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Alkylating agents from sugars. Alkyl hexopyranoside derivatives as carrier systems for chlorambucil☆

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Abstract

Chlorambucil derivatives involving alkyl 2-aminodeoxy sugars have been synthesized in good yield by coupling the chlorambucil moiety to positions C-2 or C-3 of the sugar, directly or via a spacer. The starting material was easily available from 2-acetamido-2-deoxy-D-glucose. The final compounds were tested for cytotoxicity, and some of those that presented the best results were studied for inhibition of cell proliferation.

Keywords: Alkylating agents; Chlorambucil; 2-Aminodeoxy sugars; Drug carrier; Cytotoxicity; Inhibition of cell proliferation

1. Introduction

Alkylating agents have been pioneering drugs in the treatment of malignant metastases, but their selectivity for neoplastic tissues is generally low [2]. An approach for improving such selectivity is to incorporate the alkylating agent in a more selective carrier, amino acid [3] or carbohydrate [4], as carcinoma cells in rapid growth have a great demand for these primary metabolites. At the same time, it is useful to introduce lipophilic moieties in the carrier in order to improve the cell membrane crossing.

In Part 1 of this series [1], we reported the synthesis of alkylating agents of which the active moiety is a derivative of cyclophosphamide bonded to the sugar moiety of alkyl 2-amino-2-deoxy-D-allopyranoside and which differ in the lipophilic strength of the alkyl group.

In the present report, we now describe the synthesis of alkyl hexopyranoside derivatives in which the alkylating agent chlorambucil (widely used clinically in the treatment of chronic lymphocytic leukaemia [5]) was linked at C-2 or C-3 of the sugar. Variation of the alkyl moiety of the aglycon, and the introduction of a spacer between the carrier and the chlorambucil, enables the hydrophilic–lipophilic balance (HLB) of these compounds to be modulated. We also studied the cytotoxic and cytostatic properties of some of these compounds against various model cell lines.

☆ Potential anticancer drugs, Part 2. For Part 1, see Ref [1].

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2. Results and discussion

Chemistry.—The appropriately protected intermediate sugars comprising the starting material to which the chlorambucil would be bonded as ester or amide were prepared from 2-acetamido-2-deoxy-D-glucose for the *gluco* and *allo* derivatives, and from methyl α-D-glucopyranoside for the *altro* derivatives. The synthesis of 1-hexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**3**) and 1-hexyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**4**) from **1** [6], using procedures described in the literature [1,7,8] is shown in Scheme 1. Elemental analyses, and MS and NMR spectroscopy data, confirmed the proposed structures.

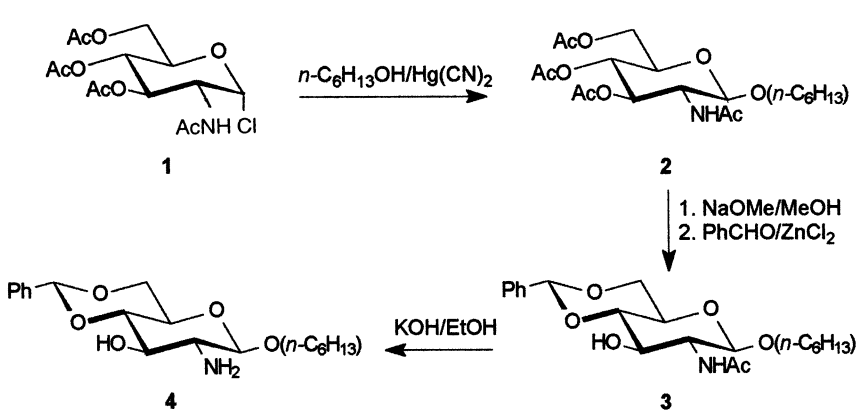
Compound **3** and its analogues **5** and **6** [1] are the key intermediates for synthesis of the compounds in which chlorambucil bonds directly to C-3 of the sugar moiety via an ester function (Scheme 2). Acylation at O-3 of **3**, **5**, and **6** was confirmed by the downfield chemical shift of the signals corresponding to H-3 in the ^{1}H NMR spectra of **7**, **8** and **9** (5.35, 5.33 and 5.28 ppm, respectively). In addition, for each of **7**, **8** and **9**, the NMR spectra showed the characteristic signals corresponding to the chlorambucil moiety (6.9, 6.5, 2.5 and 2.3 ppm approximately, in ^{1}H spectra, and 53 and 40 ppm approximately, in ^{13}C spectra). Removal of the benzylidene group in **7–9** gave diols **10–12** in almost quantitative yield, the structures of which were confirmed by NMR spectroscopy.

The coupling of chlorambucil at C-2 of the sugar via an amide bond requires the preparation of **4** (Scheme 1) and its *gluco* analogues **13–15** [9], as well as the *allo* derivative **16** [1]. The reaction of **4**, **13–16** with chlorambucil, activated by the pentachlorophenyl trichloroacetate procedure [10], in THF gave the corresponding amides **17–21** (Scheme 3). These compounds were isolated in good yield; in no case was acylation of the hydroxyl at C-3 observed. The ^{1}H NMR spectrum of **17** shows two doublets at 7.73 ppm for NH and at 5.23 ppm for OH-3, as significant signals, besides those already mentioned for the chlorambucil moiety. Hydrolysis of the benzylidene group gave **22–26**. The ^{1}H NMR spectrum of **22** shows characteristic signals at 7.57 (d, NH), 4.94 (bs, OH-4), 4.83 (d, OH-3), and 4.49 ppm (t, OH-6).

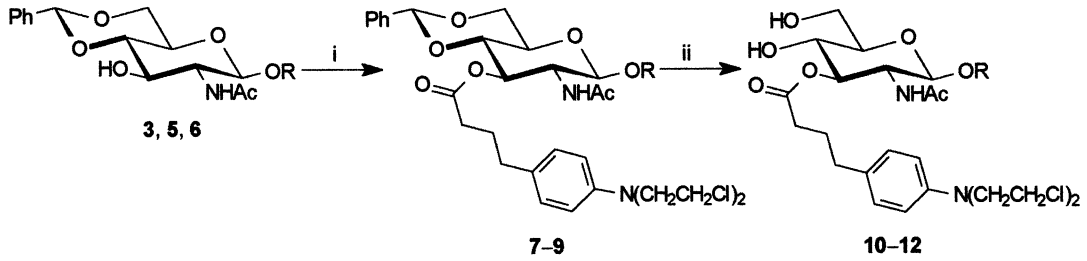
Following a widely applied strategy [11], we also prepared compounds **29**, **35** and **36** in which 2-aminoethanol (**29** and **35**) or 3-aminopropanol (**36**) were used as spacers between the sugar residue and chlorambucil.

The 2-aminoethanol spacer was introduced at C-3 of the *gluco* derivative **29** (Scheme 4) by the reaction of the deprotonated (NaH) alcohol **3** with bromoacetonitrile to give **27**. Reduction of the cyano group [12] in **27** ($CoCl_2$–$NaBH_4$/MeOH) provided the corresponding amino derivative that was directly acylated with chlorambucil (DMAP–DCC) thus leading to **28**. In turn, removal of the benzylidene group in **28** gave the expected diol **29**. Elemental analyses, NMR and mass spectrometry confirmed the structure and purity of **27–29**.

Alternatively, the *altro* derivatives **35** and **36** were obtained starting from oxirane **30** [13]



Scheme 1.

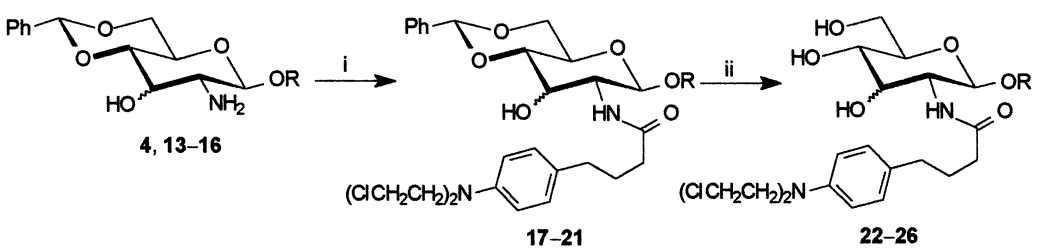


Scheme 2.

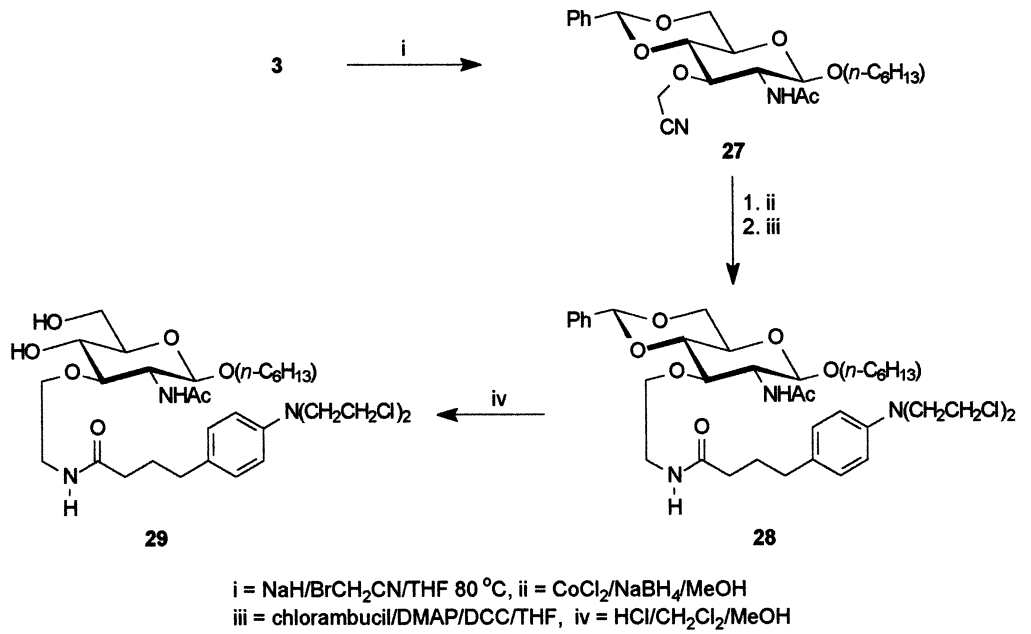
(Scheme 5). The ring-opening reaction, using the amino group of 2-aminoethanol or 3-aminopropanol as nucleophile and lithium perchlorate as catalyst [14], led to **31** and **32**. Acylation of the latter compounds with chlorambucil (DMAP–DCC/THF) led to the esters **33** and **34**. It is noteworthy that this reaction did not produce *N*-acylation, probably because of the axial arrangement of the nitrogen atom. Hydrolysis of the benzylidene group in **33** and **34** led to **35** and **36**. Introduction of the chlorambucil moiety in the esters **33** and **34** was confirmed by the downfield chemical shift of the spacer CH_2O (4.1 ppm) in the ^1H NMR spectra.

Biological assays.—In a previous study [15], we tested the cytotoxicity of an array of 12 compounds, all derived from chlorambucil. Cytotoxic effect was tested in two carcinoma-derived cells (ML-1210 and KB-07) and fibroblast cells (used as control of normal, healthy cells). From that study, we selected cyclohexyl glycosides **11** and **23** for their higher cytotoxicity to fibroblasts, in comparison with the other compounds that show similar toxicity for both carcinoma cells and fibroblasts. We interpreted these results as a sign of preference to carcinoma cells by these compounds. This preference could be due to the compound's higher affinity to some cellular receptor or transporter on the carcinoma cells, originated by the incorporation of glucosamine, as has been reported for similar compounds [4], and is reflected by the higher viability of fibroblast cells compared to the carcinoma cells.

To gain more insight into these phenomena, we now report the cytotoxicity of the two compounds **11** and **23**, using two different test procedures: (i) the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) assay [16], based on the measurement of the activity of mitochondrial enzymes (dehydrogenases) in viable cells, and: (ii) the 5-bromo-2′-deoxyuridine (BrdU) assay [17], based on the incorporation of BrdU instead of thymidine into DNA during DNA synthesis. The incorporation of BrdU into DNA is easily detected using a monoclonal antibody against BrdU and an enzyme-conjugate second antibody. The effect of various concentrations of **11** and **23** on the proliferation of ML-1210 and KB-



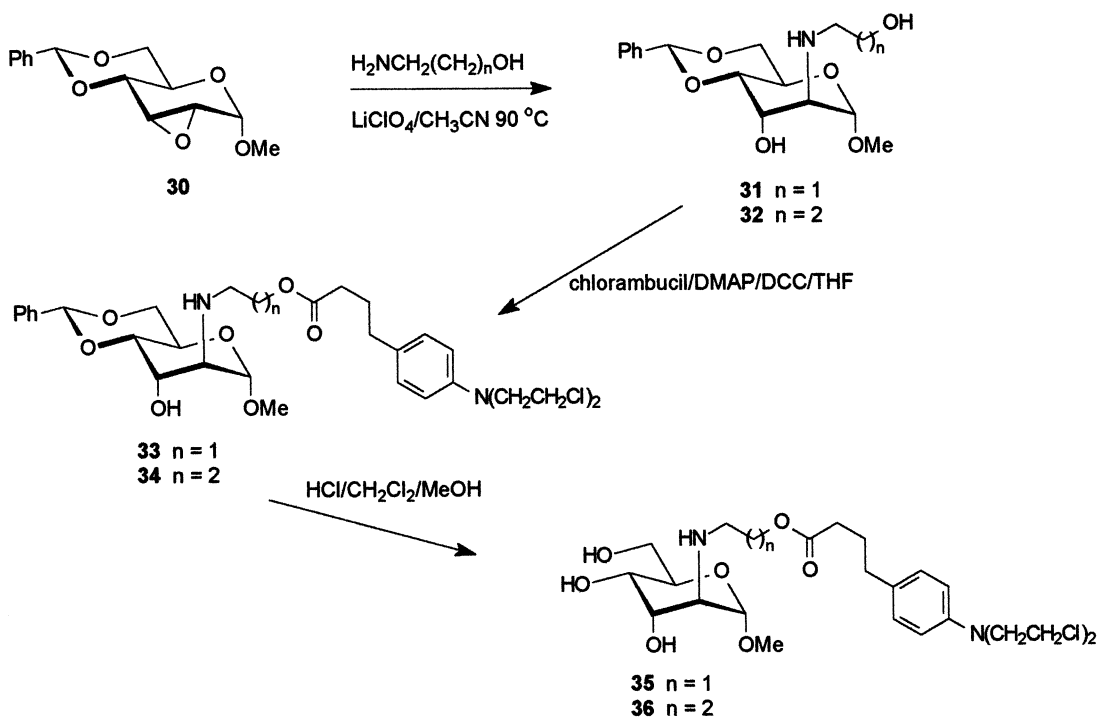
Scheme 3.



Scheme 4.

07 cells after 24, 48, and 96 h is shown in Tables 1 and 2.

From the data in Tables 1 and 2, we observed that proliferation inhibition by both compounds and on both cell lines is time- and dose-dependent. Cell proliferation at low concentration (1, 10 mM) shows a statistically significant difference ($P < 0.05$) in proliferation inhibition with time. At high concentration (100 mM), no statistically significant difference was observed between 24 and 96 h of culture. This could be due to the existence of a threshold concentration above which the cell does not import more product. These



Scheme 5.

results indicate that, for both compounds, a repeated low-dose treatment may be more efficient than a single-dose treatment at high concentration.

Calculation of the dose (concentration, mM in this study) that causes a 50% inhibition of proliferation (IC_{50}) is a good parameter for comparing efficiency between compounds that show the same or similar actions. From the data shown in Table 3, we observed that the best inhibitor of cell proliferation for the two cell lines used in this study is compound **11**, as shown by its lower IC_{50}. The estimation of IC_{50}, for **11** and **23** shows that the IC_{50} values are independent of the cell line and of the assay used, showing always a lower value for **11** than for **23** and chlorambucil (see Table 3). That means that with a lower dose it can achieve the same effect, reducing the overloading of detoxification systems, and reducing the side effects generally associated with chemotherapy.

These results make compound **11** a candidate for further studies in in vivo systems, such as in Meta-Mouse™ [18,19]. The greater hydrophilicity of **11**, as deduced from its higher solubility in aqueous media, make it a candidate for the chemotherapy of cerebral tumors which require more hydrophilic, and less myelotoxic antitumor agents than the highly hydrophobic alkylating drugs in use today [4].

3. Experimental

Chemistry

General methods. Evaporations were conducted under reduced pressure. Preparative chromatography was performed on Silica Gel 60 (E. Merck). Kieselgel 60 F_{254} (E. Merck) was used for TLC. Melting points are uncorrected. Optical rotations were obtained on a Bellingham and Stanley Ltd. P-20 polarimeter at 25 °C. Mass spectra were recorded on Kratos MS-80-RFA and Micromass AUTOSPECQ mass spectrometers at 70 eV for EI and 150 eV for CI. FAB mass spectra were recorded using a thioglycerol matrix. NMR spectra were recorded at 25 °C on a Bruker AC-200 spectrometer at 200 MHz for ^1H and 50 MHz for ^{13}C, and on a Bruker AMX-500 spectrometer at 500 MHz for ^1H. The chemical shifts are reported in ppm on the δ scale relative to Me_4Si.

*1-Hexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**2**). A soln of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride [6] (**1**) (18.3 g, 50 mmol) in dry $MeNO_2$ (40 mL) was added dropwise, with exclusion of moisture, to a stirred mixture of 1-hexanol in anhyd toluene (40 mL) containing 4 Å molecular sieves (30 g) and $Hg(CN)_2$ (12.5 g). The mixture was stirred overnight at room temperature. When TLC showed that all of the chloride had reacted, the mixture was diluted with EtOAc and filtered through a pad (12 cm diameter × 1 cm height) of alumina. The organic layer was washed with an aq satd soln of $NaHCO_3$ and water, then dried (Na_2SO_4) and concentrated. Suspension of the residue with stirring in hexane for 1 h gave a solid that was filtered and washed with hexane. Recrystallization from EtOH gave the pure peracetate **2**: 20.5 g (95%); mp 138–140 °C; $[\alpha]_D$ −10.4° (*c* 1.7, CH_2Cl_2); CIMS: *m*/*z* 432 (18%) [MH^+]; ^1H NMR (200 MHz, $CDCl_3$): δ 5.99 (d, 1 H, $J_{2,NH}$ 8.7 Hz, N–H), 5.25 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3), 4.99 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 4.63 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.20 (dd, 1 H, $J_{5,6}$ 4.8, $J_{6,6'}$ 12.2 Hz, H-6), 4.06 (dd, 1 H, $J_{5,6'}$ 2.5, $J_{6,6'}$ 12.3 Hz, H-6′), 3.8–3.6 (m, 3 H, H-2, H-5, OC*H*HR), 3.40 (m, 1 H, OCH*H*R), 2.00, 1.96, 1.95, 1.87 (4s, 4CH_3CO), 1.5–1.2 [$(CH_2)_4$], 0.80 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.8, 170.7, 170.2, 169.3 (4C=O), 100.6 (C-1), 72.3 (C-4), 71.5 (C-3), 69.8 (OCH_2R), 68.7 (C-5), 62.2 (C-6), 54.6 (C-2), 31.4, 29.2, 25.4, 22.9 [$(CH_2)_4$], 23.1 (CH_3CON), 20.7, 20.6, 20.5 (3CH_3COO), 13.9 (CH_3). Anal. Calcd for $C_{20}H_{33}NO_9$: C, 55.67; H, 7.71; N, 3.25. Found: C, 55.35; H, 7.57; N, 3.26.

*1-Hexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**3**). To a soln of 1-hexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**2**) (8.6 g, 20 mmol) in MeOH (200 mL) was added a soln of NaOMe (4.0 mmol) in MeOH (20 mL). After 30 min at room temperature, the mixture was neutralized by addition of Dowex 50 resin

(H^+ form), filtered, and evaporated. The solid obtained was dissolved in benzaldehyde (100 mL), and $ZnCl_2$ (5 g) was added. The mixture was stirred overnight and then poured into 1:1 hexane–water (500 mL) with stirring. The precipitate was filtered, washed with water and with hexane, and then recrystallized from EtOH: 7.2 g (91%); mp 267–269 °C; $[\alpha]_D$ −76.7° (*c* 0.8, DMF); CIMS: *m/z* 394 (42%) [MH^+]; ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 7.80 (d, 1 H, $J_{2,NH}$ 8.6 Hz, N–H), 7.5–7.3 (m, 5 H, Ph), 5.58 (s, 1 H, PhC*H*), 5.25 (d, 1 H, $J_{3,OH}$ 5.2 Hz, O–H), 4.45 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.18 (dd, 1 H, $J_{5,6e}$ 4.7, $J_{6e,6a}$ 10.4 Hz, H-6_e), 3.8–3.2 (m, 7 H, H-2, H-3, H-4, H-5, H-6_a, OCH_2R), 1.79 (s, 3 H, CH_3CON), 1.5–1.2 [$(CH_2)_4$], 0.85 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 168.9 (C=O), 137.8, 128.9, 128.0, 126.4 (Ph), 101.6 (Ph*C*H), 100.6 (C-1), 81.3 (C-4), 70.4 (C-3), 68.7 (OCH_2R), 67.9 (C-6), 65.9 (C-5), 56.2 (C-2), 31.0, 29.0, 25.0, 22.1 [$(CH_2)_4$], 23.0 (CH_3CON), 13.9 (CH_3). Anal. Calcd for $C_{21}H_{31}NO_6$: C, 64.10; H, 7.94; N, 3.56. Found: C, 63.86; H, 7.86; N, 3.51.

*1-Hexyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**4**). A soln of hexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**3**) (3.15 g, 8.0 mmol), in a hot mixture of KOH (15 g) and 96% EtOH (50 mL), was heated under reflux for 6 h. The hot soln was poured carefully into hot water (400 mL). After being cooled to room temperature, the precipitate was kept overnight at −10 °C. The solid was filtered, washed with water, dried, and then crystallized from EtOH: 2.6 g (92%); mp 109–111 °C; $[\alpha]_D$ −54.7° (*c* 0.9, CH_2Cl_2); CIMS: *m/z* 352 (19%) [MH^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5 H, Ph), 5.51 (s, 1 H, PhC*H*), 4.30 (dd, 1 H, $J_{5,6e}$ 4.8, $J_{6e,6a}$ 10.5 Hz, H-6_e), 4.20 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.0–3.3 (m, 6 H, H-3, H-4, H-5, H-6_a, OCH_2R), 2.78 (t, 1 H, $J_{1,2} = J_{2,3}$ 7.9 Hz, H-2), 1.7–1.1 [$(CH_2)_4$], 0.87 (t, 3 H, *J* 6.7 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.1, 129.2, 128.3, 126.2 (Ph), 104.5 (C-1), 101.9 (Ph*C*H), 81.4 (C-4), 73.2 (C-3), 70.4 (OCH_2R), 68.7 (C-6), 66.4 (C-5), 57.8 (C-2), 31.6, 29.5, 25.6, 22.5 [$(CH_2)_4$], 14.0 (CH_3). Anal. Calcd for $C_{19}H_{29}NO_5$: C, 64.93; H, 8.32; N, 3.98. Found: C, 64.58; H, 8.37; N, 3.89.

*Alkyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(4-{4-[bis(2-chloroethyl)amino]phenyl}butanoyl)-2-deoxy-β-*D*-glucopyranosides* (**7**–**9**). To a soln of alkyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**3**, **5**, **6**) (2.0 mmol) in distilled and dried THF (40 mL), chlorambucil (0.9 g, 3.0 mmol), DMAP (0.02 g, 0.16 mmol), and DCC (0.7 g, 3.3 mmol) were added. The reaction mixture was stirred overnight at room temperature. Dichloromethane was added and the solid was filtered. The organic layer was washed successively with aq 1 N AcOH and water, then dried (Na_2SO_4) and concentrated to give a solid. The product obtained was purified by flash chromatography on silica gel using a mixture of CH_2Cl_2–MeOH as eluent.

1-Hexyl derivative **7**. 1.2 g (87%); mp 161–163 °C; $[\alpha]_D$ −46.5° (*c* 0.2, CH_2Cl_2); FABMS: *m/z* 701 (100%) [MNa^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.4–7.2 (m, 5 H, Ph), 6.98, 6.51 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 6.65 (d, 1 H, $J_{2,NH}$ 9.4 Hz, N–H), 5.46 (s, 1 H, PhC*H*), 5.35 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 4.4–4.1 (m, 3 H, H-1, H-2, H-6_e), 3.8–3.4 (m, 12 H, H-4, H-5, H-6_a, OC*H*HR, $N(CH_2CH_2Cl)_2$), 3.14 (m, 1 H, OCH*H*R), 2.50 (t, 2 H, *J* 7.4 Hz, CH_2Ar), 2.35 (t, 2 H, *J* 7.2 Hz, CH_2COO), 1.92 (s, CH_3CON), 1.85 (m, 2 H, Ar–CH_2–CH_2–CH_2–COO), 1.6–1.1 [$(CH_2)_4$], 0.86 (t, 3 H, *J* 6.7 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.2 (COO), 170.0 (CON), 144.2, 136.9, 129.9, 129.4, 128.8, 128.0, 125.7, 111.9 (2Ar), 102.1 (Ph*C*H), 101.0 (C-1), 78.8 (C-4), 72.2 (C-3), 69.9 (OCH_2R), 68.5 (C-6), 65.7 (C-5), 53.7 (C-2), 53.3 (NCH_2), 40.3 (CH_2Cl), 33.5, 33.3, 31.4, 29.2, 26.7, 25.3, 22.5 [7(CH_2)], 23.1 (CH_3CON), 13.9 (CH_3). Anal. Calcd for $C_{35}H_{48}Cl_2N_2O_7$: C, 61.85; H, 7.12; N, 4.12. Found: C, 61.87; H, 6.80; N, 4.06.

Cyclohexyl derivative **8**. 1.2 g (82%); mp 187–189 °C; $[\alpha]_D$ −83.1° (*c* 0.4, CH_2Cl_2); FABMS: *m/z* 699 (31%) [MNa^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5 H, Ph), 6.98, 6.51 (2d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 6.03 (d, 1 H, $J_{2,NH}$ 9.4 Hz, N–H), 5.48 (s, 1 H, PhC*H*), 5.33 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.8 Hz, H-3), 4.54 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.26 (dd, 1 H, $J_{5,6e}$ 4.6, $J_{6e,6a}$ 10.2 Hz, H-6_e), 4.03 (m, 1 H, H-2), 3.7–3.4 (m, 12 H, H-4, H-5, H-6_a, OCH,

Table 1
Inhibitory effect (%) of **11** and **23** on the proliferation of ML-1210 cells determined by the MTT and BrdU assays

Product		1 mM	10 mM	20 mM	50 mM	100 mM
ML-1210 (MTT assay)						
11	(24)	16.3 ± 1.7	32.1 ± 3.2	40.3 ± 2.3	40.1 ± 1.6	49.0 ± 3.2
	(48)	21.8 ± 2.4	37.5 ± 1.7	42.5 ± 1.7	43.5 ± 3.8	53.1 ± 2.8
	(96)	24.2 ± 3.0	38.1 ± 4.2	43.1 ± 3.8	44.2 ± 2.4	53.0 ± 2.7
23	(24)	1.0 ± 0.2	17.1 ± 2.3	28.5 ± 2.3	29.2 ± 2.8	33.2 ± 2.1
	(48)	4.3 ± 1.2	20.4 ± 1.7	32.0 ± 1.3	33.0 ± 1.6	36.1 ± 3.2
	(96)	10.2 ± 3.2	26.2 ± 3.2	34.3 ± 2.4	37.4 ± 2.3	38.4 ± 1.9
ML-1210 (BrdU assay)						
11	(24)	22.2 ± 3.4	34.1 ± 3.2	35.3 ± 4.1	40.1 ± 4.9	45.1 ± 2.7
	(48)	24.6 ± 5.3	35.3 ± 2.4	37.0 ± 3.3	42.4 ± 2.6	44.0 ± 4.1
	(96)	27.1 ± 2.6	36.1 ± 1.3	44.2 ± 2.7	43.8 ± 3.7	46.8 ± 4.5
23	(24)	1.3 ± 0.3	10.2 ± 1.3	20.3 ± 1.0	26.3 ± 2.4	36.3 ± 2.4
	(48)	4.2 ± 1.1	13.2 ± 2.6	24.2 ± 2.3	28.4 ± 3.1	35.7 ± 5.3
	(96)	8.1 ± 1.7	20.4 ± 1.8	30.1 ± 2.8	30.8 ± 1.8	36.8 ± 1.8

$N(CH_2CH_2Cl)_2$), 2.49 (t, 2 H, *J* 7.3 Hz, CH_2Ar), 2.34 (t, 2 H, *J* 7.3 Hz, CH_2COO), 1.91 (s, CH_3CON), 1.8–1.1 [6(CH_2)]; ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.1 (COO), 169.9 (CON), 144.3, 137.0, 130.1, 129.6, 129.0, 128.2, 126.0, 112.0 (2Ar), 101.3 (Ph*C*H), 100.4 (C-1), 78.8 (C-4), 77.3 (OCH), 71.9 (C-3), 68.7 (C-6), 66.1 (C-5), 54.7 (C-2), 53.5 (NCH_2), 40.4 (CH_2Cl), 33.6, 33.4, 33.1, 31.4, 26.8, 25.4, 23.6, 23.4 [8(CH_2)], 23.3 (*C*H_3CON). Anal. Calcd for $C_{35}H_{46}Cl_2N_2O_7$: C, 62.03; H, 6.84; N, 4.13. Found: C, 61.98; H, 6.96; N, 4.12.

1-Octyl derivative **9**. 1.2 g (88%); mp 168–170 °C; $[\alpha]_D$ −65.6° (*c* 0.3, CH_2Cl_2); FABMS: *m/z* 729 (100%) [MNa^+]; ^1H NMR (200 MHz, $CDCl_3$): δ 7.5–7.2 (m, 5 H, Ph), 6.96, 6.50 (2d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.79 (d, 1 H, $J_{2,NH}$ 9.5 Hz, N–H), 5.49 (s, 1 H, PhC*H*), 5.28 (t, 1 H, $J_{2,3} = J_{3,4}$ 10.0 Hz, H-3), 4.44 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.31 (dd, 1 H, $J_{5,6e}$ 4.8, $J_{6e,6a}$ 10.4 Hz, H-6_e), 4.07 (m, 1 H, H-2), 3.9–3.4 (m, 12 H, H-4, H-5, H-6_a, OC*H*HR, $N(CH_2CH_2Cl)_2$), 3.2 (m, 1 H, OCH*H*R), 2.48 (t, 2 H, *J* 7.3 Hz, CH_2Ar), 2.32 (t, 2 H, *J* 7.2 Hz, CH_2COO), 2.0–1.8 (m, 2 H, Ar–CH_2–CH_2–CH_2–COO), 1.92 (s, CH_3CON), 1.7–1.1 [(CH_2)$_6$], 0.85 (t, 3 H, *J* 6.9 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.0 (COO), 169.9 (CON), 144.3, 136.9, 130.2, 129.4, 129.0, 128.2, 126.0, 112.1 (2Ar), 102.2 (Ph*C*H), 101.3 (C-1), 78.7 (C-4), 71.8 (C-3), 70.1 (OCH_2R), 68.6 (C-6), 65.3 (C-5), 54.4 (C-2), 53.5 (NCH_2), 40.5 (CH_2Cl), 33.7, 33.6, 33.5, 31.8, 29.4, 29.3, 26.8, 25.8, 22.6 [9(CH_2)], 23.3 (*C*H_3CON), 14.1 (CH_3). Anal. Calcd for $C_{37}H_{52}Cl_2N_2O_7$: C, 62.79; H, 7.40; N, 3.96. Found: C, 62.86; H, 7.38; N, 3.85.

*Alkyl 2-acetamido-3-*O*-(4-{4-[bis(2-chloroethyl)amino]phenyl}butanoyl)-2-deoxy-β-*D*-glucopyranosides* (**10–12**). To a soln of alkyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(4-{4-[bis-(2-chloroethyl)amino]phenyl}butanoyl)-2-deoxy-β-D-glucopyranoside (**7–9**) (1.0 mmol) in 5:1 CH_2Cl_2–MeOH (60 mL) was added conc HCl (0.5 mL) and the soln was then heated under reflux for 2 h with stirring. After being cooled to room temperature, the mixture was neutralized by addition of Amberlite IRA-440C resin (OH^- form), filtered, and evaporated. The solid was purified by flash chromatography on silica gel using CH_2Cl_2–MeOH mixtures as eluents.

1-Hexyl derivative **10**. 0.5 g (90%); mp 73–75 °C; $[\alpha]_D$ −7.8° (*c* 1.0, DMF); FABMS: *m/z* 613 (100%) [MNa^+]; ^1H NMR (200 MHz, Me_2SO-d_6): δ 7.82 (d, 1 H, $J_{2,NH}$ 9.4 Hz, N–H), 7.02, 6.66 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.25 (d, 1 H, $J_{4,OH}$ 5.8 Hz, OH-4), 4.83 (t, 1 H, $J_{2,3} = J_{3,4}$ 10.4 Hz, H-3), 4.58 (t, 1 H, $J_{6,OH}$ 5.8 Hz, OH-6), 4.37 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 2.43 (t, 2 H, *J* 7.4 Hz, CH_2Ar), 2.20 (t, 2 H, *J* 7.3 Hz, CH_2COO), 1.68 (s, CH_3CON), 1.5–1.1 [(CH_2)$_4$], 0.84 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 174.2 (COO), 168.7 (CON), 144.4, 129.6, 129.3, 111.8 (*p*-C_6H_4), 100.5 (C-1), 76.6 (C-4), 75.9 (C-3), 68.4

Table 2
Inhibitory effect (%) of **11** and **23** on the proliferation of KB-07 cells determined by the MTT and BrdU assays

Product		1 mM	10 mM	20 mM	50 mM	100 mM
KB-07 (MTT assay)						
11	(24)	8.3 ± 2.4	20.3 ± 3.2	37.3 ± 1.9	40.2 ± 3.2	48.2 ± 2.7
	(48)	12.4 ± 1.6	23.8 ± 1.6	37.8 ± 2.6	44.3 ± 1.9	50.2 ± 2.4
	(96)	17.5 ± 1.3	27.4 ± 2.4	39.3 ± 3.8	44.8 ± 2.8	51.8 ± 1.9
23	(24)	4.3 ± 0.8	20.2 ± 2.4	30.8 ± 2.8	32.8 ± 1.8	43.2 ± 2.4
	(48)	6.2 ± 1.2	23.1 ± 4.3	34.3 ± 3.7	34.1 ± 3.5	47.1 ± 3.1
	(96)	9.1 ± 2.3	26.7 ± 1.7	35.7 ± 2.4	33.8 ± 2.6	47.7 ± 1.8
KB-07 (BrdU assay)						
11	(24)	3.6 ± 1.3	12.3 ± 1.3	17.3 ± 1.4	20.0 ± 1.4	33.2 ± 1.8
	(48)	6.2 ± 0.9	14.6 ± 1.7	23.4 ± 2.1	23.1 ± 3.2	35.1 ± 3.2
	(96)	6.7 ± 1.4	18.3 ± 2.3	24.8 ± 1.6	23.0 ± 1.7	34.8 ± 1.7
23	(24)	1.0 ± 0.1	1.4 ± 0.3	8.2 ± 2.1	17.2 ± 2.3	22.8 ± 1.6
	(48)	1.2 ± 0.2	3.5 ± 0.2	10.4 ± 2.2	23.4 ± 2.8	25.1 ± 2.4
	(96)	2.4 ± 0.3	8.2 ± 1.0	12.7 ± 1.8	24.0 ± 3.2	24.8 ± 3.1

(OCH_2R), 67.9 (C-5), 60.6 (C-6), 53.1 (C-2), 52.2 (NCH_2), 41.1 (CH_2Cl), 33.2, 31.0, 28.9, 26.7, 25.1, 22.1 [7(CH_2)], 22.7 (CH_3CON), 13.9 (CH_3). Anal. Calcd for $C_{28}H_{44}Cl_2N_2O_7$: C, 56.85; H, 7.50; N, 4.74. Found: C, 56.80; H, 7.37; N, 4.63.

Cyclohexyl derivative **11**. 0.5 g (92%); mp 79–81 °C; $[\alpha]_D$ −3.4° (*c* 0.6, DMF); FABMS: *m/z* 611 (100%) [MNa^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.02, 6.58 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 6.26 (d, 1 H, $J_{2,NH}$ 8.6 Hz, N–H), 5.18 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.8 Hz, H-3), 4.70 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 2.50 (t, 2 H, *J* 7.3 Hz, CH_2Ar), 2.37 (t, 2H, *J* 7.3 Hz, CH_2COO), 1.91 (s, CH_3CON); ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.6 (COO), 170.4 (CON), 144.3, 130.2, 129.6, 112.1 (*p*-C_6H_4), 99.3 (C-1), 77.5 (C-4), 75.3 (OCH), 75.2 (C-3), 69.2 (C-5), 62.1 (C-6), 54.7 (C-2), 53.5 (NCH_2), 40.5 (CH_2Cl), 33.8, 33.6, 33.2, 31.6, 26.8, 25.4, 23.8, 23.6 [8(CH_2)], 23.2 (CH_3CON). Anal. Calcd for $C_{28}H_{42}Cl_2N_2O_7$: C, 57.04; H, 7.18; N, 4.75. Found: C, 56.76; H, 7.30; N, 4.63.

1-Octyl derivative **12**. 0.6 g (94%); mp 81–83 °C; $[\alpha]_D$ −7.1° (*c* 0.5, CH_2Cl_2); FABMS: *m/z* 641 (100%) [MNa^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.01, 6.58 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.75 (d, 1 H, $J_{2,NH}$ 9.0 Hz, N–H), 5.10 (t, 1 H, $J_{2,3} = J_{3,4}$ 10.2 Hz, H-3), 4.52 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 2.52 (t, 2 H, *J* 7.2 Hz, CH_2Ar), 2.35 (t, 2 H, *J* 7.2 Hz, CH_2COO), 1.92 (s, CH_3CON), 1.6–1.1 [$(CH_2)_6$], 0.83 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.7 (COO), 170.3(CON), 144.4, 130.2, 129.6, 112.1 (*p*-C_6H_4), 101.1 (C-1), 75.3 (C-4), 75.3 (C-3), 69.9 (OCH_2R), 69.3 (C-5), 62.2 (C-6), 54.3 (C-2), 53.5 (NCH_2), 40.5 (CH_2Cl), 33.8, 33.6, 31.7, 29.4, 29.3, 26.8, 25.8, 22.6 [9(CH_2)], 23.3 (CH_3CON), 14.1 (CH_3). Anal. Calcd for $C_{30}H_{48}Cl_2N_2O_7$: C, 58.15; H, 7.81; N, 4.52. Found: C, 57.84; H, 7.68; N, 4.36.

Alkyl 4,6-O-benzylidene-2-(4-{4-[bis-(2-chloroethyl)amino]phenyl}butanamido)-2-deoxy-β-D-hexopyranosides (**17–21**). A soln of chlorambucil pentachlorophenol ester (0.83 g, 1.5 mmol) in distilled and dried THF (20 mL) was added to a soln of alkyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-hexopyranoside (**4**, **13–16**) (2.0 mmol) in the same solvent (40 mL) and the mixture was stirred at room

Table 3
Value of IC_{50} (mM) for **11**, **23** and chlorambucil

	11	**23**	Chlorambucil
Cell line KB-07			
MTT assay	96.7 ± 7.8	133 ± 10.9	437.8 ± 32.6*
BrdU assay	695 ± 16.9	791 ± 198.6	nd[a]
Cell line ML-1210			
MTT assay	88.0 ± 20.8	176.6 ± 39.1	628.34 ± 122.3**
BrdU assay	242 ± 75.8	291.5 ± 71.2	nd

[a] nd, not determined.
* $P < 0.005$.
** $P < 0.05$.

temperature for 12 h. Then, a new portion of reactive soln (1.5 mmol) was added and the mixture was stirred for a further 12 h. The reaction was diluted with CH_2Cl_2, washed successively with aq 5% soln of NaOH and water, then dried (Na_2SO_4) and concentrated to dryness. The solid obtained was purified by flash chromatography on silica gel using CH_2Cl_2–MeOH mixtures as eluents.

1-Hexyl glucopyranoside derivative **17**. 1.0 g (72%); mp 195–197 °C; $[\alpha]_D$ −6.9° (*c* 0.3, DMF); FABMS: *m/z* 659 (100%) [MNa^+]; ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 7.73 (d, 1 H, $J_{2,NH}$ 8.2 Hz, N–H), 7.5–7.3 (m, 5 H, Ph), 7.03, 6.62 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.58 (s, 1 H, PhC*H*), 5.23 (d, 1 H, $J_{3,OH}$ 5.3 Hz, OH), 4.45 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.18 (dd, 1 H, $J_{5,6e}$ 4.7, $J_{6e,6a}$ 10.2 Hz, H-6_e), 2.40 (t, 2 H, *J* 7.3 Hz, CH_2Ar), 2.15 (t, 2 H, *J* 7.3 Hz, CH_2CON), 1.8–1.1 [5(CH_2)], 0.83 (t, 3H, *J* 6.7 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 171.8 (C=O), 144.3, 137.7, 129.3, 128.8, 128.0, 126.3, 111.8 (2Ar), 101.6 (Ph*C*H), 100.6 (C-1), 81.3 (C-4), 70.4 (C-3), 68.7 (OCH_2R), 67.9 (C-6), 66.0 (C-5), 56.0 (C-2), 52.2 (NCH_2), 41.1 (CH_2Cl), 35.3, 33.5, 31.0, 29.1, 27.5, 25.0, 22.0 [7(CH_2)], 13.9 (CH_3). Anal. Calcd for $C_{33}H_{46}Cl_2N_2O_6$: C, 62.16; H, 7.27; N, 4.39. Found: C, 62.07; H, 7.49; N, 4.51.

Cyclohexyl glucopyranoside derivative **18**. 1.0 g (76%); mp 163–165 °C; $[\alpha]_D$ −37.2° (*c* 0.6, DMF); FABMS: *m/z* 657 (85%) [MNa^+]; ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 7.71 (d, 1 H, $J_{2,NH}$ 8.4 Hz, N–H), 7.5–7.3 (m, 5 H, Ph), 7.00, 6.64 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.57 (s, 1 H, PhC*H*), 5.21 (d, 1 H, $J_{3,OH}$ 5.3 Hz, OH), 4.59 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.18 (dd, 1 H, $J_{5,6e}$ 4.5, $J_{6e,6a}$ 10.0 Hz, H-6_e), 2.08 (t, 2 H, *J* 7.2 Hz, CH_2CON), 1.8–1.0 [6(CH_2)]; ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 171.8 (C=O), 144.3, 137.8, 130.0, 129.3, 128.8, 128.0, 126.3, 111.9 (2Ar), 100.6 (Ph*C*H), 99.9 (C-1), 81.4 (C-4), 75.8 (OCH), 70.3 (C-3), 68.0 (C-6), 65.9 (C-5), 56.5 (C-2), 52.2 (NCH_2), 41.1 (CH_2Cl), 35.3, 33.5, 32.9, 31.1, 27.3, 25.2, 23.2, 22.9 [8(CH_2)]. Anal. Calcd for $C_{33}H_{44}Cl_2N_2O_6$: C, 62.37; H, 6.63; N, 4.30. Found: C, 62.36; H, 6.98; N, 4.41.

1-Octyl glucopyranoside derivative **19**. 1.2 g (88%); mp 193–195 °C; $[\alpha]_D$ −50.8° (*c* 0.3, DMF); FABMS: *m/z* 687 (100%) [MNa^+]; ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 7.72 (d, 1 H, $J_{2,NH}$ 8.4 Hz, N–H), 7.5–7.3 (m, 5 H, Ph), 7.01, 6.64 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.58 (s, 1 H, PhC*H*), 5.22 (d, 1 H, $J_{3,OH}$ 5.3 Hz, OH), 4.46 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.18 (dd, 1 H, $J_{5,6e}$ 4.7, $J_{6e,6a}$ 10.4 Hz, H-6_e), 2.40 (t, 2 H, *J* 7.3 Hz, CH_2Ar), 2.06 (t, 2 H, *J* 7.3 Hz, CH_2CON), 1.75 (m, 2 H, Ar–CH_2–CH_2–CH_2–CON), 1.5–1.1 [(CH_2)$_6$], 0.83 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 171.8 (C=O), 144.3, 137.8, 130.0, 129.2, 128.8, 128.0, 126.3, 111.8 (2Ar), 101.6 (Ph*C*H), 100.6 (C-1), 81.4 (C-4), 70.4 (C-3), 68.7 (OCH_2R), 67.9 (C-6), 66.0 (C-5), 56.0 (C-2), 52.2 (NCH_2), 41.1 (CH_2Cl), 35.3, 33.5, 31.2, 29.1, 28.8, 28.7, 27.4, 25.4, 22.2 [9(CH_2)], 13.9 (CH_3). Anal. Calcd for $C_{35}H_{50}Cl_2N_2O_6$: C, 63.15; H, 7.57; N, 4.21. Found: C, 63.11; H, 7.62; N, 4.19.

1-Dodecyl glucopyranoside derivative **20**. 1.2 g (79%); mp 150–152 °C; $[\alpha]_D$ −67.0° (*c* 1.1, DMF); FABMS: *m/z* 743 (18%) [MNa^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.6–7.3 (m, 5 H, Ph), 7.10, 6.60 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.67 (d, 1 H, $J_{2,NH}$ 8.9 Hz, N–H), 5.55 (s, 1 H, PhC*H*), 4.70 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.30 (dd, 1 H, $J_{5,6e}$ 4.6, $J_{6e,6a}$ 9.9 Hz, H-6_e), 4.12 (t, 1 H, $J_{5,6a} = J_{6e,6a}$ 9.9 Hz, H-6_a), 2.55 (t, 2 H, *J* 7.3 Hz, CH_2Ar), 2.20 (t, 2 H, *J* 7.2 Hz, CH_2CON), 1.91 (m, 2 H, Ar–CH_2–CH_2–CH_2–CON), 1.7–1.1 [(CH_2)$_{10}$], 0.85 (t, 3 H, *J* 6.4 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.2 (C=O), 144.3, 137.0, 130.7, 129.7, 129.3, 128.3, 126.1, 112.1 (2Ar), 101.9 (Ph*C*H), 100.5 (C-1), 81.7 (C-4), 71.2 (C-3), 70.1 (OCH_2R), 68.6 (C-6), 66.2 (C-5), 59.3 (C-2), 53.2 (NCH_2), 40.4 (CH_2Cl), 36.0, 33.9, 31.9, 29.7, 29.6, 29.4, 29.3, 27.4, 25.9, 22.7 [13(CH_2)], 14.1 (CH_3). Anal. Calcd for $C_{39}H_{58}Cl_2N_2O_6$: C, 64.90; H, 8.10; N, 3.88. Found: C, 65.15; H, 8.01; N, 3.50.

1-Dodecyl allopyranoside derivative **21**. 1.2 g (79%); mp 156–158 °C; $[\alpha]_D$ −51.8° (*c* 0.8, DMF); FABMS: *m/z* 743 (42%) [MNa^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5 H, Ph), 7.06, 6.60 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.85 (d, 1 H, $J_{2,NH}$ 8.9 Hz, N–H), 5.57 (s, 1 H, PhC*H*), 4.63 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.36 (dd, 1 H, $J_{5,6e}$ 4.6, $J_{6e,6a}$ 9.9 Hz, H-6_e), 4.2 (m, 2 H, H-2, H-3), 2.55 (t, 2 H, *J* 7.3 Hz, CH_2Ar), 2.20 (t, 2 H, *J* 7.2 Hz, CH_2CON),

1.91 (m, 2 H, Ar–CH_2–CH_2–CH_2–CON), 1.7–1.1 [$(CH_2)_{10}$], 0.87 (t, 3 H, *J* 6.4 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.2 (C=O), 144.3, 137.0, 130.7, 129.7, 129.3, 128.3, 126.1, 112.1 (2Ar), 101.7 (Ph*C*H), 100.3 (C-1), 78.7 (C-4), 69.9 (OCH_2R), 69.1 (C-6), 68.9 (C-3), 63.4 (C-5), 53.6 (NCH_2), 51.8 (C-2), 40.5 (CH_2Cl), 36.0, 33.9, 31.9, 29.7, 29.6, 29.4, 29.3, 27.4, 25.9, 22.7 [13(CH_2)], 14.1 (CH_3). Anal. Calcd for $C_{39}H_{58}Cl_2N_2O_6$: C, 64.90; H, 8.10; N, 3.88. Found: C, 64.68; H, 7.75; N, 3.81.

*Alkyl 2-(4-{4-[bis(2-chloroethyl)amino]-phenyl} butanamido)-2-deoxy-β-*D*-hexopyranosides* (**22–26**). Reaction of alkyl 4,6-*O*-benzylidene-2-(4-{4-[bis(2-chloroethyl)amino]-phenyl}butanamido)-2-deoxy-β-D-hexopyranoside (**17–21**) (1.0 mmol) in 2:3 CH_2Cl_2–MeOH (50 mL) with HCl, as described for the preparation of **10–12**, gave a crude solid, which was purified by column chromatography using CH_2Cl_2–MeOH mixtures as eluents to give a pure compound (**22–26**).

1-Hexyl glucopyranoside derivative **22**. 0.5 g (83%); mp 153–155 °C; $[\alpha]_D$ −17.8° (*c* 0.3, DMF); FABMS: *m*/*z* 571 (100%) [MNa^+]; ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 7.57 (d, 1 H, $J_{2,NH}$ 8.4 Hz, N–H), 7.02, 6.62 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 4.94 (bs, 1 H, OH-4), 4.83 (d, 1 H, $J_{3,OH}$ 5.0 Hz, OH-3), 4.49 (t, 1 H, $J_{6,OH}$ 5.2 Hz, OH-6), 4.25 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 2.40 (t, 2 H, *J* 7.6 Hz, CH_2Ar), 2.04 (t, 2 H, *J* 7.6 Hz, CH_2CON), 1.72 (m, 2 H, Ar–CH_2–CH_2–CH_2–CON), 1.5–1.1 [$(CH_2)_4$], 0.80 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 171.7 (C=O), 144.3, 130.1, 129.3, 111.8 (*p*-C_6H_4), 101.0 (C-1), 77.0 (C-4), 74.2 (C-3), 70.5 (C-5), 68.3 (OCH_2R), 61.1 (C-6), 55.2 (C-2), 52.2 (NCH_2), 41.1 (CH_2Cl), 35.4, 33.6, 31.1, 29.1, 27.5, 25.1, 22.1 [7(CH_2)], 13.9 (CH_3). Anal. Calcd for $C_{26}H_{42}Cl_2N_2O_6$: C, 56.83; H, 7.70; N, 5.10. Found: C, 57.21; H, 7.61; N, 4.87.

Cyclohexyl glucopyranoside derivative **23**. 0.5 g (86%); mp 137–139 °C; $[\alpha]_D$ −20.2° (*c* 0.6, DMF); FABMS: *m*/*z* 569 (100%) [MNa^+]; ^{1}H NMR (500 MHz, Me_2SO-d_6): δ 7.55 (d, 1 H, $J_{2,NH}$ 8.4 Hz, N–H), 7.05, 6.60 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 4.38 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.03 (m, 1 H, H-3), 2.44 (t, 2 H, *J* 7.3 Hz, CH_2Ar), 2.03 (t, 2 H, *J* 7.3 Hz, CH_2CON), 1.71 (m, 2 H, Ar–CH_2–CH_2–CH_2–CON), 1.6–1.1 [$(CH_2)_5$]; ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 171.7 (C=O), 144.3, 130.1, 129.3, 111.9 (*p*-C_6H_4), 99.9 (C-1), 76.9 (C-4), 75.1 (OCH), 74.1 (C-3), 70.8 (C-5), 61.1 (C-6), 55.7 (C-2), 52.2 (NCH_2), 41.1 (CH_2Cl), 35.3, 33.6, 33.0, 31.0, 27.3, 25.3, 23.3, 23.0 [8(CH_2)]. Anal. Calcd for $C_{26}H_{40}Cl_2N_2O_6$: C, 57.04; H, 7.36; N, 5.12. Found: C, 57.13; H, 7.41; N, 4.94.

1-Octyl glucopyranoside derivative **24**. 0.5 g (88%); mp 143–145 °C; $[\alpha]_D$ −15.4° (*c* 0.4, DMF); FABMS: *m*/*z* 599 (100%) [MNa^+]; ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 7.58 (d, 1 H, $J_{2,NH}$ 8.4 Hz, N–H), 7.01, 6.63 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 4.23 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 2.40 (t, 2 H, *J* 7.6 Hz, CH_2Ar), 2.04 (t, 2 H, *J* 7.6 Hz, CH_2CON), 1.70 (m, 2 H, Ar–CH_2–CH_2–CH_2–CON), 1.5–1.1 [$(CH_2)_6$], 0.82 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 171.7 (C=O), 144.3, 130.1, 129.3, 111.8 (*p*-C_6H_4), 101.0 (C-1), 77.0 (C-4), 74.2 (C-3), 70.7 (C-5), 68.3 (OCH_2R), 61.0 (C-6), 55.2 (C-2), 52.2 (NCH_2), 41.1 (CH_2Cl), 35.4, 33.6, 31.3, 29.1, 28.9, 28.7, 27.5, 25.5, 22.1 [9(CH_2)], 14.0 (CH_3). Anal. Calcd for $C_{28}H_{46}Cl_2N_2O_6$: C, 58.23; H, 8.03; N, 4.85. Found: C, 57.99; H, 7.92; N, 4.79.

1-Dodecyl glucopyranoside derivative **25**. 0.5 g (86%); mp 165–167 °C; $[\alpha]_D$ −18.0° (*c* 1.1, DMF); FABMS: *m*/*z* 655 (63%) [MNa^+]; ^{1}H NMR (200 MHz, Me_2SO-d_6, D_2O, 100 °C): δ 7.01, 6.68 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 4.35 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 2.10 (t, 2 H, *J* 7.3 Hz, CH_2CON), 1.75 (m, 2 H, Ar–CH_2–CH_2–CH_2–CON), 1.5–1.1 [$(CH_2)_{10}$], 0.83 (t, 3 H, *J* 6.7 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 171.7 (C=O), 144.3, 130.1, 129.2, 111.8 (*p*-C_6H_4), 101.0 (C-1), 77.0 (C-4), 74.2 (C-3), 70.7 (C-5), 68.3 (OCH_2R), 61.0 (C-6), 55.2 (C-2), 52.3 (NCH_2), 40.9 (CH_2Cl), 35.0, 33.6, 31.3, 29.2, 29.1, 29.0, 28.9, 28.7, 27.5, 25.5, 22.0 [13(CH_2)], 14.0 (CH_3). Anal. Calcd for $C_{32}H_{54}Cl_2N_2O_6$: C, 60.65; H, 8.59; N, 4.42. Found: C, 60.83; H, 8.48; N, 4.45.

1-Dodecyl allopyranoside derivative **26**. 0.6 g (90%); mp 133–135 °C; $[\alpha]_D$ −28.0° (*c* 0.5, DMF); FABMS: *m*/*z* 655 (100%) [MNa^+]; ^{1}H NMR (500 MHz, Me_2SO-d_6): δ 7.42 (d, 1 H, $J_{2,NH}$ 8.3 Hz, N–H), 7.05, 6.60 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.05 (s, 1 H, OH-4), 4.65 (s, 1 H,

OH-3), 4.55 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 2.45 (m, 2 H, CH_2Ar), 2.15 (m, 2 H, CH_2CON), 1.75 (m, 2 H, Ar–CH_2–CH_2–CH_2–CON), 1.5–1.1 [$(CH_2)_{10}$], 0.85 (t, 3 H, *J* 6.7 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 171.3 (C=O), 144.3, 130.1, 129.2, 111.8 (*p*-C_6H_4), 98.7 (C-1), 74.3 (C-4), 70.1 (C-3), 68.2 (OCH_2R), 67.4 (C-5), 61.3 (C-6), 52.3 (NCH_2), 52.2 (C-2), 41.1 (CH_2Cl), 35.0, 33.6, 31.3, 29.2, 29.1, 29.0, 28.9, 28.7, 27.5, 25.5, 22.0 [13(CH_2)], 13.9 (CH_3). Anal. Calcd for $C_{32}H_{54}Cl_2N_2O_6$: C, 60.65; H, 8.59; N, 4.42. Found: C, 60.55; H, 8.44; N, 4.32.

*1-Hexyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-cyanomethyl-2-deoxy-β-*D*-glucopyranoside* (**27**). To a soln of 1-hexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**3**) (0.8 g, 2.0 mmol) in distilled and dried dioxane (60 mL), NaH (1.2 g, 50 mmol) was added and stirred for 30 min at room temperature. Then, $BrCH_2CN$ (0.48 g, 4.0 mmol) was added and the mixture stirred for a further 1 h. The excess NaH was removed by the dropwise addition of water, and the solution was poured into water (400 mL), giving a precipitate. The solid was filtered, washed with water, dried (Na_2SO_4), and crystallized from EtOH to give 0.8 g (95%) of **27**: mp 237–239 °C; $[\alpha]_D$ −41.2° (*c* 0.7, DMF); CIMS: *m*/*z* 433 (40%) [MH^+]; ^1H NMR (200 MHz, Me_2SO-d_6): δ 8.01 (d, 1 H, $J_{2,NH}$ 8.8 Hz, N–H), 7.5–7.3 (m, 5 H, Ph), 5.66 (s, 1 H, PhC*H*), 4.62 (d, 1 H, J_{gem} 16.6 Hz, OC*H*-HCN), 4.56 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.49 (d, 1 H, J_{gem} 16.6 Hz, OCH*H*CN), 4.22 (dd, 1 H, $J_{5,6e}$ 4.9, $J_{6e,6a}$ 10.1 Hz, H-6_e), 1.82 (s, 3 H, CH_3CON), 1.6–1.2 [$(CH_2)_4$], 0.85 (t, 3 H, *J* 6.8 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 169.1 (C=O), 137.4, 128.8, 128.1, 126.1 (Ph), 117.4 (CN), 101.1 (Ph*C*H), 100.1 (C-1), 80.3 (C-4), 79.5 (C-3), 68.9 (OCH_2R), 67.8 (C-6), 65.3 (C-5), 57.1 (OCH_2CN), 54.1 (C-2), 31.0, 29.0, 25.0, 22.1 [$(CH_2)_4$], 23.0 (CH_3CON), 13.9 (CH_3). Anal. Calcd for $C_{23}H_{32}N_2O_6$: C, 63.87; H, 7.46; N, 6.48. Found: C, 63.87; H, 7.27; N, 6.49.

*1-Hexyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-[2-(4-{4-[bis(2-chloroethyl)amino]phenyl}-butanamido)ethyl]-2-deoxy-β-*D*-glucopyranoside* (**28**). To a soln of 1-hexyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-cyanomethyl-2-deoxy-β-D-glucopyranoside (**27**) (0.65 g, 1.5 mmol) and $CoCl_2 \cdot 6H_2O$ (0.71 g, 3.0 mmol) in MeOH (250 mL), $NaBH_4$ (0.57 g, 15 mmol) was added in small portions and the mixture was stirred for 1 h at room temperature. The solvent was evaporated, and the residue was suspended in CH_2Cl_2 (100 mL) with stirring for 30 min. The solid was removed by filtration, and the organic phase was concentrated to a small vol (20 mL). Chlorambucil (0.46 g, 1.5 mmol), DMAP (0.01 g, 0.08 mmol) and DCC (0.35 g, 1.7 mmol) were added and stirred overnight at room temperature. The solid was filtered, and the filtrate was diluted with CH_2Cl_2, washed successively with aq 1 N AcOH and water, dried (Na_2SO_4), and concentrated to dryness. The product obtained was purified by flash chromatography on silica gel using 4:1 hexane–EtOAc and then 40:1 CH_2Cl_2–MeOH as eluents to give 1.0 g (93%) of **28**: mp 202–204 °C; $[\alpha]_D$ −19.8° (*c* 0.4, CH_2Cl_2); FABMS: *m*/*z* 744 (100%) [MNa^+]; ^1H NMR (200 MHz, Me_2SO-d_6): δ 7.94 (d, 1 H, $J_{2,NH}$ 7.4 Hz, N–H), 7.5–7.3 (m, 6 H, CH_2NH, Ph), 6.99, 6.61 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.62 (s, 1 H, PhC*H*), 4.48 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.20 (dd, 1 H, $J_{5,6e}$ 4.8, $J_{6e,6a}$ 10.1 Hz, H-6_e), 2.36 (t, 2 H, *J* 7.2 Hz, CH_2Ar), 1.94 (t, 2 H, *J* 7.3 Hz, CH_2CON), 1.70 (m, 2 H, Ar–CH_2–CH_2–CH_2–CON), 1.79 (s, 3 H, CH_3CON), 1.5–1.2 [$(CH_2)_4$], 0.85 (t, 3 H, *J* 6.6 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 171.8, 169.1 (2C=O), 144.3, 137.6, 129.9, 129.2, 128.8, 128.1, 126.0, 111.8 (2Ar), 101.4 (Ph*C*H), 100.1 (C-1), 80.5 (C-4), 78.8 (C-3), 70.0 (OCH_2CH_2NHCO), 68.8 (OCH_2R), 67.8 (C-6), 65.6 (C-5), 54.6 (C-2), 52.2 (NCH_2-CH_2Cl), 41.1 (NCH_2CH_2Cl), 34.7 (OCH_2CH_2-NHCO), 33.6, 30.9, 29.0, 27.2, 25.0, 22.1 [7(CH_2)], 22.8 (CH_3CON), 13.9 (CH_3). Anal. Calcd for $C_{37}H_{53}Cl_2N_3O_7$: C, 61.49; H, 7.39; N, 5.81. Found: C, 61.69; H, 7.42; N, 5.79.

*1-Hexyl 2-acetamido-3-*O*-[2-(4-{4-[bis(2-chloroethyl)amino]phenyl}butanamido)ethyl]-2-deoxy-β-*D*-glucopyranoside* (**29**). Reaction of 1-hexyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-[2-(4-{4-[bis(2-chloroethyl)amino]phenyl}-butanamido)ethyl]-2-deoxy-β-D-glucopyranoside (**28**) (0.72 g, 1.0 mmol) in 3:1 CH_2Cl_2–MeOH (40 mL) with HCl, as described for the preparation of **10**–**12**, gave a solid which was

purified by column chromatography using 20:1 CH_2Cl_2–MeOH as eluent, to give 0.6 g (90%) of **29**: mp 138–140 °C; $[\alpha]_D$ −5.71° (*c* 0.4, CH_2Cl_2); FABMS: *m/z* 656 (100%) [MNa^+]; ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 7.85 (d, 1 H, $J_{2,NH}$ 8.9 Hz, N–H), 7.57 (t, 1 H, *J* 4.8 Hz, CH_2NH), 7.02, 6.62 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 4.27 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 2.40 (t, 2 H, *J* 7.2 Hz, CH_2Ar), 2.05 (t, 2 H, *J* 7.1 Hz, CH_2CON), 1.76 (s, 3 H, CH_3CON), 1.70 (m, 2 H, Ar–CH_2–CH_2–CH_2–CON), 1.5–1.2 [$(CH_2)_4$], 0.84 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 172.1, 169.0 (2C=O), 144.4, 130.0, 129.4, 111.9 (2Ar), 100.8 (C-1), 83.3 (C-4), 76.7 (C-3), 70.3 (OCH_2CH_2NHCO), 69.5 (C-5), 68.4 (OCH_2-R), 60.8 (C-6), 54.4 (C-2), 52.3 (NCH_2CH_2Cl), 41.2 (NCH_2CH_2Cl), 34.9 (OCH_2CH_2NHCO), 33.7, 31.1, 29.0, 27.4, 25.1, 22.2 [7(CH_2)], 22.9 (CH_3CON), 14.0 (CH_3). Anal. Calcd for $C_{30}H_{49}Cl_2N_3O_7$: C, 56.78; H, 7.78; N, 6.62. Found: C, 56.87; H, 7.75; N, 6.52.

*Methyl 4,6-*O*-benzylidene-2-deoxy-2-(3-hydroxypropylamino)-α-*D*-altropyranoside* (**32**). A soln of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside [13] (**30**) (2.64 g, 10.0 mmol) and $LiClO_4$ (2.13 g, 20.0 mmol) in MeCN (40 mL) was heated under stirring at 90 °C, and 3-aminopropanol (3.1 mL, 40.0 mmol) was added. The mixture was stirred until completion of the reaction (TLC, 24 h). It was then left to cool to room temperature and poured into ice-water with stirring. The precipitate obtained was filtered and crystallized from EtOH–water: 3.0 g (87%); mp 150–152 °C; $[\alpha]_D$ +68.4° (*c* 0.6, CH_2Cl_2); CIMS: *m/z* 340 (100%) [MH^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5 H, Ph), 5.59 (s, 1 H, PhC*H*), 4.62 (s, 1 H, H-1), 4.27 (dd, 1 H, $J_{5,6e}$ 4.9, $J_{6e,6a}$ 9.7 Hz, H-6_e), 4.15 (m, 2 H, H-3, H-5), 3.77 (m, 4 H, H-4, H-6, $NHCH_2CH_2CH_2OH$), 3.40 (s, 3 H, OCH_3), 2.99 (d, 1 H, $J_{2,3}$ 2.2 Hz, H-2), 2.87 (m, 2 H, $NHCH_2CH_2CH_2OH$), 1.69 (m, 2 H, $NHCH_2CH_2CH_2OH$); ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.2, 129.2, 128.2, 126.2 (Ph), 102.3 (PhCH), 101.0 (C-1), 76.7 (C-4), 69.1 (C-6), 68.2 (C-3), 63.3 ($NCH_2CH_2CH_2OH$), 60.8 (C-5), 58.4 (OCH_3), 55.6 (C-2), 47.9 (NCH_2–CH_2–CH_2OH), 31.5 (NCH_2–CH_2–CH_2OH). Anal. Calcd for $C_{17}H_{25}NO_6$: C, 60.16; H, 7.42; N, 4.13. Found: C, 60.45; H, 7.51; N, 4.10.

*Methyl 4,6-*O*-benzylidene-2-[ω-(4-{4-[bis(2-chloroethyl)amino]phenyl}butanoyloxy)alkylamino]-2-deoxy-α-*D*-altropyranoside* (**33** and **34**). A soln of methyl 4,6-*O*-benzylidene-2-deoxy-2-(ω-hydroxyalkylamino)-α-D-altropyranoside (**31** [14] and **32**) (2.0 mmol) in CH_2Cl_2 (40 mL), chlorambucil (0.61 g, 2.0 mmol), DMAP (0.02 g, 0.16 mmol) and DCC (0.5 g, 2.4 mmol) were added and stirred overnight at room temperature. The solid was removed by filtration, and the filtrate was diluted with CH_2Cl_2 and washed with aq 1 N AcOH and water, and evaporated to dryness. The solid was chromatographed by column of silica gel using 100:1 CH_2Cl_2–MeOH as eluent.

Methyl 2-[2-(4-{4-[bis(2-chloroethyl)amino]phenyl}butanoyloxy)ethylamino] derivative **33**. 1.2 g (95%); mp 104–106 °C; $[\alpha]_D$ +50.0° (*c* 0.5, CH_2Cl_2); CIMS: *m/z* 611 (100%) [MH^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5 H, Ph), 7.07, 6.57 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.56 (s, 1 H, PhC*H*), 4.62 (s, 1 H, H-1), 4.29 (dd, 1 H, $J_{5,6e}$ 5.0, $J_{6e,6a}$ 9.8 Hz, H-6_e), 4.15 (m, 3 H, H-3, $NHCH_2CH_2OCO$), 3.41 (s, 3 H, OCH_3), 3.03 (d, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 2.92 (m, 2 H, $NHCH_2CH_2OCO$), 2.54 (t, 2 H, *J* 7.3 Hz, Ar–CH_2–CH_2–CH_2–COO), 2.33 (t, 2 H, *J* 7.4 Hz, Ar–CH_2–CH_2–CH_2–COO), 1.89 (m, 2 H, Ar–CH_2–CH_2–CH_2–COO); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.1 (C=O), 144.3, 137.2, 130.3, 129.7, 129.1, 128.2, 126.2, 112.0 (2Ar), 102.3 (PhCH), 101.3 (C-1), 76.7 (C-4), 69.1 (C-6), 68.4 (C-3), 63.8 ($NHCH_2CH_2OCO$), 60.6 (C-5), 58.4 (OCH_3), 53.5 (NCH_2CH_2Cl), 49.1 (C-2), 47.0 ($NHCH_2$-CH_2OCO), 40.4 (NCH_2CH_2Cl), 33.9, 33.5, 24.9 [$(CH_2)_3$]. Anal. Calcd for $C_{30}H_{40}Cl_2N_2O_7$: C, 58.92; H, 6.59; N, 4.58. Found: C, 58.96; H, 6.66; N, 4.53.

Methyl 2-[3-(4-{4-[bis(2-chloroethyl)amino]phenyl}butanoyloxy)propylamino] derivative **34**. 1.2 g (96%); mp 90–92 °C; $[\alpha]_D$ +54.7° (*c* 0.4, CH_2Cl_2); FABMS: *m/z* 647 (100%) [MNa^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5 H, Ph), 7.07, 6.58 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 5.60 (s, 1 H, PhC*H*), 4.62 (s, 1 H, H-1), 4.31 (dd, 1 H, $J_{5,6e}$ 5.0, $J_{6e,6a}$ 9.8 Hz, H-6_e), 4.15 (t, 2 H, *J* 6.4 Hz, $NHCH_2CH_2CH_2OCO$), 4.05 (bs, 1 H, H-3), 3.42 (s, 3 H, OCH_3), 2.92

(d, 1 H, $J_{2,3}$ 2.2 Hz, H-2), 2.75 (t, 2 H, *J* 6.9 Hz, NHCH_2CH$_2$CH$_2$OCO), 2.53 (t, 2 H, *J* 7.5 Hz, Ar–CH_2–CH$_2$–CH$_2$–COO), 2.30 (t, 2 H, *J* 7.6 Hz, Ar–CH$_2$–CH$_2$–CH_2–COO), 1.95 (m, 4 H, NHCH$_2$CH_2CH$_2$OCO, Ar–CH$_2$–CH_2–CH$_2$–COO); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.6 (C=O), 144.3, 137.2, 130.4, 129.7, 129.1, 128.3, 126.2, 112.1 (2Ar), 102.3 (PhCH), 101.5 (C-1), 76.8 (C-4), 69.2 (C-6), 68.4 (C-3), 62.1 (NHCH$_2$CH$_2$CH$_2$OCO), 60.7 (C-5), 58.5 (OCH_3), 53.5 (NCH$_2$CH$_2$Cl), 49.1 (C-2), 45.1 (NHCH$_2$CH$_2$CH$_2$OCO), 40.4 (NCH$_2$CH$_2$Cl), 34.0, 33.5, 29.3, 24.9 [4(CH_2)]. Anal. Calcd for $C_{31}H_{42}Cl_2N_2O_7$: C, 59.52; H, 6.77; N, 4.48. Found: C, 59.81; H, 6.88; N, 4.47.

Methyl 2-[ω-(4-{4-[bis(2-chloroethyl)-amino]-phenyl}butanoyloxy)alkylamino]-2-deoxy-α-D-altropyranoside (**35** and **36**). Methyl 4,6-*O*-benzylidene-2-[ω-(4-{4-[bis(2-chloroethyl)-amino]phenyl}butanoyloxy)alkylamino]-2-deoxy-α-D-altropyranoside **33** and **34** (1.0 mmol) in 5:1 CH_2Cl_2–MeOH (60 mL) were hydrolysed as described for the preparation of **10–12**, to give **35** and **36**, respectively, which were purified by flash chromatography using 30:1 CH_2Cl_2–MeOH as eluent.

Methyl 2-[2-(4-{4-[bis(2-chloroethyl)-amino]-phenyl}butanoyloxy)ethylamino] derivative **35**. 0.5 g (93%); oil; FABMS: *m*/*z* 545 (100%) [MNa$^+$]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.15, 6.58 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 4.64 (s, 1 H, H-1), 4.11 (t, 2 H, *J* 5.5 Hz, NHCH$_2$CH_2OCO), 3.37 (s, 3 H, OCH_3), 2.98 (d, 1 H, $J_{2,3}$ 2.0 Hz, H-2), 2.89 (t, 2 H, *J* 5.3 Hz, NHCH_2CH$_2$OCO), 2.52 (t, 2 H, *J* 7.3 Hz, Ar–CH_2–CH$_2$–CH$_2$–COO), 2.30 (t, 2 H, *J* 7.4 Hz, Ar–CH$_2$–CH$_2$–CH_2–COO), 1.82 (m, 2 H, Ar–CH$_2$–CH_2–CH$_2$–COO); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.5 (C=O), 144.2, 130.3, 129.6, 112.0 (*p*-C_6H_4), 100.5 (C-1), 69.8 (C-4), 69.0 (C-3), 64.2 (C-5), 63.5 (C-6), 62.2 (NHCH$_2$CH$_2$OCO), 59.1 (OCH_3), 55.4 (C-2), 53.4 (NCH$_2$CH$_2$Cl), 46.4 (NHCH$_2$CH$_2$OCO), 40.4 (NCH$_2$CH$_2$Cl), 33.8, 33.3, 26.6 [(CH_2)$_3$]. Anal. Calcd for $C_{23}H_{36}Cl_2N_2O_7$: C, 52.78; H, 6.93; N, 5.35. Found: C, 52.94; H, 7.12; N, 5.29.

Methyl 2-[3-(4-{4-[bis(2-chloroethyl)-amino]-phenyl}butanoyloxy)propylamino] derivative **36**. 0.5 g (91%); oil; FABMS: *m*/*z* 559 (100%) [MNa$^+$]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.06, 6.58 (2 d, 4 H, *J* 8.6 Hz, *p*-C_6H_4), 4.66 (s, 1 H, H-1), 4.12 (t, 2 H, *J* 6.4 Hz, NHCH$_2$CH$_2$CH_2OCO), 3.42 (s, 3 H, OCH_3), 2.96 (d, 1 H, $J_{2,3}$ 2.1 Hz, H-2), 2.70 (t, 2 H, *J* 6.7 Hz, NHCH_2CH$_2$CH$_2$OCO), 2.52 (t, 2 H, *J* 7.5 Hz, Ar–CH_2–CH$_2$–CH$_2$–COO), 2.29 (t, 2 H, *J* 7.5 Hz, Ar–CH$_2$–CH$_2$–CH_2–COO), 1.86 (m, 4 H, NHCH$_2$CH_2CH$_2$OCO, Ar–CH$_2$–CH_2–CH$_2$–COO); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.7 (C=O), 144.3, 130.4, 129.6, 112.1 (*p*-C_6H_4), 100.6 (C-1), 69.8 (C-4), 68.8 (C-3), 64.4 (C-5), 62.7 (C-6), 62.0 (NHCH$_2$CH$_2$CH$_2$OCO), 59.1 (OCH_3), 55.5 (C-2), 53.5 (NCH$_2$CH$_2$Cl), 44.5 (NHCH$_2$-CH$_2$CH$_2$OCO), 40.5 (NCH$_2$CH$_2$Cl), 33.9, 33.5, 29.3, 26.6 [4(CH_2)]. Anal. Calcd for $C_{24}H_{38}Cl_2N_2O_7$: C, 53.63; H, 7.13; N, 5.21. Found: C, 53.87; H, 7.22; N, 5.04.

Biological assays. Materials and methods

Culture of cell line. Dr Berta Sanchez of the Department of Immunology of the Virgen del Rocio Hospital, Seville (Spain) kindly supplied all the cell lines used in this study. The promyelocytic cell line ML-1210 was isolated from peripheral blood of a mouse with promyelocytic leukaemia. The cell line KB-07, from human origin, was isolated from a nasopharyngeal carcinoma of a 36-year-old woman. The fibroblast cell was a standard cell line.

Cell lines were subcultured in minimum essential medium (MEM) (Gibco, Grand Island, NY) containing 10% foetal calf serum (FCS) (Boehringer Mannheim, Germany) and 1% antibiotic (penicillin and streptomycin)/antimycotic (amphotericin B) solution (Gibco) at 37 °C in an atmosphere of air with 5% added CO_2.

Cell proliferation assays. The effects of compounds **11** and **23**, and chlorambucil as control, were determined (i) by directly counting the number of living cells, and (ii) by two colorimetric assays: (a) the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) assay based on the activities of mitochondrial enzymes in viable cells, and (b) the bromouridine (BrdU) assay based on the measurement of BrdU incorporated during DNA synthesis.

For the cell count test, all the cell lines were placed into a 96-well culture plate at a seeding density of 2.5×10^5 cells/well. After 24 h, the complete medium was removed and replaced by MEM containing 10% FCS, and then assayed (**11**, **23** and chlorambucil) at final concentrations of 10, 50, 100, 200, 400, and 1000 mg/mL. The living cells were counted after 24 h incubation using staining Trypan Blue.

Flat 96-well culture plates, seeded at a density of 5.0×10^5 cells/well, were used to test the effects of the assayed products by the MTT and BrdU assays. These treatments and concentrations of assayed products were coordinated with the cell counting method. The colorimetric MTT assay was similar to that originally described by Mosmann [16], using MTT dissolved at 5 mg/mL in phosphate-buffered saline solution. Exactly 24 h after replacement of the medium with fresh MEM containing the assayed products, 10 mL of MTT solution were added directly to the medium and cells were incubated for an additional 2 h. After removal of the medium, 100 mL of 0.04 M hydrochloric acid in 2-propanol were added to each well for solubilization of the formazan crystals, and the absorbance of the plates was measured on a microculture plate reader using a test wavelength of 550 nm and a reference wavelength of 630 nm. The BrdU assay was performed as described by the supplier (Boehringer, Mannheim) [17]. Briefly, cells growing in 96-well microtitre tissue culture plates for 24 h are labelled by the addition of BrdU during 6 h of growth. After removal of the labelling medium, the cells are fixed and the DNA is denatured; anti-BrdU-POD antibody is added and binds to the incorporated BrdU. The immunocomplexes are detected by adding the POD substrate 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and measuring the absorbance at 405 nm using an ELISA reader.

The concentration of product causing 50% inhibition of cell proliferation (IC_{50}) was calculated using a computer program.

Statistics. Data were compared using Student's t-test; a P value < 0.05 compared with the non-treatment group (control) was considered significant. Values are expressed as the mean $\pm$ S.D. of the mean.

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