
administration. Our primary findings also initiated the design and synthesis of new amide derivatives **5** and **6** by analogy with the work of Mergen et al on the anti-convulsant drug valproic acid [18].

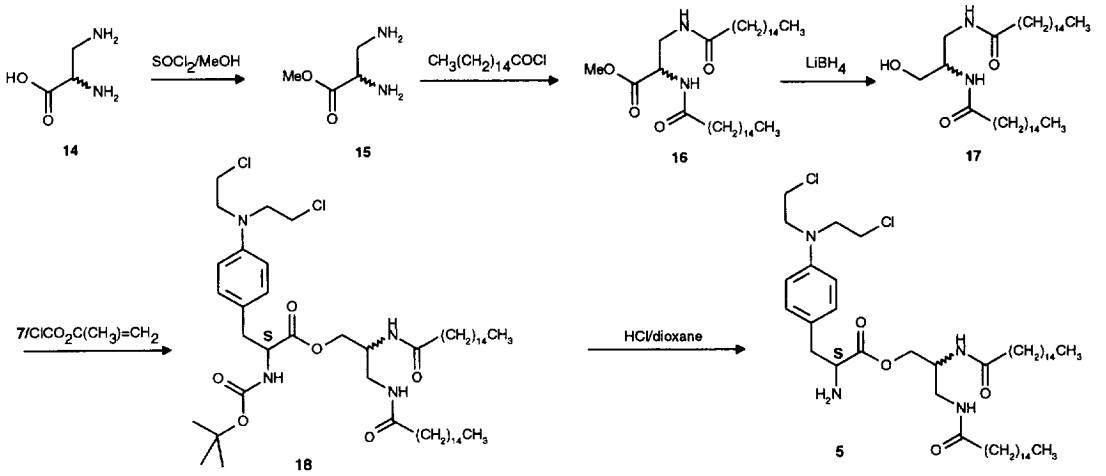
Chemistry

All compounds tested were synthesised from a common *N-tert*-butoxycarbonyl intermediate **7**. (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) mediated coupling between the commercially available, racemic primary alcohols **8–10** and the *N*-protected amino-acid **7** yielded the 1,2-dimyristoyl, 1,2-dipalmitoyl and 1,2-distearoyl glycerol derivatives **11–13**, respectively (scheme 1). Liberation of the amine function by treatment with a saturated solution of hydrogen chloride in dioxane provided the respective diastereomers **2–4** as their hydrochloride salts. No attempt was made to prepare or separate the diastereomers of **2–4**. HPLC studies suggested that adequate separation conditions would be difficult to achieve and, above all, our previous work on nicotinic acid derivatives [16, 17], where the corresponding pure enantiomers were prepared by a stereoselective synthesis, had shown that the chirality of the glycerol moiety did not influence compound absorption as the pancreatic lipase had no apparent stereospecificity.

The 1,2- and 1,3-dipalmitoyl diamide derivatives **5** and **6** were prepared as more biostable analogues of triester **3**. The penultimate synthon of **5**, alcohol **17**, was synthesised from the commercially available racemic mixture of 2,3-diaminopropionic acid **14** (scheme 2). Methyl ester formation under methanol/thionyl chloride conditions yielded the dihydrochloride salt **15**, which smoothly provided the diamide



Scheme 1.

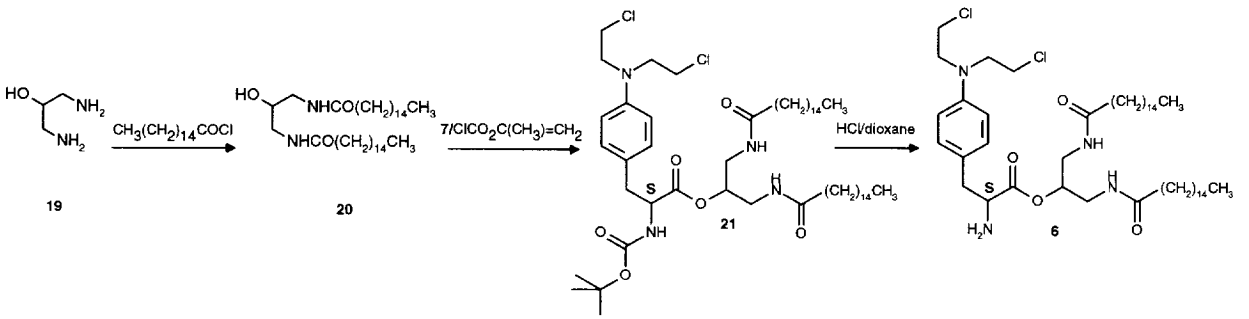


Scheme 2.

derivative **16** on reaction with palmitoyl chloride in the presence of triethylamine. Reduction of **16** with lithium borohydride gave the required alcohol **17** in an overall yield of 84%. After several unsatisfactory attempts to condense **7** with **17** using BOP and analogues, isopropenyl chloroformate was successfully employed to activate the carboxyl of **7**. The ensuing condensation reaction with **17** was enhanced by the presence of DMAP [19] and provided the protected amino-acid **18** in average yield. Using the same method as for **2–4**, compound **5** was obtained after a clean amine deprotection employing hydrogen chloride in dioxane. The *N,N'*-dipalmitoyl-1,3-diamino-2-propanol derivative **6** was isolated applying similar procedures and starting from the known alcohol **20** synthesised from 1,3-diaminopropan-2-ol **19** according to the method of Mergen [19] (scheme 3).

Pharmacology

All in vivo tests were conducted according to the protocols of Geran et al [20]. Our preliminary studies looked at the effect of acute intraperitoneal administration of compounds **2–4** on groups of P388 leukaemia cell inoculated female mice (table I) [21]. The P388 tumour employed typically develops along the lymphatic system after sc implantation. Follow up secondary studies examined the effect of **2–4** (table II) and analogues **5** and **6** (table III) after per os administration. Results are evaluated as the percentage of median survival time for the treated animals T over the control group C (T/C%), the mean body weight change (BWC) during the initial 5 or 6 days which is indicative of toxicity [20], and survival rate on day 60.



Scheme 3.

Table I. Dose–effect of compounds **1–4** after acute ip administration (day 1) on CDF1 mice ip inoculated with 10^6 P388 leukaemia cells (day 0).

Product	*Dose (mg/kg)*	*Dose (mmol/kg)*	*BWC*[a] *(g)*	*T/C*[b] *(%)*	*Survival rate on day 60*
1	15	0.049	+0.1	>560	7/8 (88%)
	30	0.098	–4.2	93	2/8 (25%)
2	40.8	0.049	+0.4	>560	7/8 (88%)
	81.7	0.098	–2.7	>560	8/8 (100%)
3	43.9	0.049	+0.2	245	2/5 (40%)
	87.8	0.098	–0.5	>560	5/5 (100%)
4	46.6	0.049	+0.5	199	0/5 (0%)
	93.3	0.098	–1.0	273	0/5 (0%)
Control	–	–	+2.6	100	0/24 (0%)

[a]BWC (body weight change) is the mean body weight variation between day 6 and day 1; [b]T/C is the ratio of the median survival time of treated animals T to the median survival time of untreated animals C in percentage.

Table II. Dose–effect of compounds **1–4** after acute po administration (day 1) on CDF1 mice ip inoculated with 10^6 P388 leukaemia cells (day 0).

Product	*Dose (mg/kg)*	*Dose (mmol/kg)*	*BWC*[a] *(g)*	*T/C*[b,c] *(%)*	*MST*[d] *(days)*
1	12.5	0.041	+1.1	154	16.0
	25	0.082	+0.1	176	18.3
	50	0.164	–2.9	212	22.0
	100	0.328	–4.9	69	7.2
2	34	0.041	+1.3	163	17.0
	68	0.081	–0.9	192	20.0
	136	0.163	–4.8	87	9.0
	272	0.325	–5.3	71	7.4
3	36.3	0.041	+0.7	171	17.8
	72.7	0.081	–1.1	195	20.3
	145.4	0.163	–4.5	75	7.8
	290.7	0.326	–5.3	74	7.7
4	38.6	0.041	+2.2	147	15.3
	77.2	0.081	+0.1	195	20.3
	154.3	0.163	–3.9	233	24.3
	308.6	0.325	–4.4	74	7.7
Control	–	–	+3.6	100	10.4

[a]BWC (body weight change) is the mean body weight variation between day 5 and day 1; [b]T/C is the ratio of the median survival time of treated animals T to the median survival time of untreated animals C in percentage; [c]in all experiments there were no surviving mice on day 60; [d]median survival time.

Table III. Dose–effect of compounds **1**, **5** and **6** after acute po administration (day 1) on CDF1 mice ip inoculated with 10^6 P388 leukaemia cells (day 0).

Product	*Dose (mg/kg)*	*Dose (mmol/kg)*	*BWC*[a] *(g)*	*T/C*[b,c] *(%)*	*MST*[d] *(days)*
1	25	0.082	–1.0	228	21.3
	50	0.164	–5.7	82	7.7
	100	0.328	–6.0	76	7.1
5	70	0.079	–0.9	204	19.0
	140	0.157	–5.1	77	7.2
	280	0.314	–5.8	76	7.1
6	70	0.079	+0.7	129	12.0
	140	0.157	–0.3	153	14.3
	280	0.314	–0.2	226	21.0
Control	–	–	+3.6	100	9.3

[a]BWC (body weight change) is the mean body weight variation between day 6 and day 1; [b]T/C is the ratio of the median survival time of treated animals T to the median survival time of untreated animals C in percentage; [c]in all experiments there were no surviving mice on day 60; [d]median survival time.

Results and discussion

When administered ip, the myristoyl derivative **2** was shown to be effectively the same as **1** at the equimolar dose of 15 mg/kg, the palmitoyl **3** and stearoyl **4** being less effective (table I). At 30 mg/kg equivalent dose, the stearoyl derivative **4** was more effective than **1** when considering T/C values but not survival rate on day 60, and was less toxic than **1** with regard to BWC and T/C. However, both **2** and **3** proved to be

far superior to **1** when considering a 100% compared to 25% survival rate on day 60 (0% for the control group of mice), a greatly increased T/C and, for the derivative **3**, little preliminary indication of significant toxicity when considering average weight change (–0.5 vs –4.2 g for **1**) and T/C values.

Disappointingly, oral administration of products **2–4** failed to provide any significant benefits over the equivalent acute doses of melphalan administered by the same route, albeit that some median survival times (MST) were slightly more favourable (table II). In fact, as **2–4** gave similar profiles to **1** after oral administration, it was considered possible that the derivatives underwent a biodegradation in the digestive tract preventing the desired absorption of the drug derivative. In an attempt to rectify this problem, we looked at stabilisation of the least toxic derivative **3** by replacement of the two palmitoyl-carrying ester functions with palmitoyl-carrying amide bonds. This kind of transformation has been employed by Mergen [18] with the aim of improving the brain bioavailability of valproic acid and the amino-acid glycine. Hence, the novel 1,2-disubstituted compound **5** and its 1,3-counterpart **6** were synthesised and then profiled after oral administration (table III).

Derivative **5** duplicated results obtained for melphalan **1**, again suggesting an early degradation in the digestive tract to the parent amino-acid. Species **6**, less effective than **1** and **5** for 25 mg/kg equivalent dosing, notably increased MST at 50 and 100 mg/kg equivalents effectively doubling and tripling the remaining life-span after initial P388 cell inoculation, respectively. However, considering BWC, it should be noted that the favourable action of **6** may be by virtue of its decreased toxicity which allows the efficacy of higher doses to be fully expressed.

Conclusion

Although compound **6** does show some advantages over melphalan, we feel that it does not sufficiently enhance T/C or MST to take it further. From the melphalan derivatives **2–4** formulated and tested, we may conclude that esterification to the α-position of long-chain diacyl glycerols, a prodrug approach that we applied successfully to nicotinic acid [16, 17], will not be a therapeutically advantageous approach in the case of oral administration of melphalan. An apparent in vivo instability of these compounds in the intestinal tract needs to be remedied, perhaps by chemical modification of an ester for an amide linkage between the amino-acid and the classically lymphotropic-carrying component [9], before it is worth performing further pharmacological evaluations.

Experimental protocols

Chemistry

General procedures

^{1}H-NMR spectra were recorded on Bruker 200 or 400 MHz spectrometers and are given in ppm relative to internal TMS. Infrared spectra were recorded on a Bruker Fourier transform spectrometer in Nujol emulsions. All new substances were monospot by TLC and exhibited spectroscopic data consistent with the assigned structures. Elemental analyses were performed on a Carlo Erba 1108 instrument and analyses indicated by the symbols of the elements are within ±0.4% of theoretical values. Melting points were obtained on a Reichert hot stage microscope and are not corrected. Silica gel 60, Merck 230–400 mesh, was used with chromatography at atmospheric pressure. TLC were performed on precoated 5 x 10 cm Merck silica gel F254 plates (layer thickness 0.25 mm).

Melphalan **1** and 1,2-dimyristoyl-*rac*-glycerol **8** were obtained from Sigma, France, and 1,2-dipalmitoyl-*rac*-glycerol **9** and 1,2-distearoyl-*rac*-glycerol **10** from Fluka, France.

(S)-N-tert-Butoxycarbonyl-4-[bis(2-chloroethyl)amino]phenylalanine 7

Di-*tert*-butyl dicarbonate (2.40 g, 11 mmol) was added to an ice-cooled mixture of melphalan **1** (3.05 g, 10 mmol), water (10 mL), dioxane (20 mL) and 1 N NaOH (11 mL). After stirring for 16 h at rt, the mixture was concentrated and the residue taken up in water, acidified to pH 2 and extracted with ethyl acetate. The organic phase was washed with water and brine, dried over anhydrous sodium sulphate, filtered and concentrated to yield the known compound **7** [14] as a paste (3.85 g, 95%). IR: 3317, 1751, 1624, 769 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.35 (s, 9H, $C(CH_3)_3$), 2.75 (dd, 1H, PhC*H*a), 2.95 (dd, 1H, PhC*H*b), 3.7 (m, 8H, $N(CH_2CH_2)_2$), 3.9 (m, 1H, C*H*CO), 6.6 (d, 2H, arom *meta*), 7.0 (d, 2H, arom *ortho*).

1,2-Dimyristoyl-3-[(S)-N-tert-butoxycarbonyl-4-[bis(2-chloroethyl)amino]phenylalanyl]glycerol 11

(*S*)-*N*-*tert*-Butoxycarbonyl-4-[bis(2-chloroethyl)amino]phenylalanine **7** (3.24 g, 8 mmol), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (3.54 g, 8 mmol), 1-hydroxybenzotriazole (1.08 g, 8 mmol) and 1,2-dimyristoyl-*rac*-glycerol **8** (4.10 g, 8 mmol) were combined in CH_2Cl_2 (50 mL). *N*,*N*-Diisopropylethylamine (2.787 mL, 16 mmol) was added and the mixture stirred at rt overnight. The reaction was diluted with CH_2Cl_2 and the organic phase washed with $NaHCO_3$ and brine, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (eluent CH_2Cl_2) to yield oil **11** (3.74 g, 52%). An analytically pure sample was obtained by rinsing, followed by decanting, with isopropanol: IR: 3442–3388, 1745, 1718 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.9 (m, 6H, $2((CH_2)_{12}CH_3)$), 1.25 (m, 40H, $2((CH_2)_{10}CH_3)$), 1.4 (s, 9H, $C(CH_3)_3$), 1.6 (m, 4H, $2(OCOCH_2CH_2)$), 2.3 (m, 4H, $2(OCOCH_2)$), 3.0 (m, 2H, CH_2Ph), 3.6–3.7 (2m, 4H + 4H, $2(CH_2CH_2Cl)$), 4.05–4.4 (m, 4H, OCH_2CHCH_2O), 4.55 (m, 1H, N*H*), 4.9 (br s, 1H, NC*H*CO), 5.2 (m, 1H, OCH_2CHCH_2O), 6.6 (d, 2H, arom), 7.0 (d, 2H, arom). Anal $C_{49}H_{84}Cl_2N_2O_8$ (C calcd 65.38, found 64.81%, H, Cl, N).

1,2-Dipalmitoyl-3-[(S)-N-tert-butoxycarbonyl-4-[bis(2-chloroethyl)amino]phenylalanyl]glycerol 12

The procedure for compound **11** was followed using 1,2-dipalmitoyl-*rac*-glycerol **9** (4.55 g, 8 mmol) to yield low melting solid **12** (2.83 g, 37%), mp 36 °C. IR: 3443, 1746, 1720, 1520,

1167 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.9 (m, 6H, 2($(CH_2)_{14}CH_3$)), 1.1–1.4 (m, 48H, 2($(CH_2)_{12}CH_3$)), 1.4 (s, 9H, C(CH_3)$_3$), 1.6 (m, 4H, 2($OCOCH_2CH_2$)), 2.3 (m, 4H, 2($OCOCH_2$)), 3.0 (m, 2H, CH_2Ph), 3.5–3.8 (m, 8H, 2(CH_2CH_2Cl)), 4.0–4.4 (m, 4H, OCH_2CHCH_2O), 4.5 (m, 1H, NH), 4.9 (m, 1H, NCHCO), 5.2 (m, 1H, OCH_2CHCH_2O), 6.6 (d, 2H, arom), 7.0 (d, 2H, arom). Anal $C_{53}H_{92}Cl_2N_2O_8$ (C, H, Cl, N).

1,2-Distearoyl-3-[(S)-N-tert-butoxycarbonyl-4-[bis(2-chloroethyl)amino]phenylalanyl]glycerol ***13***

The procedure for compound **11** was employed using 1,2-distearoyl-*rac*-glycerol (4.99 g, 8 mmol) to yield solid **13** (1.46 g, 18%), mp 52 °C. IR: 3442, 1745, 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.85 (m, 6H, 2($(CH_2)_{16}CH_3$)), 1.1–1.4 (m, 56H, 2($(CH_2)_{14}CH_3$)), 1.4 (s, 9H, C(CH_3)$_3$), 1.6 (m, 4H, 2($OCOCH_2CH_2$)), 2.3 (m, 4H, 2($OCOCH_2$)), 2.95 (m, 2H, CH_2Ph), 3.55–3.75 (m, 8H, 2(CH_2CH_2Cl)), 4.0–4.45 (m, 4H, OCH_2CHCH_2O), 4.55 (m, 1H, NH), 4.9 (m, 1H, NCHCO), 5.2 (m, 1H, OCH_2CHCH_2O), 6.6 (d, 2H, arom), 7.0 (d, 2H, arom). Anal $C_{57}H_{100}Cl_2N_2O_8$ (C, H, Cl, N).

1,2-Dimyristoyl-3-[(S)-4-[bis(2-chloroethyl)amino]phenylalanyl]glycerol ***2***

1,2-Dimyristoyl-3-{(*S*)-*N-tert*-butoxycarbonyl-4-[bis(2-chloroethyl)amino]phenylalanyl}glycerol **11** (900 mg, 1 mmol) was stirred with a saturated solution of HCl in dioxane (10 mL) over 10 min at rt. Excess HCl gas and dioxane was removed under a stream of argon. The residue was then washed several times with pentane before drying under vacuum to provide **2** as a white hydrochloride salt (803 mg, 96%), mp 91–92 °C. IR: 3200–2000, 1753 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 0.85 (m, 6H, 2($(CH_2)_{12}CH_3$)), 1.1–1.35 (m, 40H, 2($(CH_2)_{10}CH_3$)), 1.5 (m, 4H, 2($OCOCH_2CH_2$)), 2.35 (m, 4H, 2($OCOCH_2$)), 2.95 (m, 2H, CH_2Ph), 3.7 (m, 8H, 2(CH_2CH_2Cl)), 3.95–4.4 (m, 5H, OCH_2CHCH_2O, NCHCO), 5.15 (m, 1H, OCH_2CHCH_2O), 6.7 (d, 2H, arom), 7.05 (d, 2H, arom). Anal $C_{44}H_{76}Cl_2N_2O_6$•HCl (C calcd 63.18 found 62.65%, H, Cl, N).

1,2-Dipalmitoyl-3-[(S)-4-[bis(2-chloroethyl)amino]phenylalanyl]glycerol ***3***

Using 1,2-dipalmitoyl-3-{(*S*)-*N-tert*-butoxycarbonyl-4-[bis(2-chloroethyl)amino]phenylalanyl}glycerol **12** (956 mg, 1 mmol) as starting substrate the same procedure was employed as for **2**, to yield **3** as a white hydrochloride salt (669 mg, 75%), mp 82–84 °C. IR: 3100–2200, 2029, 1753 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 0.85 (m, 6H, 2($(CH_2)_{12}CH_3$)), 1.1–1.40 (m, 48H, 2($(CH_2)_{12}CH_3$)), 1.5 (m, 4H, 2($OCOCH_2CH_2$)), 2.25 (m, 4H, 2($OCOCH_2$)), 3.0 (m, 2H, CH_2Ph), 3.7 (m, 8H, 2(CH_2CH_2Cl)), 3.9–4.4 (m, 5H, OCH_2CHCH_2O, NCHCO), 5.15 (m, 1H, OCH_2CHCH_2O), 6.7 (d, 2H, arom *meta*), 7.05 (d, 2H, arom *ortho*). Anal $C_{48}H_{84}Cl_2N_2O_6$•HCl (C, H, Cl, N).

1,2-Distearoyl-3-[(S)-4-[bis(2-chloroethyl)amino]phenylalanyl]glycerol ***4***

Using the procedure employed for the formation of **2** and starting from 1,2-distearoyl-3-{(*S*)-*N-tert*-butoxycarbonyl-4-[bis(2-chloroethyl)amino]phenylalanyl}glycerol **13** (1.01 g, 1 mmol), compound **4** was produced as the hydrochloride salt (655 mg, 69%), mp 79–81 °C. IR: 3000–2300, 2000, 1753 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 0.8 (t, 6H, 2($(CH_2)_{16}CH_3$)), 1.2 (m, 56H, 2($(CH_2)_{14}CH_3$)), 1.5 (m, 4H, 2($OCOCH_2CH_2$)), 2.1–2.3 (m, 4H, 2($OCOCH_2$)), 3.0 (m, 2H, CH_2Ph), 3.7 (m, 8H, 2(CH_2CH_2Cl)), 4.0–4.4 (m, 5H, OCH_2CHCH_2O, NCHCO), 5.15 (m, 1H, OCH_2CHCH_2O), 6.7 (d, 2H, arom), 7.05 (d, 2H, arom). Anal $C_{52}H_{92}Cl_2N_2O_6$•HCl• 0.7% H_2O (C, H, Cl, N).

2,3-Diaminopropionic acid methyl ester dihydrochloride ***15***

Compound **15** was synthesised using a modification of the procedure described by Egbertson [22]. The hydrochloride salt of 2,3-diaminopropionic acid **14** (5.00 g, 35.6 mmol) was combined with methanol (200 mL) and cooled in an ice/water bath. Thionyl chloride (10.4 mL, 142 mmol) was added dropwise over 30 min and the mixture allowed to warm to rt before heating to reflux overnight. The mixture was concentrated and the residue stirred with a large volume of pentane. Filtration and drying yielded the title compound **15** as its analytically pure, white dihydrochloride salt (6.79 g, 100%), mp 177–178 °C decomp [22].

2,3-Bishexadecanoylaminopropionic acid methyl ester ***16***

2,3-Diaminopropionic acid methyl ester dihydrochloride **15** (3.82 g, 20 mmol), dichloromethane (200 mL) and triethylamine (13.64 mL, 0.1 mol) were stirred at rt for 10 min and then cooled to 0 °C. A solution of palmitoyl chloride (10.96 mL, 40 mmol) in dichloromethane (10 mL) was added over 2 min, the mixture left with stirring for 30 min and then filtered. The filtrate was washed with 1 N HCl; 1 N NaOH, water and brine, dried over $MgSO_4$ and concentrated to yield 2,3-bishexadecanoylaminopropionic acid methyl ester **16** (11.42 g, 96%), mp 99–101 °C. IR: 3000–2400, 1703, 941 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.85 (t, 6H, 2(CH_2CH_3)), 1.15–1.35 (m, 48H, 2($(CH_2)_{12}CH_3$)), 1.6 (m, 4H, 2($COCH_2CH_2$)), 2.1–2.3 (2t, 4H, 2($COCH_2$)), 3.65 (m, 2H, CH_2NH), 3.8 (s, 3H, OCH_3), 4.65 (m, 1H, CHNH), 6.05 (t, 1H, CH_2NH), 6.75 (d, 1H, CHNH). Anal $C_{36}H_{70}N_2O_4$ (C, H, N).

2,3-Bishexadecanoylamino-1-propanol ***17***

Lithium borohydride (0.6 g, 27.5 mmol) and tetrahydrofuran (60 mL) were combined and cooled to 0 °C. 2,3-Bishexadecanoylaminopropionic acid methyl ester **16** (6.0 g, 10 mmol) was added cautiously in small portions and then the mixture allowed to warm to rt over 1.5 h. The reaction was hydrolysed using H_2O (20 mL) and a white solid filtered off, washed with H_2O and dried under vacuum to yield the alcohol **17** (4.93 g, 87%), mp 107–108 °C. IR: 3327, 1637 cm^{-1}; ^{1}H-NMR ($CDCl_3$) (it is possible that the OH of **17** resonates between 1.4–1.8 ppm, however the corresponding signal is masked by the aliphatic proton resonances): δ 0.9 (m, 6H, (CH_2CH_3)), 1.25 (m, 48H, 2($(CH_2)_{12}CH_3$)), 1.6 (m, 4H, 2($COCH_2CH_2$)), 2.2 (m, 4H, 2($COCH_2$)), 3.25 (m, 1H, CHHN), 3.4–3.7 (m, 3H, OCH_2 and CHHN), 3.85 (m, 1H, OCH_2CH), 6.05 (t, exchangeable by D_2O, 1H, CH_2NHCO), 6.3 (d, exchangeable by D_2O, 1H, CHNHCO). Anal $C_{35}H_{70}N_2O_3$ (C, H, N).

N,N'-Dipalmitoyl-1-[(S)-N''-tert-butoxycarbonyl-4-[bis(chloroethyl)amino]phenylalanyl]oxo-2,3-diaminopropane ***18***

(*S*)-*N-tert*-Butoxycarbonyl-4-[bis(2-chloroethyl)amino]phenylalanine **7** (3.24 g, 8 mmol), 2,3-bishexadecanoylamino-1-propanol **17** (4.99 g, 8.8 mmol), triethylamine (1.23 mL, 8.8 mmol) and 4-dimethylaminopyridine (538 mg, 4.4 mmol) were combined and stirred for 20 min in tetrahydrofuran (40 mL) at 35 °C. Isopropenyl chloroformate (962 μL, 8.8 mmol) was added and the mixture stirred for a further 1.5 h. The reaction mixture was concentrated and taken up in 100 mL of dichloromethane. After washing with water, 1 N $NaHCO_3$ and brine, the organic phase was concentrated and the residue purified by column chromatography (CH_2Cl_2/EtOH 9:1) providing the title compound **18** as a white solid (4.28 g, 56%), mp 79–80 °C. IR: 3309, 1750, 1643, 1547 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.9 (m, 6H, 2(CH_2CH_3)), 1.15–1.4 (m, 48H, 2($(CH_2)_{12}CH_3$)), 1.4 (s, 9H, C(CH_3)$_3$), 1.6 (m, 4H,

2($COCH_2CH_2$)), 2.15 (m, 4H, 2($COCH_2$)), 2.8–3.6 (2m, 2H + 3H, CH_2Ph + OCH_2CHCH_2), 3.6–3.8 (m, 8H, $N(CH_2CH_2Cl)_2$), 4.0–4.5 (2m, 1H + 2H, NCHCO + OCH_2CH), 4.9 (d, 1H, NHCOO), 6.0–6.5 (broad m, exchangeable by D_2O, 2H, 2(CH_2CONH)), 6.65 (d, 2H, arom *meta*), 7.05 (d, 2H, arom *ortho*). Anal $C_{53}H_{94}Cl_2N_4O_6$ (C calcd 66.71, found 67.80%, Cl calcd 7.43, found 6.68%, H, N).

N,N'-Dipalmitoyl-1-{(S)-4-[bis(chloroethyl)amino]phenylalanyl}oxo-2,3-diaminopropane hydrochloride **5**

Compound **18** (3.82 g, 4.0 mmol) was stirred at rt with a saturated solution of HCl in dioxane (40 mL). The required product was isolated as described for **2** providing the white hydrochloride salt **5** (2.49 g, 70%), mp 84–85 °C. IR: 3294, 3000–2000, 1747, 1643, 1543 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 0.85 (m, 6H, 2(CH_2CH_3)), 1.15–1.35 (m, 48H, 2($(CH_2)_{12}CH_3$)), 1.5 (m, 4H, 2($COCH_2CH_2$)), 2.1 (m, 4H, 2($COCH_2$)), 3.05 (m, 2H, CH_2Ph), 3.15 (m, 2H, CH_2NH), 3.7 (m, 8H, $N(CH_2CH_2Cl)_2$), 4.0–4.2 (m, 4H, OCH_2CH and H_2NCH), 6.75 (d, 2H, arom *meta*), 7.1 (d, 2H, arom *ortho*), 7.6–7.9 (m, exchangeable by D_2O, 2H, 2(NHCO)), 8.3–8.5 (m, 3H, NH_3^+). Anal $C_{48}H_{86}Cl_2N_4O_4$•HCl (C, H, N, calcd for Cl^- 3.98, found 4.40%).

N,N'-Dipalmitoyl-2-{(S)-N''-tert-butoxycarbonyl-4-[bis(chloroethyl)amino]phenylalanyl}oxo-1,3-diaminopropane **21**

1,3-Bishexadecanoylamino-2-propanol **20** (3.74 g, 6.6 mmol), prepared in 60% yield according to the procedure of Mergen et al [19], was combined with (*S*)-*N*-*tert*-butoxycarbonyl-4-[bis(2-chloroethyl)amino]phenylalanine **7** (2.43 g, 6 mmol), triethylamine (1.76 mL, 12.6 mmol) and 4-dimethylaminopyridine (440 mg, 3.6 mmol) in toluene (80 mL) and heated to 65 °C for 15 min. Isopropenyl chloroformate (721 μL, 6.6 mmol) was added dropwise and the mixture stirred for a further 1.3 h before removing the volatiles under reduced pressure. The residue was taken up in dichloromethane and washed with water, 1 N $NaHCO_3$ and saturated NaCl. The organic phase was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (CH_2Cl_2/EtOH 98:2) providing the title compound **21** as a white solid (3.09 g, 54%), mp 88–89 °C. IR: 3429, 3359, 1736, 1693, 1523 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.9 (m, 6H, 2(CH_2CH_3)), 1.1–1.6 (m + s, 48H + 9H, 2($(CH_2)_{12}CH_3$) + $C(CH_3)_3$), 1.85 (m, 4H, 2($COCH_2CH_2$)), 2.15–2.3 (m, 4H, 2($COCH_2$)), 2.8–3.2 (m, 2H, CH_2Ph), 3.2–3.6 (m, 4H, 2(CH_2NH)) 3.6–3.8 (m, 8H, $N(CH_2CH_2Cl)_2$), 4.3 (m, 1H, NCHCO), 4.7–4.9 (m, 1H, OCH), 4.95 (d, 1H, CHNH), 6.2–6.65 (m, 2H, 2(CH_2NH)), 6.65 (d, 2H, arom *meta*), 7.05 (d, 2H, arom *ortho*). Anal $C_{53}H_{94}Cl_2N_4O_6$ (C, H, Cl, N).

1,3-N,N'-Dipalmitoyl-2-{(S)-4-[bis(chloroethyl)amino]phenylalanyl}oxo-1,3-diaminopropane **6**

Compound **21** (2.86 g, 3 mmol) was deprotected using the procedure described for **2**, employing a saturated solution of HCl in dioxane (30 mL). The required compound **6** was obtained as a white hydrochloride salt (2.00 g, 75%), mp 128–130 °C. IR: 3500–3100, 3000–2000, 1751, 1649, 1530 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 0.85 (m, 6H, 2(CH_2CH_3)), 1.1–1.4 (m, 48H, 2($(CH_2)_{12}CH_3$)), 1.5 (m, 4H, 2($COCH_2CH_2$)), 2.1 (m, 4H, 2($COCH_2$)), 2.9–3.5 (m, 6H, CH_2Ph + 2(CH_2NH)), 3.7 (m, 8H, $N(CH_2CH_2Cl)_2$), 4.1 (m , 1H, NCHCO), 4.85 (m, 1H, OCH), 6.7 (d, 2H, arom), 7.1 (d, 2H, arom *ortho*), 7.8–8.0 (m, 2H, 2(CH_2NH)), 8.3–8.5 (m, 2H, $CHNH_2$). Anal $C_{48}H_{86}Cl_2N_4O_4$•HCl (C, H, Cl, N).

Pharmacology

All pharmacological tests were conducted according to the published protocols of Geran et al [20]. On day 1, 10^6 Leukaemia cells (P388) were injected into the peritoneal cavity of CDF1 female mice weighing between 18 and 22 mg. Products were administered in a single dose, ip or po, on day 1. Product activity was assessed by the ratio of median survival time in the treated group of mice to median survival time in the untreated group (T/C%). In accord with Geran et al [20], a T/C ratio superior or equal to 125% is required to consider a product active, and a T/C inferior or equal to 95 % indicates the presence of a general toxicity.

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References

1 Calabresi P, Chabner BA (1990) In: *The Pharmacological Basis of Therapeutics Eighth Edition* (Goodman Gilman A, Rall TW, Nies AS, Taylor P, eds) Pergamon Press, USA, 1209
2 Reece PA, Dale BM, Morris RG et al (1987) *Cancer Chemother Pharmacol* 20, 256–258
3 Furner RL, Brown RK (1980) *Cancer Treat Rep* 64, 559–574
4 Miller L, Deffie AM, Bose R (1992) *Biochem Pharmacol* 43, 1154–1158
5 Cornford EM, Young D, Paxton JW, Finlay GJ, Wilson WR, Pardridge WM (1992) *Cancer Res* 52, 138–143
6 Kerr DE, Senter PD, Burnett WV, Hirschberg DL, Hellstrom I, Hellstrom KE (1990) *Cancer Immunol Immunother* 31, 202–206
7 Vrudhula VM, Senter PD, Fischer KJ, Wallace PM (1993) *J Med Chem* 36, 919–923
8 Kumar R, Billimoria JD (1978) *J Pharm Pharmacol* 30, 754–758
9 Garzon-Aburbeh A, Poupaert JH, Claesen M, Dumont P, Atassi G (1983) *J Med Chem* 26, 1200–1203
10 Garzon-Aburbeh A, Poupaert JH, Claesen M, Dumont P (1986) *J Med Chem* 29, 687–691
11 Sugihara J, Furuuchi S, Ando H, Takashima K, Harigaya S (1988) *J Pharmacobio Dyn* 11, 555–562
12 Sugihara J, Furuuchi S, Nakano K, Harigaya S (1988) *J Pharmacobio Dyn* 11, 369–376
13 Saraiva Goncalves JC, Razzouk C, Poupaert JH, Dumont P (1989) *J Chromatogr* 494, 389–396
14 Deverre JR, Loiseau P, Puisieux F et al (1992) *Arzneim-Forsch/Drug Res* 42, 1153–1156
15 Loiseau PM, Deverre JR, El Kihel L, Gayral P, Letourneux Y (1994) *J Chemother* 6, 230–237
16 Cordi A, Lacoste JM, Duhault J et al (1995) *Arzneim-Forsch/Drug Res* 45, 997–1001
17 Cordi AA, Duhault J, Laudignon N, Castagne N (1996) *Drugs Future* 21, 490–492
18 Mergen F, Lambert DM, Goncalves Saraiva JC, Poupaert J, Dumont P (1992) *J Pharm Pharmacol* 43, 815–816
19 Mergen F, Lambert DM, Poupaert JH, Bidaine A, Dumont P (1991) *Chem Phys Lip* 59, 267–272
20 Geran RI, Greenberg NH, Macdonald MM, Schumacher AM, Abott BJ (1972) *Cancer Chemother Rep Part 3*, 3, 1–103
21 Tsuruo T, Naganuma K, Iida H, Tsukagoshi S (1980) *Cancer Res* 40, 4758–4763
22 Egbertson MS, Homnick CF, Hartman GD (1993) *Syn Comm* 23, 703–709