Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 325–334

BIOORGANIC & MEDICINAL CHEMISTRY

New Nitrogen Mustards Structurally Related to (L)-Carnitine

Ludovic Faissat, Katja Martin, Claude Chavis, Jean-Louis Montéro and Marc Lucas*

ENSCM, Laboratoire de Chimie Biomoléculaire, UMR 5032, 8 Rue de l'Ecole Normale, 34296 Montpellier Cédex 05, France

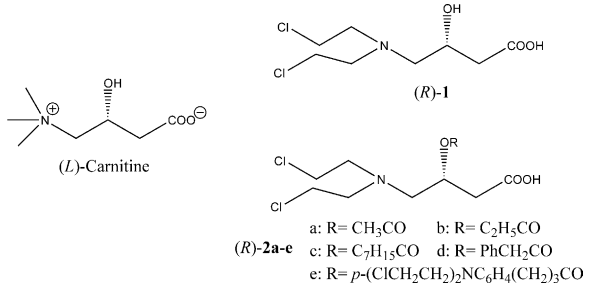
Received 10 July 2002; accepted 19 September 2002

Abstract—Enantiopure nitrogen mustards which mimic (L)-carnitine framework are prepared by a multi-step synthesis from the (*R*)-di-*tert*-butyl malate and their antitumor properties evaluated.

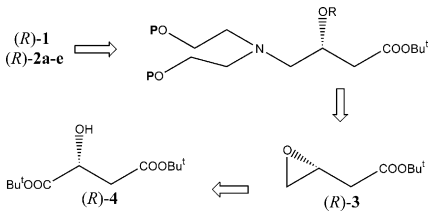
The nitrogen mustards are among the earliest more effective antitumor drugs used in human cancer chemotherapy.[1] Actually, Mechlorethamine and Chlorambucil are two of the most currently used anticancer agents, and these cytotoxic drugs are believed to exert their biological activity by inducing interstrand cross-links in the major groove of DNA and this represents the most toxic of all alkylation events.[2,3] Numerous nitrogen mustard structural modifications have been made during the last 30 years in order to increase their cytotoxicity and their target affinity. In this respect, we designed new nitrogen mustards structurally related to (L)-carnitine devoted to the specific tumor cells mitochondrial DNA target. This idea was supported by the fact that (L)-carnitine and some of its *O*-alkyl derivatives have been demonstrated to play a crucial role in the transport and the β-oxidation of long-chain fatty acids into mitochondria[4–6] and that a number of reports indicated that mitochondria are potentially attractive targets for multiple classes of DNA damaging agent.[7–10] An increase in the pool of mitochondria[11] and a strong expression of the carnitine transporter[12] in cancer cells provides an opportunity for tumor mitochondrial DNA-selective alkylation.

We herein disclose the synthesis of the new structurally modified carnitine analogues (*R*)-**1** and (*R*)-**2a–e** (Scheme 1), where the trimethylammonium moiety is replaced by a bis(2-chloroethyl)amine, keeping the required L-configuration to maintain as much as possible the molecular recognition properties of (L)-carnitine; the in vivo protonation of the bis(2-chloroethyl)amino group or the nitrogen quaternarisation during the formation of the requisite alkylating aziridinium cation will give the necessary positive ion related to the trimethylammonium moiety of carnitine. The acyl groups are introduced on the alcoholic function of mustard **1** in order to enhance the lipophilicity of these molecules (**2a–e**) and their cell penetration and because of the lack of toxicity of some *O*-acyl carnitine derivatives when orally administrated as human nutritional supplements has been demonstrated.

Our retrosynthetic plan (Scheme 2) follows the route proposed by Boots et al.[13] for the preparation of (*R,S*)-carnitine and its key step is the regioselective ring-



Scheme 1.



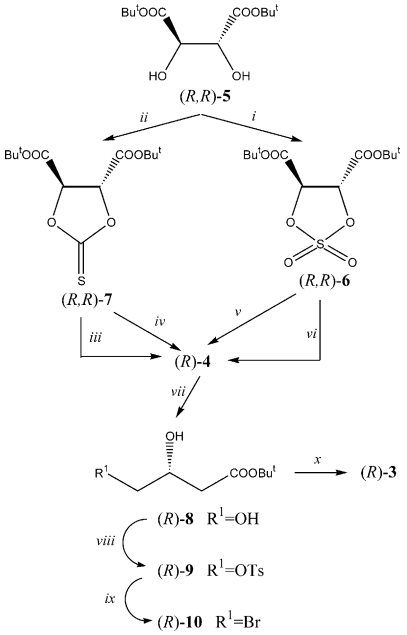
Scheme 2.

*Corresponding author. Tel.: +33-4-6714-4342; fax: +33-4-6714-4344; e-mail: lucas@univ-montp2.fr

0968-0896/03/$ - see front matter
PII: S0968-0896(02)00458-3

opening of the chiral epoxyester (*R*)-**3** by a protected bis(hydroxyethyl)amine used as a masked nitrogen mustard moiety. The *tert*-butyl carboxylate moiety of **3** was chosen to prevent amide formation during the epoxide ring-opening step. Moreover basic conditions are no longer required during the last ester hydrolysis step which could lead to a possible partial racemisation of the target molecules. The enantiopure *tert*-butyl epoxybutyrate (*R*)-**3** was obtained by a multi-step sequence from the di-*tert*-butyl malate (*R*)-**4** (Scheme 3).

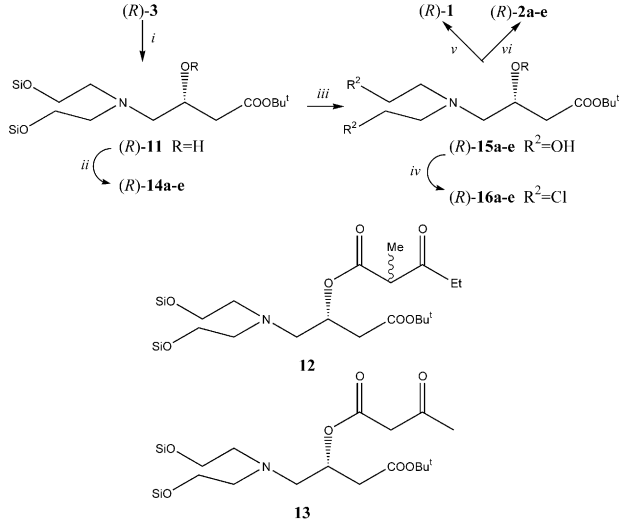
The direct esterification of (*R*)-malic acid was rather disappointing and afforded the corresponding di-*tert*-butyl malate in very poor yield;[14–16] thus, the cyclic sulfate (*R,R*)-**6** and thioxocarbonate (*R,R*)-**7** derived from natural tartaric acid were selected as useful intermediates known[17–20] to give efficient access to the unnatural (*R*)-dialkyl malates (Me, Et, Bu^n, Pr^i). The (*R,R*)-di-*tert*-butyl tartrate **5**[21] was converted by standard procedures[22,23] into the cyclic sulfate (*R,R*)-**6** and thioxocarbonate (*R,R*)-**7** already described by us[24] in, respectively, 82 and 60% yields (Scheme 3). We then subjected **6** to a deoxygenation reaction[23] using either sodium borohydride or sodium cyanoborohydride followed by a mild acid catalysed hydrolysis[25] of the resulting β-sulfate intermediate, in order to afford the (*R*)-di-*tert*-butyl malate **4** in 56 and 80% yields, respectively. Moreover, the less reactive cyclic thioxocarbonate **7** was also assessed for its suitability toward two radical deoxygenation reactions using either tri-*n*-butyltin hydride/AIBN[17] or magnesium iodide,[19] and obtained the (*R*)-di-*tert*-butyl malate **4** in lower yields, 25 and 32%, respectively. It thus appeared that the cleaner reaction and the best yields were obtained with the sodium cyanoborohydride reduction of the cyclic sulfate (*R,R*)-**6**. These results demonstrate that the transformation of the cyclic sulfate and thioxocarbonate of dialkyl tartrates into the corresponding malates can be successfully applied to di-*tert*-butyl tartrate.



Scheme 3. Reagents and conditions: (i), (ii) see ref 24; (iii) $NaBH_4$, DMA, rt, then concd H_2SO_4 (cat.), H_2O (0.5–1.0 equiv), THF, 56%; (iv) $NaBH_3CN$, THF, reflux, then concd H_2SO_4 (cat.), H_2O (0.5–1.0 equiv), THF, 80%; (v) $HSnBu_3$, AIBN, benzene, reflux, 25%; (vi) MgI_2, CH_3CN, reflux, 32%; (vii) $Me_2S.BH_3$, $NaBH_4$ (cat.), THF, then EtOH, PTSA, 55%; (viii) TsCl, Et_3N, CH_2Cl_2, 0 °C; (ix) LiBr, 2-butanone, reflux, 66% (two steps from **8**); (x) Ag_2O, DME, reflux, 37%.

In addition, it is known that the regioselective Saito reduction of α-hydroxy esters proceeds fairly well with methyl,[26] ethyl,[27] isopropyl[27] and benzyl[28] esters, but to the best of our knowledge, no attempts have been reported in the literature with substrates containing a bulkier *tert*-butyl ester. The Saito reduction conditions (borane-methyl sulfide complex/sodium borohydride cat.) gave cleanly the *tert*-butyl dihydroxy-3,4-butyrate (*R*)-**8** in 55% yield from (*R*)-**4**. Monotosylation of the diol **8** produced the 4-tosyloxy derivative (*R*)-**9** which failed to afford the epoxide **3** when subjected to alkaline conditions (potassium carbonate, methanol), but instead gave the *tert*-butyl 4-hydroxy butenoate, a result in accordance with earlier reports[29–32] about the ability of β,γ-epoxy esters to isomerise easily into α,β-unsaturated γ-hydroxyesters. This problem was overcome by converting the tosylate **9** into the bromohydrin (*R*)-**10** (66% from **8**) which finally gave successfully the desired epoxide by using silver(I) oxide/DME (37%);[32] the epoxy ester (*R*)-**3** displayed an optical rotation in good accordance with the value reported by Liu et al.,[33] who recently described an efficient hydrolytic kinetic resolution of racemic epoxide **3** by using the (*R,R*)-salen Co(III) Jacobsen catalyst.[34]

The silica gel-mediated ring opening of epoxide (*R*)-**3** (Scheme 4) by the bis(*tert*-butyldiphenylsilyloxyethyl)-amine[24] gave the γ-amino β-hydroxyester (*R*)-**11** in 50% yield. At this stage, different acyl groups (acetyl, pro-



Si= TPDPS; a: R= CH_3CO; b: R= C_2H_5CO; c: R= $C_7H_{15}CO$; d: R= $PhCH_2CO$; e: R= *p*-$(ClCH_2CH_2)_2NC_6H_4(CH_2)_3CO$

Scheme 4. Reagents and conditions: (i) bissilylated amine, SiO_2, CH_3CN, reflux, 50%; (ii) anhydride or acid chloride, Et_3N, DMAP (cat.), CH_2Cl_2, reflux; 58–95% for **14a–e**; $PhCH_2COOH$ or Chlorambucil, 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, CH_2Cl_2, rt for **14d** (76%) and **14e** (52%); (iii) TBAF/THF/imidazole, rt; (iv) CH_2Cl_2, Et_3N, DMAP, PTSCl, rt, 34–51% (two steps from **14a–e**); (v) aqueous HCl 2.4 M, THF, rt, 75%; (vi) TFA, CH_2Cl_2, rt, 50–76%.

pionyl, capryloyl, phenylacetyl and Chlorambucil-1-yl) were introduced using appropriate anhydrides (mixed anhydrides in the case of **14d–e**) or acid chlorides and afforded the expected *O*-acyl aminohydroxyesters (*R*)-**14a–e** in yields ranging from 52 to 95%. It is worth noting that reactions performed with capryloyl and propionyl chlorides gave competitively a by-product (up to 15%) whose structure was assigned by ^{1}H and ^{13}C nmr to a diastereomeric mixture of β-ketoesters **12** (formula shown for the propionyl moiety), presumably resulting from a partial acylation of the alcoholic moiety through the very reactive β-lactone dimer by-product generated from ketene under the reaction conditions used with acyl chlorides.[35,36] On the other hand, the same experimental conditions used with acetyl chloride were unfavourable for the generation of diketene[37] itself from the very volatile ketene and this explains the observed exclusive formation of the *O*-acetyl aminohydroxyester (*R*)-**14a** and the lack of acetylacetonate derivative **13** from (*R*)-**11**.

As substantial deacylation occurred when the desilylation of ethers (*R*)-**14a–e** was carried out with TBAF/THF, the reaction was performed by using modified conditions, that is TBAF/THF/imidazole[38] which gave the bis(2-hydroxyethyl)amine derivatives (*R*)-**15a–e** which were prone to very fast acyl migration on standing. Therefore, they were immediately converted into the corresponding dichlorides (*R*)-**16a–e** in 22–51% yields by a classical procedure[39] (*p*-toluenesulfonyl chloride/DMAP). A final acidic hydrolysis step, gave according to the experimental conditions, either the fully deprotected nitrogen mustard (*R*)-**1** (20% aq HCl/anisole)[40] (75% yield) or the *O*-acyl nitrogen mustards (*R*)-**2a–e** (TFA/anisole)[41] (50–87% yields).

Biological Results

The in vitro antitumor activities of the nitrogen mustards **2a–e** were evaluated against the A375 human melanoma, HT29 resistant type colon carcinoma and MCF7 human breast carcinoma cells and were compared with the cytotoxic activity of Melphalan and Chlorambucil, chosen as references (Table 1). The parent nitrogen mustard **1** containing a free hydroxylic function showed a low cytotoxicity (IC_{50} 870 μM) against A375 cell line whereas its *O*-acyl derivatives **2b–e** exhibited a significant inhibitory activity, especially **2e** with a IC_{50} of 193 μM close to that of Chlorambucil (IC_{50} 202 μM). A fairly good correlation appears between the antitumor activity of compounds **2b–e** and the lipophilicity (log P)[42] of their acyl chain; the presence of either a long alkyl chain (C_7H_{15}, **2c**) or a very lipophilic moiety (Chlorambucil, **2e**) proved to be effective in increasing the cytotoxic activity by a factor up to 4 when compared with the parent compound **1**. These results may account for the fact that these nitrogen mustards would enter the cells by a diffusion rather than an active process via a carnitine receptor. Moreover, the increased lipophilicity brought by the *tert*-butyl ester function in **16c** depleted its activity by comparison with activity of the parent compound **2c** pointing out the upmost importance of the free acid function (as in Melphalan and Chlorambucil structures).

Table 1. In vitro cytotoxic activities[a]

Compound	Log P[b]	A375 IC_{50} (μM)	HT29 IC_{50} (μM)	MCF7 IC_{50} (μM)
Melphalan	0.09	33	>1000	390
Chlorambucil	3.70	202	>1000	>1000
1	0.62	870	>1000	>1000
2a	2.08	>1000	>1000	>1000
2b	2.62	573	>1000	>1000
2d	3.87	403	>1000	>1000
2c	5.27	312	723	589
2e	5.96	193	753	258
16c	6.96	>1000	>1000	>1000

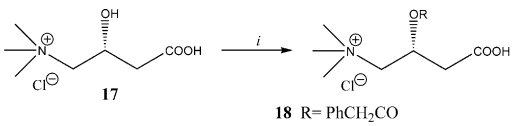
[a]Three independent experiments were realised for each compound.
[b]Log P values were calculated with ACD/log P calculator program.

Nitrogen mustards **2c** and **2e** showed significant cytotoxicity against MCF7 cell line with IC_{50} values of 589 and 258 μM, respectively much better than Chlorambucil which was found inactive in our conditions (1 h drug exposure) compared with a recent reported IC_{50} value of 1.7 μM (24 h drug exposure).[43] The two nitrogen mustard analogues **2c** and **2e** exhibited interesting inhibitory activities with IC_{50} values of 723 and 753 μM, respectively, against the HT29 cell line where both Melphalan and Chlorambucil were inactive.

Since the structural modification brought by the nitrogen mustard moiety of **2a–e** to the trimethylammonium core of natural carnitine seemed to preclude any recognition of these molecules with the carnitine receptor, we planned to evaluate the ability of the latter to act as a carrier for two active principles, NaPa (sodium phenylacetate)[44–46] and Chlorambucil. Thus, the two analogues **18** and **19** (Scheme 5) were prepared in order to check the potential use of the natural L-carnitine for prodrug development.

Thus direct acylation[47] of the commercially available L-carnitine chloride **17** with the phenylacetyl and Chlorambucil chlorides afforded the compounds **18** and **19** which unfortunately exhibited a very disappointing lack of antitumoral activity against A375, HT29 and MCF7 cell lines.

In summary, we have proposed a convenient multi-step synthesis of new enantiopure nitrogen mustards which mimic the (L)-carnitine framework. The broad antitumor activities of the lipophilic compounds **2c**,**e** against A375, HT29 and MCF7 cell lines, appears promising for a future prospect in the design of new structural analogues. As nitrogen mustards are very important anticancer drugs, our synthetic and biological



17

18 R= $PhCH_2CO$
19 R= *p*-$(ClCH_2CH_2)_2NC_6H_4(CH_2)_3CO$

Scheme 5. Reagents and conditions: (i) $PhCH_2COOH$ or Chlorambucil, $SOCl_2$, 60–80 °C, **17**.

studies bring valuable breakthroughs for further work in this area.

Experimental

Elemental analyses were performed by the 'Service de Microanalyse de l'Ecole Supérieure de Chimie de Montpellier'. ^{1}H NMR spectra were determined on a Bruker 200 spectrometer (200 MHz frequency) and ^{13}C NMR spectra on a Bruker AC 400 spectrometer (100.6 MHz frequency). Mass spectra were obtained with a JEOL JMS-DX-300 by the FAB ionisation method (positive mode) with *p*-nitrobenzyl alcohol (NBA) as the matrix; Optical rotations were measured in a 0.1 dm cell on a Perkin–Elmer 241 polarimeter. Petroleum spirit refers to the fraction boiling in the range 40–65 °C.

(*R*)-Di-*tert*-butyl malate 4 from the cyclic thioxocarbonate 7.[24] **Method A.** A mixture of **7** (0.2 g, 0.66 mmol), tri-*n*-butyltin hydride (0.2 cm^3, 0.72 mmol) and AIBN (cat.) dissolved in benzene (1 cm^3) were heated under reflux under nitrogen for 1 h. To the soln cooled to rt was added *tert*-butanol (10 cm^3) and silica gel 60 (37–70 μm) (100 mg) then the solution was stirred for 30 min and evaporated to dryness. The residue was chromatographed on silica gel with dichloromethane–methanol (99.5:0.5) as the eluent and the fractions were pooled, evaporated and dissolved in acetonitrile, washed with *n*-hexanes and concd under vacuum. Chromatography on silica gel with dichloromethane–methanol (99.5:0.5) as the eluent afforded the *title compound* (41 mg, 25%).

Method B. A solution of thioxocarbonate **7** (50 mg, 0.16 mmol) and MgI_2 (91.7 mg, 0.33 mmol) in acetonitrile (1.5 cm^3) was refluxed for 45 min. To the reaction was added at rt a satd soln of sodium bisulfide followed by an extraction with dichloromethane. The organic phase was washed with a satd solution of NaCl and dried over Na_2SO_4 and concd under vacuum. Chromatography as above afforded the *title compound* (13 mg, 32%).

From the cyclic sulfate 6.[24]. **Method A.** To the sulfate (50 mg, 0.15 mmol) dissolved in DMA (2 cm^3) was added at 0 °C sodium borohydride (5.8 mg, 0.15 mmol). After 1 h at rt, the solution was evaporated to dryness. THF (2 cm^3) and a mixture of concd H_2SO_4 (cat.)–H_2O (2–3 μL) were added at 0 °C. After 2 h at rt, a satd soln of $NaHCO_3$ was added until pH 8 and the aqueous phase extracted with diethyl ether, the organic phase washed with a saturated solution of NaCl, dried over Na_2SO_4 and evaporated under vacuum. Chromatography on silica gel as above afforded the *title compound* (21 mg, 56%).

Method B. To the sulfate (2 g, 6 mmol) dissolved in THF (60 cm^3) was added sodium cyanoborohydride (tech., 85%) (0.91 g, 12.4 mmol). The soln was refluxed for 15 h and after addition at 0 °C of THF (40 cm^3) and of the mixture concd H_2SO_4 (cat.)–H_2O (120 μL), the reaction was stirred for 3 h at rt, neutralized with a satd soln of $NaHCO_3$, extracted with diethyl ether, washed with brine, dried over Na_2SO_4 and evaporated under vacuum. Chromatography on silica gel as above afforded the *title compound* (1 g, 70%); R_f 0.7 (dichloromethane); $[\alpha]_D^{20}$ + 7.0° ± 0.2 (*c* 5.0 chloroform) (lit.,[15,16] no reported data); δ_H (200 MHz; $CDCl_3$) 1.47 (9H, s, 4-Bu^t), 1.50 (9H, s, 1-Bu^t), 2.65 (1H, d, $J_{3'\text{-}2}$ 5.7, $J_{3'\text{-}3}$ 16.5 Hz, 3′-H), 2.76 (1H, d, $J_{3\text{-}2}$ 4.6, $J_{3\text{-}3'}$ 16.5 Hz, 3-H), 3.23 (1H, d, $J_{OH\text{-}2}$ 5.7 Hz, OH) and 4.31 (1H, td, $J_{2\text{-}3'}$ 5.7, $J_{2\text{-}OH}$ 5.7, $J_{2\text{-}3}$ 4.6 Hz, 2-H); δ_C (100 MHz; $CDCl_3$) 28.4 (3C, C*Me*$_3$), 28.5 (3C, C*Me*$_3$), 40.3 (1C, 3-C), 67.9 (1C, 2-C), 81.8 (1C, 4-*C*Me$_3$), 83.1 (1C, 1-*C*Me$_3$), 170.2 (1C, 4-CO) and 173.2 (1C, 1-CO); *m*/*z* (NBA) 247 $[M+H]^+$, 269 $[M+Na]^+$, 493 $[2M+H]^+$, 515 $[2M+Na]^+$ (Found: C, 58.4; H, 9.2. $C_{12}H_{22}O_5$ requires C, 58.5; H, 9.0%).

(*R*)-*tert*-Butyl 3,4-dihydroxybutyrate 8. To the (*R*)-di-*tert*-butyl malate **4** (5.15 g, 20.9 mmol) dissolved in THF (130 cm^3) was added borane dimethylsulfide (10 mol dm^{-3} in THF) (2.3 cm^3, 23.0 mmol). After 30 min at rt sodium borohydride (39.7 mg, 1.0 mmol) was added and the soln stirred until completion on tlc (4 h). Addition of ethanol (50 cm^3) and *p*-toluene sulfonic acid (PTSA) (143 mg, 1.0 mmol) and stirring for 3 h was followed by co-evaporation several times with benzene–ethanol (1:1). Chromatography on silica gel with dichloromethane–methanol (9:1) as the eluent afforded the *title compound* as an oil (2.2 g, 60%); R_f 0.42 [dichloromethane–methanol (95:5)]; $[\alpha]_D^{20}$ + 20.7° ± 0.7 (*c* 1.5 chloroform); δ_H (200 MHz; $CDCl_3$) 1.49 (9H, s, Bu^t), 2.45 (1H, dd, $J_{2\text{-}2'}$ 16.5, $J_{2\text{-}3}$ 4.8 Hz, 2-H), 2.51 (1H, dd, $J_{2'\text{-}2}$ 16.5, $J_{2'\text{-}3}$ 7.7 Hz, 2′-H); 3.52 (1H, dd, $J_{4\text{-}4'}$ 11.3, $J_{4\text{-}3}$ 6.2 Hz, 4-H), 3.68 (1H, dd, $J_{4'\text{-}4}$ 11.3, $J_{4'\text{-}3}$ 3.4 Hz, 4′-H) and 4.11 (1H, m, 3-H); δ_C (100 MHz; $CDCl_3$) 27.3 (3C, C*Me*$_3$), 37.5 (1C, 2-C), 64.7 (1C, 4-C), 67.5 (1C, 3-C), 80.6 (1C, *C*Me$_3$) and 171.2 (1C, CO); *m*/*z* (NBA) 177 $[M+H]^+$, 199 $[M+Na]^+$, 103 $[M+H-OBu^t]^+$ (Found: C, 54.7; H, 8.9. $C_8H_{16}O_4$ requires C, 54.5; H, 9.1%).

(*R*)-*tert*-Butyl 3-hydroxy-4-bromobutyrate 10. To a stirred solution of *p*-toluenesulfonyl chloride (0.32 g, 1.7 mmol) in pyridine (1 cm^3) was added at 0 °C dropwise a solution of (*R*)-*tert*-butyl 3,4-dihydroxybutyrate **8** (0.2 g, 1.1 mmol) in pyridine (1 cm^3). After 24 h at 0 °C, the soln was concd under reduced pressure and extracted with diethyl ether, washed with an aqueous soln of citric acid (5%) and a satd soln of $NaHCO_3$ until alkaline pH and then with brine. The organic phase was dried over Na_2SO_4 and concd under reduced pressure to give the crude (*R*)-*tert*-butyl 3-hydroxy-4-tosyloxy butyrate **9** which was used further without purification. To the crude tosylate dissolved in 2-butanone (5 cm^3) was added lithium bromide (395 mg, 4.5 mmol) and the soln refluxed for 30 min. The mixture was diluted with dichloromethane and the soln washed with water and a satd soln of NaCl and then dried over Na_2SO_4. After evaporation of the solvents under reduced pressure the crude product was chromatographed on silica gel with petroleum ether–ethyl acetate (97:3) as the eluent to afford the *title compound* as an oil (173 mg, 66%); R_f 0.53 [petroleum ether–ethyl acetate (85:15)]; $[\alpha]_D^{20}$ +

15.2° ±0.8 (*c* 1.4 chloroform); δ_H (200 MHz; $CDCl_3$) 1.48 (9H, s, Bu^t), 2.58 (1H, dd, $J_{2\text{-}3}$ 5.0, $J_{2\text{-}2'}$ 16.7 Hz, 2-H), 2.60 (1H, dd, $J_{2'\text{-}3}$ 7.1, $J_{2'\text{-}2}$ 16.7 Hz, 2′-H), 3.45 (1H, dd, $J_{4'\text{-}3}$ 5.5, $J_{4'\text{-}4}$ 10.4 Hz, 4′-H), 3.52 (1H, dd, $J_{4\text{-}3}$ 5.14, $J_{4\text{-}4'}$ 10.4 Hz, 4-H) and 4.19 (1H, m, 3-H); δ_C (100 MHz; $CDCl_3$) 28.4 (3C, C*Me*$_3$), 37.7 (1C, 4-C), 40.7 (1C, 2-C), 68.1 (1C, 3-c), 82.2 (1 C, *C*Me$_3$) and 171.6 (1C, CO); *m*/*z* (NBA) 239–241 $[M+H]^+$ (Found: C, 40.0; H, 6.5. $C_8H_{15}BrO_3$ requires C, 40.2; H, 6.3%).

(*R*)-*tert*-Butyl 3,4-epoxybutyrate 3. A soln of (*R*)-*tert*-butyl 3-hydroxy-4-bromobutyrate **10** (378 mg, 1.6 mmol) and Ag_2O (375 mg, 1.6 mmol) in DME (5 cm^3) was refluxed for 12 h. The cooled soln was filtered and concd under reduced pressure and the residue chromatographed on silica gel with ethyl acetate (0–4%) in petroleum ether as the eluent to give the *title compound* as a colourless oil (92 mg, 37%); R_f 0.75 [petroleum ether–diethyl ether (85:15)]; $[\alpha]_D^{20}$ + 5.2° ±0.2 (*c* 5.3 chloroform), {lit.,[33] $[\alpha]_D^{20}$ + 4.7° (*c* 1.5 chloroform)}; δ_H (200 MHz; $CDCl_3$) 1.47 (9H, s, Bu^t), 2.40 (2H, m, 2×2-H), 2.45–2.75 (2H, m, 2×4-H) and 3.15 (1H, m, 3-H); δ_C (100 MHz; $CDCl_3$) 27.8 (3C, C*Me*$_3$), 39.0 (1C, 2-C), 46.4 (1C, 3-C), 48.0 (1C, 4-C), 80.9 (1C, *C*Me$_3$) and 169.5 (1C, CO); *m*/*z* (NBA) 159 $[M+H]^+$.

(*R*)-*tert*-Butyl 3-hydroxy-4-[*N*,*N*-bis(2-*tert*-butyldiphenylsilyloxyethyl)amino]butyrate 11. A stirred solution of epoxyester **3** (100 mg, 0.63 mmol), bis(*tert*-butyldiphenylsilyloxyethyl)amine (386 mg, 0.66 mmol) and silica gel 60 (37–70 μm) (100 mg) in acetonitrile (10 cm^3) was refluxed for 12 h. After evaporation of the solvent under reduced pressure, the residual oil was chromatographed on silica gel with diethyl ether (0–5%) in petroleum ether as the eluent to give the *title compound* as a colourless oil (234 mg, 50%); R_f 0.62 [(petroleum ether–ethyl acetate (85:15)]; $[\alpha]_D^{20}$ −22.6° ±0.7 (*c* 1.6 chloroform); δ_H (200 MHz; $CDCl_3$) 0.97 (18H, s, 2×SiBu^t), 1.40 (9H, s, CO_2Bu^t), 2.25 (1H, dd, $J_{2\text{-}3}$ 5.5, $J_{2\text{-}2'}$ 15.3 Hz, 2-H), 2.39 (1H, dd, $J_{2'\text{-}3}$ 7.7, $J_{2'\text{-}2}$ 15.3 Hz, 2′-H), 2.42 (1H, m, 4′-H), 2.61 (1H, m, 4-H), 2.73 (4H, m, 2×CH_2N), 3.66 (4H, m, 2×CH_2O), 3.95 (1H, m, 3-H) and 7.39–7.66 (20H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 27.2 (6C, 2×SiC*Me*$_3$), 28.1 (3C, CO_2C*Me*$_3$), 41.1 (1C, 2-C), 57.0 (2C, 2×CH_2N), 61.2 (1C, 4-C), 62.5 (2C, 2×CH_2O), 66.0 (1C, 3-C), 81.0 (3C, 3×*C*Me$_3$), 128.1 (8C, C$_m$ aromatics), 130.0 (4C, C$_p$ aromatics), 134.1 (4C, C$_q$ aromatics), 139.9 (8C, C$_o$ aromatics) and 171.5 (1C, CO); *m*/*z* (NBA) 740 $[M+H]^+$ (Found: C, 71.1; H, 8.5. $C_{44}H_{61}NO_5Si_2$ requires C, 71.4; H, 8.3%).

General procedure for the preparation of *O*-acyl aminohydroxyesters 14a–c. To a cooled soln (0 °C) of the hydroxyester **11** (2.8 g, 3.8 mmol), triethylamine (1.06 cm^3, 7.6 mmol) and DMAP (cat.) in dichloromethane (55 cm^3) was added dropwise anhydride (acetic, propionic, capryloyl) (5.7 mmol). The reaction was then refluxed for 12 h, cooled and the organic phase washed with a soln. of $NaHCO_3$ and brine then dried over Na_2SO_4. After evaporation of the solvent, the crude oil was chromatographed on silica gel with ethyl acetate (0–5%) in petroleum ether as the eluent to afford the expected *O*-acyl hydroxyesters **14a–c**.

Preparation of *O*-acyl aminohydroxyesters 14d–e (mixed anhydride method). To a cooled soln (0 °C) of phenylacetic acid or Chlorambucil (1.2 mmol) and triethylamine (6.6 mmol) in dichloromethane (25 cm^3) was added dropwise 2,4,6-trichlorobenzoyl chloride (6.6 mmol). After 2 h at rt, hydroxyester **11** (5.5 mmol) in dichloromethane (20 cm^3) was added and the reaction refluxed for 10 h, cooled and the organic phase washed with a solution of $NaHCO_3$ and brine then dried over Na_2SO_4. After evaporation of the solvent, the crude oil was chromatographed on silica gel with petroleum ether–ethyl acetate (95:5) as the eluent to afford the expected *O*-acyl hydroxyesters **14d–e**.

(*R*)-*tert*-Butyl 3-acetyloxy-4-[*N*,*N*-bis(2-*tert*-butyldiphenylsilyloxyethyl)amino]butyrate 14a. The *title compound* was obtained according to the aforementioned procedure from acetic anhydride in 95% yield as an oil; R_f 0.76 [petroleum ether–diethyl ether (85:15)]; $[\alpha]_D^{20}$ + 4.0° ±0.7 (*c* 10.7 chloroform); δ_H (200 MHz; $CDCl_3$) 1.04 (18H, s, 2×SiBu^t), 1.41 (9H, s, CO_2Bu^t), 1.95 (3H, s, MeCO), 2.52–2.75 (4H, m, 2×2-H and 2×4-H), 2.72 (4H, t, $J_{NCH_2CH_2O}$ 6.4 Hz, 2×CH_2N), 3.64 (4H, t, $J_{OCH_2CH_2N}$ 6.4 Hz, 2×CH_2O), 5.16 (1H, m, 3-H) and 7.38–7.67 (20H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 21.5 (1C, *Me*CO), 27.2 (6C, 2×SiC*Me*$_3$), 28.5 (3C, CO_2C*Me*$_3$), 39.0 (1C, 2-C), 57.5 (2 C, 2×CH_2N), 58.5 (1C, 4-C), 62.9 (2C, 2×CH_2O), 69.9 (1C, 3-C), 81.0 (3C, 3×*C*Me$_3$), 128.1 (8C, C$_m$ aromatics), 130.0 (4C, C$_p$ aromatics), 134.1 (4C, C$_q$ aromatics), 136.0 (8C, C$_o$ aromatics), 170.3 (1C, CO) and 170.5 (1C, CO); *m*/*z* (NBA) 782 $[M+H]^+$ (Found: C, 70.8; H, 8.3. $C_{46}H_{63}NO_6Si_2$ requires C, 70.6; H, 8.1%).

(*R*)-*tert*-Butyl 3-propionyloxy-4-[*N*,*N*-bis(2-*tert*-butyldiphenylsilyloxyethyl)amino]butyrate 14b. The *title compound* was obtained according to the aforementioned procedure from propionic anhydride in 80% yield as an oil; R_f 0.62 [(petroleum ether–ethyl acetate (9:1)]; $[\alpha]_D^{20}$ + 5.8° ±0.2 (*c* 4.2 chloroform); δ_H (200 MHz; $CDCl_3$) 1.02 (3H, t, *J* 7.5 Hz, Me), 1.03 (18H, s, 2×SiBu^t), 1.39 (9H, s, CO_2Bu^t), 2.20 (2H, q, *J* 7.5 Hz, *C*H_2Me), 2.34 (1H, dd, $J_{2\text{-}3}$ 8.35, $J_{2\text{-}2'}$ 15.6 Hz, 2′-H), 2.49–2.73 (3H, m, 2-H et 2×4-H), 2.74 (4H, t, $J_{NCH_2CH_2O}$ 6.6 Hz, 2×CH_2N), 3.66 (4H, t, $J_{OCH_2CH_2N}$ 6.6 Hz, 2×CH_2O), 5.15 (1H, m, 3-H) and 7.30–7.60 (20H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 9.5 (1C, Me), 27.3 (6C, 2×SiC*Me*$_3$), 28.1 (1C, CO*C*H_2), 28.5 (3C, CO_2C*Me*$_3$), 39.0 (1C, 2-C), 57.4 (2C, 2×CH_2N), 58.6 (1C, 4-C), 63.0 (2C, 2×CH_2O), 69.7 (1 C, 3-C), 81.0 (3C, 3×*C*Me$_3$), 128.1 (8C, C$_m$ aromatics), 130.0 (4C, C$_p$ aromatics), 134.1 (4C, C$_q$ aromatics), 136.0 (8C, C$_o$ aromatics), 170.4 (1C, CO) and 173.9 (1C, CO); *m*/*z* (NBA) 796 $[M+H]^+$ (Found: C, 71.0; H, 8.4. $C_{47}H_{65}NO_6Si_2$ requires C, 70.9; H, 8.2%).

(*R*)-*tert*-Butyl 3-capryloyloxy-4-[*N*,*N*-bis(2-*tert*-butyldiphenylsilyloxyethyl)amino]butyrate 14c. The *title compound* was obtained according to the aforementioned procedure from capryloyl anhydride in 85% yield as an oil; R_f 0.44 [petroleum ether–ethyl acetate (95:5); $[\alpha]_D^{20}$ + 3.7° ±0.5 (*c* 1.9 chloroform); δ_H (200 MHz; $CDCl_3$) 0.87 (3H, t, *J* 6.4 Hz, Me), 1.03 (18H, s, 2×SiBu^t), 1.26

(8H, m, 4×CH_2), 1.39 (9H, s, CO_2Bu^t), 1.55 (2H, m, $CH_2\beta$), 2.19 (2H, t, J 7.7 Hz, $CH_2\alpha$), 2.29–2.81 (8H, m, 2×2-H, 2×4-H and 2×CH_2N), 3.63 (4H, t, $J_{OCH_2CH_2N}$ 6.15 Hz, 2×CH_2O), 5.18 (1H, m, 3-H) and 7.38–7.63 (20H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 14.5 (1C, Me), 23.0 (1C, CH_2), 25.1 (1C, $CH_2\beta$), 25.3 (1C, CH_2), 27.0 (3C, SiCMe_3), 27.2 (3C, SiCMe_3), 28.5 (3C, CO_2CMe_3), 29.3 (1C, CH_2), 32.1 (1C, CH_2), 34.9 (1C, $CH_2\alpha$), 39.1 (1C, 2-C), 57.4 (2C, 2×CH_2N), 58.5 (1C, 4-C), 62.9 (2C, 2×CH_2O), 69.6 (1C, 3-C), 80.9 (2C, CMe_3), 128.1 (8C, C_m aromatics), 130.1 (4C, C_p aromatics), 134.1 (4C, C_q aromatics), 136.0 (8C, C_o aromatics), 170.4 (1C, CO) and 173.3 (1C, CO); m/z (NBA) 866 $[M+H]^+$ (Found: C, 72.3; H, 8.9. $C_{52}H_{75}NO_6Si_2$ requires C, 72.1; H, 8.7%).

(*R*)-*tert*-Butyl 3-phenylacetyloxy-4-[*N*,*N*-bis(2-*tert*-butyldiphenylsilyloxyethyl)amino]butyrate 14d. The *title compound* was obtained according to the aforementioned procedure from phenylacetic acid by the mixed anhydride method in 76% yield as an oil; R_f 0.73 [petroleum ether–ethyl acetate (9:1); $[\alpha]_D^{20}$ +4.7°±0.2 (*c* 0.7 chloroform); δ_H (200 MHz; $CDCl_3$) 1.05 (18H, s, 2×SiBu^t), 1.37 (9H, s, CO_2Bu^t), 2.37 (1H, dd, $J_{2\text{-}3}$ 8.35, $J_{2\text{-}2'}$ 15.8 Hz, 2′-H), 2.62 (3H, m, 2-H and 2×4-H), 2.69 (4H, m, AA′BB′ system, $J_{AB}+J_{AB'}$ 12.5 Hz, 2×CH_2N), 3.52 (2H, s, $COCH_2$), 3.62 (4H, m, AA′BB′ system, J_{BA} + $J_{BA'}$ 12.5 Hz, 2×CH_2O), 5.20 (1H, m, 3-H) and 7.19–7.60 (25H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 27.2 (6C, 2×SiCMe_3), 28.4 (3C, CO_2CMe_3), 38.9 (1C, 2-C), 41.8 (1C, COCH_2), 57.3 (2C, 2×CH_2N), 58.4 (1C, 4-C), 62.9 (2C, 2×CH_2O), 70.3 (1C, 3-C), 81.0 (3C, 3×CMe_3), 127.6 (2C, C_m aromatics), 128.1 (8C, C_m aromatics), 128.3 (2C, C_p aromatics), 129.6 (2C, C_o aromatics), 130.0 (4C, C_p aromatics), 133.6 (1C, C_q aromatic), 134.1 (4C, C_q aromatics), 136.0 (8C, C_o aromatics), 170.2 (1C, CO) and 171.0 (1C, CO); m/z (NBA) 859 $[M+H]^+$ (Found: C, 73.0; H, 8.2. $C_{52}H_{67}NO_6Si_2$ requires C, 72.8; H, 7.9%).

(*R*)-*tert*-Butyl 3-(4-{4-[bis(2-chloroethyl)amino]phenyl}-butyryloxy)-4-[*N*,*N*-bis(2-*tert*-butyldiphenylsilyloxyethyl)amino]butyrate 14e. The *title compound* was obtained according to the aforementioned procedure from Chlorambucil by the mixed anhydride method in 52% yield as an oil; R_f 0.68 [petroleum ether–ethyl acetate (9:1); $[\alpha]_D^{20}$ + 6.1°±0.2 (*c* 0.6 chloroform); δ_H (200 MHz; $CDCl_3$) 1.02 (18H, s, 2×SiBu^t), 1.38 (9H, s, CO_2Bu^t), 1.83 (2H, m, $CH_2CH_2CH_2$), 2.21 (2H, t, $J_{CH_2CH_2}$ 7.2 Hz, $COCH_2$), 2.34 (1H, dd, $J_{2\text{-}3}$ 8.35, $J_{2\text{-}2'}$ 15.6 Hz, 2′-H), 2.50 (2H, t, $J_{CH_2CH_2}$ 7.2 Hz, CH_2Ph), 2.55–2.74 (7H, m, 2-H, 2×4-H and 2×CH_2N), 3.64 (10H, m, 2×NCH_2CH_2Cl and 2×CH_2O), 5.19 (1H, m, 3-H), 6.60 (2H, d, J_{CHCH} 8.6 Hz, aromatics), 7.08 (2H, d, J_{CHCH} 8.6 Hz, aromatics) and 7.38–7.64 (20H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 26.7 (1C, $CH_2CH_2CH_2$), 26.9 (6C, 2×SiCMe_3), 28.1 (3C, CO_2CMe_3), 33.7 (1C, $COCH_2$), 34.0 (1C, CH_2Ph), 38.7 (1C, 2-C), 40.6 (2C, 2×CH_2Cl), 53.7 (2C, 2×NCH_2CH_2Cl), 57.1 (2C, 2×CH_2N), 58.3 (1C, 4-C), 63.6 (2C, 2×CH_2O), 69.4 (1C, 3-C), 80.6 (3C, 3×CMe_3), 112.3 (2C, 2×C aromatics), 127.8 (8C, C_m aromatics), 129.8 (2C, 2×C aromatics), 129.9 (1C, C_q aromatic), 130.8 (4C, C_p aromatics), 134.9 (4C, C_q aromatics), 135.6 (8C, C_o aromatics), 144.4 (1C, C_q aromatic), 170.0 (1C, CO) and 172.6 (1C, CO); m/z (NBA) 1025–1027 $[M+H]^+$ (Found: C, 67.7; H, 7.6. $C_{58}H_{78}Cl_2N_2O_6Si_2$ requires C, 67.9; H, 7.7%).

General procedure for the desilylation of silylated ethers 14a–e and for the chloration of bis(2-hydroxyethyl)amine derivatives 15a–e. To a mixture of the disilylated ethers **14a–e** (2.86 mmol) and imidazole (1.36 g, 20 mmol) dissolved in THF (40 cm^3), was added TBAF (1 mol dm^{-3} in THF) (17.2 cm^3, 17.2 mmol) and the soln stirred at rt for 30 min then concd under reduced pressure. The obtained crude material was quickly purified on Florisil (60–100 mesh) with ethyl acetate (0–20%) in petroleum ether as the eluent to afford the desired bis(2-hydroxyethyl)amine derivatives **15a–e** (characterised by ^1H NMR) which are used immediately to avoid the easy migration of the acyl group from the 3-position to the primary alcohols. To these later crude diols in dichloromethane (10 cm^3) were added triethylamine (794 μL, 5.72 mmol), DMAP (419 mg, 3.43 mmol) and *p*-toluenesulfonyl chloride (1.3 g, 6.86 mmol). The solution was stirred for 8 h at rt and concentrated under reduced pressure to give a crude material which was purified on Florisil (60–100 mesh) with ethyl acetate (0–5%) in petroleum ether as the eluent to afford the expected dichloro derivatives **16a–e**.

(*R*)-*tert*-Butyl 3-acetyloxy-4-[*N*,*N*-bis(2-chloroethyl)amino]butyrate 16a. The crude diol **15a** was obtained according to the aforementioned procedure as an oil; R_f 0.48 (ethyl acetate); δ_H (200 MHz; $CDCl_3$) 1.46 (9H, s, Bu^t), 2.10 (3H, s, MeCO), 2.49 (1H, dd, $J_{2\text{-}3}$ 6.9, $J_{2\text{-}2'}$ 15.7 Hz, 2-H), 2.58 (1H, dd, $J_{2'\text{-}3}$ 5.9, $J_{2'\text{-}2}$ 15.7 Hz, 2′-H), 2.65–2.83 (6H, m, 2×CH_2N and 2×4-H), 3.63 (4H, t, $J_{OCH_2CH_2N}$ 5.4 Hz, 2×CH_2O) and 5.36 (1H, m, 3-H); the *title compound* was obtained according to the aforementioned procedure in 51% yield (two steps) as an oil; R_f 0.51 [petroleum ether–ethyl acetate (9/1)]; $[\alpha]_D^{20}$ + 0.9°±0.1 (*c* 21.7 chloroform); δ_H (200 MHz; $CDCl_3$) 1.46 (9H, s, Bu^t), 2.06 (3H, s, MeCO), 2.49 (1H, dd, $J_{2\text{-}3}$ 7.2, $J_{2\text{-}2'}$ 15.8 Hz, 2-H), 2.69 (1H, dd, $J_{2'\text{-}3}$ 5.6, $J_{2'\text{-}2}$ 15.8 Hz, 2′-H), 2.76 (2H, d, $J_{4\text{-}3}$ 6.1 Hz, 2×4-H), 2.95 (4H, m, AA′BB′ system, J_{AB} + $J_{AB'}$ 13.7 Hz, 2×CH_2N), 3.53 (4H, m, AA′BB′ system, J_{BA} + $J_{BA'}$ 13.7 Hz, 2×CH_2Cl) and 5.23 (1H, ddd, $J_{3\text{-}2}$ 7.2, $J_{3\text{-}2'}$ 5.6, $J_{3\text{-}4}$ 6.1 Hz, 3-H); δ_C (100 MHz; $CDCl_3$) 21.5 (1C, *Me*CO), 28.4 (3C, CMe_3), 38.5 (1C, 2-C), 42.3 (2C, 2×CH_2Cl), 57.6 (2C, 2×CH_2N), 57.8 (1C, 4-C), 69.5 (1C, 3-C), 81.5 (1C, CMe_3), 170.0 (1C, CO) and 170.6 (1C, CO); m/z (NBA) 342–344 $[M+H]^+$ (Found: C, 50.2; H, 7.2. $C_{14}H_{25}Cl_2NO_4$ requires C, 49.1; H, 7.4%).

(*R*)-*tert*-Butyl 3-propionyloxy-4-[*N*,*N*-bis(2-chloroethyl)-amino]butyrate 16b. The crude diol **15b** was obtained according to the aforementioned procedure as an oil; R_f 0.46 [ethyl acetate–methanol (98:2)]; δ_H (200 MHz; $CDCl_3$) 1.13 (3H, t, $J_{Me\text{-}CH_2}$ 7.7 Hz, Me), 1.43 (9H, s, Bu^t), 2.34 (2H, q, $J_{CH_2\text{-}Me}$ 7.7 Hz, CH_2), 2.51 (2H, m, 2-H), 2.57–2.81 (6H, m, 2×CH_2N and 2×4-H), 3.60 (4H, m, AA′BB′ system, J_{AB} + $J_{AB'}$ 10.3 Hz, 2×CH_2O) and 5.33 (1H, m, 3-H). The *title compound* was obtained according to the aforementioned procedure in 43%

yield (two steps) as an oil; R_f 0.57 [petroleum ether–ethyl acetate (85:15)]; $[\alpha]_D^{20}$ + 3.8° ± 0.1 (*c* 9.3 chloroform); δ_H (200 MHz; $CDCl_3$) 1.12 (3H, t, $J_{Me\text{-}CH_2}$ 7.6 Hz, Me), 1.43 (9H, s, Bu^t), 2.31 (2H, q, $J_{CH_2\text{-}Me}$ 7.6 Hz, CH_2CO), 2.47 (1H, dd, $J_{2\text{-}3}$ 7.2, $J_{2\text{-}2'}$ 15.8 Hz, 2-H), 2.67 (1H, dd, $J_{2'\text{-}3}$ 5.4, $J_{2'\text{-}2}$ 15.8 Hz, 2′-H), 2.75 (2H, d, $J_{4\text{-}3}$ 6.1 Hz, 2×4-H), 2.93 (4H, m, AA′BB′ system, J_{AB} + $J_{AB'}$ 13.8 Hz, 2×CH_2N), 3.50 (4H, m, AA′BB′ system, J_{BA} + $J_{BA'}$ 13.8 Hz, 2×CH_2Cl) and 5.20 (1H, m, 3-H); δ_C (100 MHz; $CDCl_3$) 9.5 (1C, Me), 28.1 (1C, $COCH_2$), 28.4 (3C, CMe_3), 38.5 (1C, 2-C), 42.3 (2C, 2×CH_2Cl), 57.6 (2C, 2×CH_2N), 57.7 (1C, 4-C), 69.4 (1C, 3-C), 81.4 (1C, CMe_3), 170.1 (1C, CO) and 174.0 (1C, CO); *m*/*z* (NBA) 356–358 $[M+H]^+$, 378–380 $[M+Na]^+$ (Found: C, 50.8; H, 7.5. $C_{15}H_{27}Cl_2NO_4$ requires C, 50.6; H, 7.6%).

(*R*)-*tert*-Butyl 3-capryloyloxy-4-[*N*,*N*-bis(2-chloroethyl)-amino]butyrate 16c. The crude diol **15c** was obtained according to the aforementioned procedure as an oil; R_f 0.54 [ethyl acetate–methanol (98:2)]; δ_H (200 MHz; $CDCl_3$) 0.86 (3H, m, Me), 1.26 (8H, m, 4×CH_2), 1.43 (9H, s, Bu^t), 1.60 (2H, m, $CH_2\beta$), 2.31 (2H, t, *J* 7.5 Hz, $CH_2\alpha$), 2.51 (2H, m, 2×2-H), 2.58–2.81 (6H, m, 2×CH_2N and 2×4-H), 3.60 (4H, t, $J_{OCH_2CH_2N}$ 5.2 Hz, 2×CH_2O) and 5.34 (1H, m, 3-H). The *title compound* was obtained according to the aforementioned procedure in 34% yield (two steps) as an oil; R_f 0.37 [petroleum ether–ethyl acetate (9:1)]; $[\alpha]_D^{20}$ + 3.8° ± 0.1 (*c* 8.2 chloroform); δ_H (200 MHz; $CDCl_3$) 0.91 (3H, m, Me), 1.25 (8H, m, 4×CH_2), 1.43 (9H, s, Bu^t), 1.58 (2H, m, $CH_2\beta$), 2.27 (2H, t, *J* 7.4 Hz, $CH_2\alpha$), 2.47 (1H, dd, $J_{2\text{-}3}$ 7.0, $J_{2\text{-}2'}$ 15.8 Hz, 2-H), 2.67 (1H, dd, $J_{2'\text{-}3}$ 5.5, $J_{2'\text{-}2}$ 15.8 Hz, 2′-H), 2.74 (2H, d, $J_{4\text{-}3}$ 6.1 Hz, 2×4-H), 2.93 (4H, m, AA′BB′ system, J_{AB} + $J_{AB'}$ 13.8 Hz, 2×CH_2N), 3.50 (4H, m, J_{BA} + $J_{BA'}$ 13.8 Hz, 2×CH_2Cl) and 5.24 (1H, m, 3-H); δ_C (100 MHz; $CDCl_3$) 13.0 (1C, Me), 22.7 (1C, CH_2), 23.9 (1C, $CH_2\beta$), 27.0 (3 C, CMe_3), 27.9 (1C, CH_2), 28.1 (1C, CH_2), 30.6 (1C, CH_2), 33.4 (1C, $CH_2\alpha$), 37.1 (1C, 2-C), 40.9 (2C, 2×CH_2Cl), 56.2 (2C, 2×CH_2N), 56.3 (1C, 4-C), 67.9 (1C, 3-C), 80.0 (1C, CMe_3), 168.6 (1C, CO) and 172.0 (1C, CO); *m*/*z* (NBA) 426–428 $[M+H]^+$(Found: C, 56.0; H, 8.9. $C_{20}H_{37}Cl_2NO_4$ requires C, 56.3; H, 8.7%).

(*R*)-*tert*-Butyl 3-phenylacetyloxy-4-[*N*,*N*-bis(2-chloroethyl)-amino]butyrate 16d. The crude diol **15d** was obtained according to the aforementioned procedure as an oil; R_f 0.42 [ethyl acetate–methanol (99:1)]; δ_H (200 MHz; $CDCl_3$) 1.41 (9H, s, CO_2Bu^t), 2.38–2.69 (8H, m, 2×2-H, 2×4-H and 2×CH_2N), 3.54 (4H, t, $J_{OCH_2CH_2N}$ 5.3 Hz, 2×CH_2O), 3.64 (2H, s, $COCH_2$), 5.35 (1H, m, 3-H) and 7.34–7.48 (5H, m, aromatics). The *title compound* was obtained according to the aforementioned procedure in 22% yield (two steps) as an oil; R_f 0.40 [petroleum ether–ethyl acetate (9:1)]; $[\alpha]_D^{20}$ + 1.2° ± 0.1 (*c* 2.2 chloroform); δ_H (200 MHz; $CDCl_3$) 1.41 (9H, s, Bu^t), 2.47 (1H, dd, $J_{2\text{-}3}$ 7.2, $J_{2\text{-}2'}$ 16.0 Hz, 2-H), 2.66 (1H, dd, $J_{2'\text{-}3}$ 5.7, $J_{2'\text{-}2}$ 16.0 Hz, 2′-H), 2.72 (2H, d, $J_{4\text{-}3}$ 6.0 Hz, 2×4-H), 2.86 (4H, m, AA′BB′ system, $J_{AB}+J_{AB'}$ 13.8 Hz, 2×CH_2N), 3.43 (4H, m, AA′BB′ system, $J_{BA}+J_{BA'}$ 13.8 Hz, 2×CH_2Cl), 3.60 (2H, s, $COCH_2$), 5.23 (1H, m, 3-H) and 7.20–7.40 (5H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 28.4 (3C, CMe_3), 38.4 (1C, 2-C), 41.80 (1C, $COCH_2$), 42.3 (2C, 2×CH_2Cl), 57.4 (2C, 2×CH_2N), 57.6 (1C, 4-C), 70.0 (1C, 3-C), 81.5 (1C, CMe_3), 127.5 (2C, C_m aromatics), 128.3 (1C, C_p aromatic), 129.7 (2C, C_o aromatics), 133.7 (1C, C_q aromatic), 169.9 (1C, CO) and 170.5 (1C, CO); *m*/*z* (NBA) 418–420–422 $[M+H]^+$ (Found: C, 57.2; H, 6.9. $C_{20}H_{29}Cl_2NO_4$ requires C, 57.4; H, 7.0%).

(*R*)-*tert*-Butyl 3-(4-{4-[bis(2-chloroethyl)amino]phenyl}-butyryloxy-4-[*N*,*N*-bis(2-chloroethyl)amino]butyrate 16e. The crude diol **15e** was obtained according to the aforementioned procedure as an oil; R_f 0.52 [ethyl acetate–methanol (99:1)]; δ_{Hc} (200 MHz; $CDCl_3$) 1.45 (9H, s, CO_2Bu^t), 1.87 (2H, m, $CH_2CH_2CH_2$), 2.32 (2H, t, $J_{CH_2CH_2}$ 7.2 Hz, $COCH_2$), 2.49–2.75 (10H, m, CH_2Ph, 2×2-H, 2×4-H and 2×CH_2N), 3.65 (10H, m, 2×NCH_2CH_2Cl and 2×CH_2O), 5.35 (1H, m, 3-H), 6.59 (2H, d, J_{CHCH} 8.6 Hz, aromatics), 7.04 (2H, d, J_{CHCH} 8.6 Hz, aromatics). The *title compound* was obtained according to the aforementioned procedure in 23% yield (two steps) as an oil; R_f 0.34 [petroleum ether–ethyl acetate (9:1)]; $[\alpha]_D^{20}$ + 2.5° ± 0.3 (*c* 3.7 chloroform); δ_H(200 MHz; $CDCl_3$) 1.42 (9H, s, CO_2Bu^t), 1.88 (2H, m, $CH_2CH_2CH_2$), 2.29 (2H, t, J_{CH2CH2} 7.2 Hz, $COCH_2$), 2.41–2.65 (4H, m, CH_2Ph and 2×2-H), 2.73 (2H, d, $J_{4\text{-}3}$ 5.5 Hz, 2×4-H), 2.93 (4H, m, AA′BB′ system, J_{AB} + $J_{AB'}$ 13.8 Hz, 2×CH_2N), 3.50 (4H, m, AA′BB′ system, J_{BA} + $J_{BA'}$ 13.8 Hz, 2×CH_2Cl), 3.65 (8H, m, 2×NCH_2CH_2Cl), 5.22 (1H, m, 3-H), 6.62 (2H, d, J_{CHCH} 8.6 Hz, aromatics), 7.06 (2H, d, J_{CHCH} 8.6 Hz, aromatics); δ_C (100 MHz; $CDCl_3$) 26.6 (1C, $CH_2CH_2CH_2$), 28.0 (3 C, CMe_3), 33.6 (1C, $COCH_2$), 33.9 (1C, CH_2Ph), 38.1 (1C, 2-C), 40.6 (2 C, 2×CH_2Cl), 41.8 (2C, 2×CH_2Cl), 53.6 (2C, 2×NCH_2CH_2Cl), 57.1 (2C, 2×CH_2N), 57.3 (1C, 4-C), 69.0 (1C, 3-C), 81.0 (3C, 3×CMe_3), 112.2 (2C, 2×CH aromatics), 127.0 (2C, 2×CH aromatics), 129.7 (1C, C_q aromatic), 144.4 (1C, C_q aromatic), 169.7 (1C, CO) and 172.7 (1C, CO); *m*/*z* (NBA) 585–587–589 $[M+H]^+$ (Found: C, 53.4; H, 7.1. $C_{26}H_{40}Cl_4N_2O_4$ requires C, 53.2; H, 6.9%).

(*R*)-3-Hydroxy-4-[*N*,*N*-bis(2-chloroethyl)amino]butyric acid 1. To the amino ester **16a** (100 mg, 0.29 mmol) dissolved in THF (0.3 cm^3) was added anisole (31 μL, 0.29 mmol) and aqueous HCl (2.4 mol dm^{-3}) (3 cm^3, 7.3 mmol) and the solution stirred at rt for 5 days. The mixture was diluted with diethyl ether and the soln washed with water. The resulting aqueous phase was lyophilised and the obtained crude oil chromatographed on silica gel (reversed phase C2) with THF–water (7:3) as the eluent to afford the *title compound* (54 mg, 76%); R_f 0.63 [acetone–ethyl acetate–water (2:4:1)]; $[\alpha]_D^{20}$ −29.6° ± 0.2 (*c* 5.4 water); δ_H (200 MHz; D_2O) 2.48 (1H, dd, $J_{2\text{-}3}$ 7.2, $J_{2\text{-}2'}$ 16.5 Hz, 2-H), 2.59 (1H, dd, $J_{2'\text{-}3}$ 5.1, $J_{2'\text{-}2}$ 16.5 Hz, 2′-H), 3.31 (1H, dd, $J_{4\text{-}3}$ 10.3, $J_{4\text{-}4'}$ 13.6 Hz, 4-H), 3.44 (1H, dd, $J_{4'\text{-}3}$ 2.7, $J_{4'\text{-}4}$ 13.6 Hz, 4′-H), 3.68 (4H, m, AA′BB′ system, $J_{AB}+J_{AB'}$ 11.2 Hz, 2×CH_2N), 3.89 (4H, m, AA′BB′ system, $J_{BA}+J_{BA'}$ 11.2 Hz, 2×CH_2Cl) and 4.41 (3H, m, 3-H); δ_C (100 MHz; D_2O) 37.5 (2C, 2×CH_2Cl), 39.5 (1C, 2-C), 55.7 (2C, 2×CH_2N), 58.0 (1C, 4-C), 62.2 (1C, 3-C) and 174.3 (1C, CO); *m*/*z* (NBA) 244–246 $[M+H]^+$ (Found: C, 39.6; H, 6.1. $C_8H_{15}Cl_2NO_3$ requires C, 39.4; H, 6.2%).

General procedure for the acidic hydrolysis of *tert*-butyl diesters 16a–e

To the amino esters **16a–e** (0.24 mmol) dissolved in dichloromethane (1.5 cm^3) were added anisole (26 μL, 0.24 mmol) and trifluoroacetic acid (185 μL, 2.41 mmol). The solution was stirred at rt for 10 h, concd under reduced pressure, co-evaporated with cyclohexane and the obtained crude residue was chromatographed on silica gel with ethyl acetate as the eluent to afford the corresponding amino acids **2a–e** as colourless oils.

(*R*)-3-Acetyloxy-4-[*N*,*N*-bis(2-chloroethyl)amino]butyric acid 2a. The *title compound* was obtained according to the aforementioned procedure in 76% yield as an oil; R_f 0.64 [petroleum ether–ethyl acetate (4:6)]; $[\alpha]_D^{20}$ $-17.3\pm0.9°$ (*c* 1.1 water); $[\alpha]_D^{20}$ $+1.3°\pm0.2$ (*c* 3.9 chloroform); δ_H (200 MHz; $CDCl_3$) 2.03 (3H, s, Me), 2.65 (2H, dd, $J_{2\text{-}3}$ 6.4, $J_{2\text{-}2'}$ 16.5 Hz, 2-H), 2.82 (2H, dd, $J_{2'\text{-}3}$ 6.0, $J_{2'\text{-}2}$ 16.5 Hz, 2′-H), 2.95 (2H, m, 2×4-H), 3.09 (4H, m, AA′BB′ system, $J_{AB}+J_{AB'}$ 13.3 Hz, $2\times CH_2N$), 3.59 (4H, m, AA′BB′ system, $J_{BA}+J_{BA'}$ 13.3 Hz, $2\times CH_2Cl$) and 5.28 (1H, m, 3-H); δ_C (100 MHz; D_2O) 20.8 (1C, *Me*CO), 37.0 (1C, 2-C), 37.7 (2C, $2\times CH_2Cl$), 56.5 (1C, 4-C), 56.0 (2C, $2\times CH_2N$), 66.2 (1C, 3-C), 173.7 (2C, 1-C and Me*C*O); *m*/*z* (NBA) 286–288 $[M+H]^+$(Found: C, 42.2; H, 6.1. $C_{10}H_{17}Cl_2NO_4$ requires C, 42.0; H, 6.0%).

(*R*)-3-Propionyloxy-4-[*N*,*N*-bis(2-chloroethyl)amino]butyric acid 2b. The *title compound* was obtained according to the aforementioned procedure in 67% yield as an oil; R_f 0.72 [petroleum ether–ethyl acetate (4:6)]; $[\alpha]_D^{20}$ $+6.5°\pm0.7$ (*c* 1.4 chloroform); δ_H (200 MHz; $CDCl_3$) 1.11 (3H, t, $J_{Me\text{-}CH_2}$ 7.7 Hz, Me), 2.33 (2H, q, $J_{CH_2\text{-}Me}$ 7.7 Hz, $COCH_2$), 2.66 (1H, dd, $J_{2\text{-}3}$ 6.4, $J_{2\text{-}2'}$ 16.2 Hz, 2-H), 2.81 (1H, dd, $J_{2'\text{-}3}$ 6.0, $J_{2'\text{-}2}$ 16.2 Hz, 2′-H), 3.00 (2H, d, $J_{4\text{-}3}$ 6.4 Hz, 2×4-H), 3.13 (4H, m, AA′BB′ system, $J_{AB}+J_{AB'}$ 13.2 Hz, $2\times CH_2N$), 3.61 (4H, m, AA′BB′ system, $J_{BA}+J_{BA'}$ 13.2 Hz, $2\times H_2Cl$) and 5.28 (1H, m, 3-H); δ_C (100 MHz; $CDCl_3$) 9.3 (1C, Me), 28.0 (1C, $COCH_2$), 37.0 (1C, 2-C), 41.3 (2C, $2\times CH_2Cl$), 57.1 (2C, $2\times CH_2N$), 57.4 (1C, 4-C), 68.4 (1C, 3-C), 171.8 (1C, CO) and 174.4 (1C, CO); *m*/*z* (NBA) 300–302 $[M+H]^+$, 322–324 $[M+Na]^+$ (Found: C, 43.8; H, 6.3. $C_{11}H_{19}Cl_2NO_4$ requires C, 44.0; H, 6.4%).

(*R*)-3-Capryloyloxy-4-[*N*,*N*-bis(2-chloroethyl)amino]butyric acid 2c. The *title compound* was obtained according to the aforementioned procedure in 50% yield as an oil; R_f 0.73 [petroleum ether–ethyl acetate (4:6)]; $[\alpha]_D^{20}$ $+9.0°\pm0.4°$ (*c* 2.3 chloroform); δ_H (200 MHz; $CDCl_3$) 0.81 (3H, t, *J* 7.0 Hz, Me), 1.21 (8H, m, $4\times CH_2$), 1.53 (2H, m, $CH_2\beta$), 2.23 (2H, t, *J* 7.6 Hz, $CH_2\alpha$), 2.60 (1H, dd, $J_{2\text{-}3}$ 6.4, $J_{2\text{-}2'}$ 16.4 Hz, 2-H), 2.77 (1H, dd, $J_{2'\text{-}3}$ 5.4, $J_{2'\text{-}2}$ 16.4 Hz, 2′-H), 2.85 (2H, d, $J_{4\text{-}3}$ 6.4 Hz, 2×4-H), 3.00 (4H, m, AA′BB′ system, $J_{AB}+J_{AB'}$ 12.8 Hz, $2\times CH_2N$), 3.51 (4H, m, AA′BB′ system, $J_{BA}+J_{BA'}$ 12.8 Hz, $2\times CH_2Cl$) and 5.19 (1H, m, 3-H); δ_C (100 MHz; $CDCl_3$) 14.5 (1C, Me), 23.0 (2C, $2\times CH_2$), 25.2 (1C, $CH_2\beta$), 29.3 (1C, CH_2), 32.0 (1C, CH_2), 34.7 (1C, $CH_2\alpha$), 37.1 (1C, 2-C), 41.4 (2C, $2\times CH_2Cl$), 57.2 (2C, $2\times CH_2N$), 57.5 (1C, 4-C), 68.4 (1C, 3-C), 173.7 (1C, CO) and 175.5 (1C, CO); *m*/*z* (NBA) 370–372 $[M+H]^+$, 392–394 $[M+Na]^+$ (Found: C, 51.8; H, 7.8. $C_{16}H_{29}Cl_2NO_4$ requires C, 51.9; H, 7.9%).

(*R*)-3-Phenylacetyloxy-4-[*N*,*N*-bis(2-chloroethyl)amino]-butyric acid 2d. The *title compound* was obtained according to the aforementioned procedure in 71% yield as an oil; R_f 0.57 [petroleum ether–ethyl acetate (4:6)]; $[\alpha]_D^{20}$ $+15.5°\pm0.4°$ (*c* 0.3 chloroform); δ_H (400 MHz; $CDCl_3$) 2.57 (1H, dd, $J_{2\text{-}3}$ 7.2, $J_{2\text{-}2'}$ 16.4 Hz, 2-H), 2.65 (1H, dd, $J_{2'\text{-}3}$ 6.2, $J_{2'\text{-}2}$ 13.8 Hz, 4′-H), 2.72 (1H, dd, $J_{2\text{-}3}$ 6.0, $J_{2\text{-}2'}$ 13.8 Hz, 4-H), 2.78 (1H, dd, $J_{2'\text{-}3}$ 5.4, $J_{2'\text{-}2}$ 16.4 Hz, 2-H), 2.82 (4H, m, AA′BB′ system, $J_{AB}+J_{AB'}$ 13.5 Hz, $2\times CH_2N$), 3.38 (4H, m, AA′BB′ system, $J_{BA}+J_{BA'}$ 13.5 Hz, $2\times CH_2Cl$), 3.55 (2H, $COCH_2$), 5.20 (1H, m, 3-H) and 7.17–7.28 (5H, m, aromatics); δ_C (100 MHz; $CDCl_3$) 36.9 (1C, 2-C), 41.8 (1C, $COCH_2$), 42.2 (2C, $2\times CH_2Cl$), 57.3 (2C, $2\times CH_2N$), 57.4 (1C, 4-C), 69.5 (1C, 3-C), 127.6 (2C, C_m aromatics), 129.0 (1C, C_p aromatic), 129.6 (2C, C_o aromatics), 134.0 (1C, C_q aromatic), 171.3 (1C, CO) and 175.9 (1C, CO); *m*/*z* (NBA) 362–364 $[M+H]^+$ (Found: C, 53.3; H, 6.0. $C_{16}H_{21}Cl_2NO_4$ requires C, 53.0; H, 5.8%).

(*R*)-3-(4-{4-[Bis(2-chloroethyl)-amino]phenyl}butyryloxy-4-[*N*,*N*-bis(2-chloroethyl)amino]butyric acid 2e. The *title compound* was obtained according to the aforementioned procedure in 87% yield as an oil; R_f 0.48 [petroleum ether–ethyl acetate (4:6)]; $[\alpha]_D^{20}$ $+6.9°\pm0.2°$ (*c* 0.8 chloroform); δ_H (200 MHz; $CDCl_3$) 1.81 (2H, m, $CH_2CH_2CH_2$), 2.27 (2H, t, $J_{CH_2CH_2}$ 7.4 Hz, $COCH_2$), 2.47 (2H, t, $J_{CH_2CH_2}$ 7.4 Hz, CH_2Ph), 2.62 (1H, dd, $J_{2\text{-}3}$ 6.1, $J_{2\text{-}2'}$ 16.7 Hz, 2-H), 2.73 (1H, dd, $J_{2'\text{-}3}$ 6.5, $J_{2'\text{-}2}$ 16.7 Hz, 2′-H), 3.04 (2H, d, $J_{4\text{-}3}$ 5.8 Hz, 2×4-H), 3.17 (4H, m, $2\times CH_2N$), 3.55 (4H, m, $2\times CH_2Cl$), 3.60 (8H, m, $2\times NCH_2CH_2Cl$), 5.25 (1H, m, 3-H), 6.57 (2H, d, J_{CHCH} 8.6 Hz, aromatics) and 7.00 (2H, d, J_{CHCH} 8.6 Hz, aromatics); δ_C (100 MHz; $CDCl_3$) 26.8 (1C, $CH_2CH_2CH_2$), 33.9 (1C, $COCH_2$), 34.2 (1C, CH_2Ph), 37.0 (1C, 2-C), 40.3 (2C, $2\times CH_2Cl$), 40.9 (2C, $2\times CH_2Cl$), 54.1 (2C, $2\times NCH_2CH_2Cl$), 56.8 (2C, $2\times CH_2N$), 57.3 (1C, 4-C), 67.7 (1C, 3-C), 112.8 (2C, 2×CH aromatics), 130.1 (2C, 2×CH aromatics), 130.9 (1C, C_q aromatic), 144.6 (1C, C_q aromatic), 173.5 (1C, CO) and 174.1 (1C, CO); *m*/*z* (NBA) 529 $[M+H]^+$ (Found: C, 49.5; H, 5.8. $C_{22}H_{32}Cl_4N_2O_4$ requires C, 49.8; H, 6.1%).

(*R*)-(3-Carboxy-2-phenylacetoxypropyl)trimethylammonium chloride 18. A mixture of phenyl acetic acid (506 mg, 3.7 mmol) and thionyl chloride (135 μL, 1.9 mmol) was warmed for 40 min at 80 °C. Then L-carnitine chloride **17** (300 mg, 1.9 mmol) was added and the reaction maintained at the same temperature for 45 min. After concentration under vacuum, the crude residue was chromatographed on silica gel (reverse-phase C2) with water–THF (65:35) as the eluent to afford the *title compound* as a colourless oil (426 mg, 72%); R_f 0.37 [acetone–ethyl acetate—water–acetic acid (2:2:1:2.5)]; $[\alpha]_D^{20}$ $-29.5°\pm0.4°$ (*c* 2.8 water); δ_H (200 MHz; CD_3OD) 2.70 (1H, dd, $J_{2\text{-}3}$ 7.1, $J_{2\text{-}2'}$ 17.0 Hz, 2-H), 2.79 (1H, dd, $J_{2'\text{-}3}$ 5.4, $J_{2'\text{-}2}$ 17.0 Hz, 4′-H), 2.93 (9H, s, $3\times NMe$), 3.72

(2H, s, $COCH_2$), 3.67 (1H, m, 4′-H), 3.83 (1H, dd, $J_{4\text{-}3}$ 8.51, $J_{4\text{-}4'}$ 14.5 Hz, 4-H), 5.57 (1H, m, 3-H) and 7.21–7.37 (5H, m, aromatics); δ_C (100 MHz; CD_3OD) 36.9 (1C, 2-C), 41.1 (1C, $COCH_2$), 53.5 (3C, 3×NCH_3), 65.9 (1C, 3-C), 68.2 (1C, 4-C), 127.5 (2C, C_m aromatics), 128.8 (1C, C_p aromatic), 129.6 (2C, C_o aromatics), 133.8 (1C, C_q aromatic), 171.0 (1C, CO) and 171.4 (1C, CO_2H); *m/z* (NBA) 280 $[M]^+$(Found: C, 57.2; H, 7.1. $C_{15}H_{22}ClNO_4$ requires C, 57.0; H, 7.0%).

(*R*)-[2-(4-{4-[Bis(2-chloroethyl)amino]phenyl}butyryloxy)-3-carboxypropyl]trimethylammonium chloride 19. A mixture of Chlorambucil (676 mg, 2.2 mmol) and thionyl chloride (135 μL, 1.9 mmol) was warmed for 15 min at 60 °C. Then L-carnitine chloride **17** (300 mg, 1.9 mmol) and trichloroacetic acid (450 mg) were added and the reaction maintained at the same temperature for 2 h. After concn under vacuum, the crude residue was chromatographed on silica gel (reverse-phase C2) with water–THF (65:35) as the eluent to afford the *title compound* as a colourless oil (170 mg, 19%); R_f 0.60 [acetone–ethyl acetate–water–acetic acid (2:2:1:2.5)]; $[\alpha]_D^{20}$ $-4.7° \pm 0.4°$ (*c* 4.2 water); δ_H (200 MHz; CD_3OD) 1.88 (2H, m, $CH_2CH_2CH_2$), 2.37 (2H, t, $J_{CH_2CH_2}$ 7.4 Hz, $COCH_2$), 2.54 (2H, t, $J_{CH_2CH_2}$ 7.4 Hz, CH_2Ph), 2.74 (2H, d, $J_{2\text{-}3}$ 6.0 Hz, 2-H), 3.19 (9H, s, 3×N*Me*), 3.66 (9H, m, 2×NCH_2CH_2Cl and 4′-H), 3.86 (1H, dd, $J_{4\text{-}3}$ 7.9, $J_{4\text{-}4'}$ 14.5 Hz, 4-H), 5.61 (1H, m, 3-H), 6.67 (2H, d, J_{CHCH} 8.5 Hz, aromatics) and 7.05 (2H, d, J_{CHCH} 8.5 Hz, aromatics); δ_C (100 MHz; CD_3OD) 26.6 (1C, $CH_2CH_2CH_2$), 33.4 (1C, $COCH_2$), 33.9 (1C, CH_2Ph), 36.8 (1C, 2-C), 40.7 (2C, 2×CH_2Cl), 53.3 (2C, 2×NCH_2CH_2Cl), 53.5 (3C, 3×NCH_3), 65.2 (1C, 3-C), 68.4 (1C, 4-C), 112.5 (2C, 2×CH aromatics), 129.7 (2C, 2×CH aromatics), 130.3 (1C, C_q aromatic), 145.1 (1C, C_q aromatic), 171.5 (1C, CO_2H) and 173.0 (1C, CO); *m/z* (NBA) 447–449–451 $[M]^+$ (Found: C, 52.4; H, 7.2. $C_{21}H_{33}Cl_3N_2O_4$ requires C, 52.1; H, 6.9%).

Biological studies—neutral red uptake assay[48]

Cytotoxicity tests for all compounds were carried out in triplicate on three different cell lines (all wild type) obtained from the American Type Culture Collection (Bethesda, MD, USA). Human melanoma cells (A375), colon cancer cells (HT29), both grown in DMEM and breast cancer cells (MCF7) grown in RPMI 1640, both supplemented with 10% fetal calf serum, were selected.

Cells were seeded at a density of 2×10^4 cells/well in a 96-well plate and left to adhere for 24 h at 37 °C in the presence of 5% CO_2 for attachment. Culture media containing increasing concentrations (0.1–1000 μM) of test compound was prepared and the cells exposed to it for 1 h. The drug mixture was replaced by fresh medium and the cells were grown for an additional 3 days. For the viability staining, a neutral red solution (33 mg/L) was added for 4 h and the cells were destained (15 min) with a mixture glacial acetic acid–ethanol [1:50 (v/v)]. Absorbances were read at 540 nm.

The antiproliferative activity of the drugs was expressed as IC_{50}, the concentration at which the cells occupied surface area was 50% when compared to untreated cells.

Acknowledgements

We are grateful to the Ministère de la Recherche et de la Technologie and C.N.R.S for financial support of this research. L. F. is pleased to acknowledge Mayoly Spindler Company and 'la Région Languedoc Roussillon' for providing a grant. We thank P. Cuq, A. Evrard and L. Vian of the Laboratoire de Toxicologie du Médicament (Université de Montpellier I) for their technical support during the biological testing.

References and Notes

1. Foye, W. O. *Cancer Chemotherapeutic Agents*; Massachusetts College of Pharmacy and Allied Health Sciences: Washington, DC, 1995.
2. Gniazdowski, M.; Cera, C. *Chem. Rev.* **1996**, *96*, 619.
3. Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723.
4. Fritz, I. B. Current Concepts in Carnitine Research; CRC: Boca Raton, Florida, 1992.
5. Ferrari, R.; DiMauro, S.; Sherwood, G. *L-Carnitine and its Role in Medicine: From Function to Therapy*; Academic: San Diego, 1992.
6. Ramsay, R. R.; Gandfour, R. D.; van der Leij, F. R. *Biochim. Biophys. Acta* **2001**, *1546*, 21.
7. Cavalli, L. R.; Liang, B. C. *Mutation Res. Mol. Mechanisms Mutagenesis* **1998**, *398*, 19.
8. Dacaudin, D.; Marzo, I.; Brenner, C.; Kroemer, G. *Int. J. Oncol.* **1998**, *12*, 141.
9. Costantini, P.; Jacotot, E.; Decaudin, D.; Kroemer, G. *J. Natl. Cancer Inst.* **2000**, *92*, 1042.
10. Grad, J. M.; Cepero, E.; Boise, L. H. *Drug Resist. Updates* **2001**, *4*, 85.
11. Lewis, P. D.; Baxter, P. W.; Griffiths, A. P.; Parry, J. M.; Skibinski, D. O. F. *J. Pathol.* **2000**, *192*, 562.
12. Yokogawa, K.; Miya, K.; Tamai, I.; Higashi, Y.; Nomura, M.; Miyamoto, K.-I.; Tsuji, A. *J. Pharm. Pharmacol.* **1999**, *51*, 935.
13. Boots, S. G.; Boots, M. R. *J. Pharm. Sci.* **1975**, *64*, 1262.
14. Pavlov, S.; Bogavac, M.; Arsenijevic, V. *Bull. Soc. Chim. Fr.* **1974**, 2985.
15. Kuo, C. H.; Robichaud, A. J.; Rew, D. J.; Bergstrom, J. D.; Berger, G. D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1591.
16. Sefkow, M. *Tetrahedron: Asymmetry* **2001**, *12*, 987.
17. Alpegiani, M.; Hanessian, S. *J. Org. Chem.* **1987**, *52*, 278.
18. Gao, Y.; Zepp, C. M. *Tetrahedron Lett.* **1991**, *32*, 3155.
19. Rho, H.-S. *Synth. Commun.* **1998**, *28*, 843.
20. Jang, D. O.; Song, S. H. *Tetrahedron Lett.* **2000**, *41*, 247.
21. Uray, G.; Lindner, W. *Tetrahedron* **1988**, *44*, 4357.
22. Corey, E. J.; Winter, R. A. E. *J. Am. Chem. Soc.* **1963**, *85*, 2677.
23. Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 7538.
24. Faissat, L.; Chavis, C.; Montero, J.-L.; Lucas, M. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1253.
25. Kim, B. M.; Sharpless, K. B. *Tetrahedron Lett.* **1989**, *30*, 655.
26. Saito, S.; Hasegawa, T.; Inaba, M.; Nishida, R.; Fujii, T.; Nomizu, S.; Moriwake, T. *Chem. Lett.* **1984**, 1389.
27. Saito, S.; Ishikawa, T.; Kuroda, A.; Koga, K.; Moriwake, T. *Tetrahedron* **1992**, *48*, 4067.
28. Bellamy, F. D.; Bondoux, M.; Dodey, P. *Tetrahedron Lett.* **1990**, *31*, 7323.

29. Degenhardt, C. R. *J. Org. Chem.* **1980**, *45*, 2763.
30. Bianchi, D.; Cabri, W.; Cesti, P.; Francalanci, F.; Ricci, M. *J. Org. Chem.* **1988**, *53*, 104.
31. Larchevêque, M.; Henrot, S. *Tetrahedron Lett.* **1987**, *28*, 1781.
32. Larchevêque, M.; Henrot, S. *Tetrahedron* **1990**, *46*, 4277.
33. Liu, P.; Panek, J. S. *J. Am. Chem. Soc.* **2000**, *122*, 1235.
34. Tokunaga, M.; Larrow, J. F.; Kakiuchi, F.; Jacobsen, E. N. *Science* **1997**, *277*, 936.
35. Farnum, D. G.; Johnson, J. R.; Hess, R. E.; Marshall, T. B.; Webster, B. *J. Am. Chem. Soc.* **1965**, *87*, 5191.
36. Truce, W. E.; Bailey, P. S., Jr. *J. Org. Chem.* **1969**, *34*, 1341.
37. Jarowicki, K.; Kwiatkowski, S. *Monatsh. Chem.* **1985**, *116*, 141.
38. Qin, D.; Byun, H.-S.; Bittman, R. *J. Am. Chem. Soc.* **1999**, *121*, 662.
39. Chen, K. M.; Joullié, M. M. *Tetrahedron Lett.* **1984**, *25*, 393.
40. Matsuura, F.; Hamada, Y.; Shioiri, T. *Tetrahedron* **1993**, *50*, 265.
41. Chauvel, E. N.; Coric, P.; Llorens-Cortès, C.; Wilk, S.; Roques, B. P.; Fournié-Zaluski, M.-C. *J. Med. Chem.* **1994**, *37*, 1339.
42. Hansch, C. *Acc. Chem. Res.* **1969**, *2*, 232.
43. Giraud, I.; Rapp, M.; Maurizis, J.-C.; Madelmont, J.-C. *J. Med. Chem.* **2002**, *45*, 2116.
44. Ferrandina, G.; Melichar, B.; Loercher, A.; Verschraegen, C. F.; Kudelka, A. P.; Edwards, C. L.; Scambia, G.; Kavanagh, J. J.; Abbruzzese, J. L.; Freedman, R. S. *Cancer Res.* **1997**, *57*, 4309.
45. Vasse, M.; Thibout, D.; Paysant, J.; Legrand, E.; Soria, C.; Crépin, M. *Br. J. Cancer* **2001**, *84*, 802.
46. Watanabe, M.; Sugano, S.; Imai, J.; Yoshida, K.; Onodera, R.; Amin, M. R.; Uchida, K.; Yamaguchi, R.; Tateyama, S. *Res. Vet. Sci.* **2001**, *70*, 27.
47. Ziegler, H. J.; Bruckner, P.; Binon, F. *J. Org. Chem.* **1967**, *32*, 3989.
48. Evrard, A.; Vian, L.; Aubert, C.; Cano, J. P. *Cell Biol. Toxicol.* **1996**, *12*, 345.