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Alkylating Agents from Sugars. Stereoselective Synthesis of 2,3-Diaminoglucoses from 2-Nitroalkenes, as Intermediates in the Synthesis of Carriers of Chlorambucil ☆

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Abstract: The synthesis of alkyl 2,3-diamino and alkyl 2-amino-3-thioglucopyranosides from alkyl 2-nitrosugar derivatives and amines or thiols is described. The reaction takes place through a 2-nitroalkene in good yield and with high stereoselectivity. The bonding of alkyl 2,3-diaminoglucopyranosides to chlorambucil *via* a spacer arm and an ester function is also described.

We are interested in the preparation of D-glucosamine derivatives modified at position 3 of the sugar ring. In a previous paper,[2] we have described the synthesis of saturated 2-nitrosugar derivatives and assayed the transformation of some of them into the corresponding 2-nitroalkenes.

In the present paper we described the synthesis of alkyl 2,3-diamino and 2-amino-3-thioglucopyranoside derivatives from the corresponding 2-nitrosugar derivatives as key intermediates, by reaction of amines or *tert*-butanethiol with the 2-nitroalkene intermediates generated *in situ* and subsequent reduction of the nitro group. This type of compound is generally difficult to obtain *via* substitution reactions at position 3 of the sugar.[3] Sakakibara and Sudoh[4] obtained 3-amino-2-nitro and 3-dimethylamino-2-nitro-D-glucopyranosides from 2-nitroalkenes *via* a long route.

At the same time, we are interested in the synthesis of new alkylating agents carried by sugars, as a method to obtain drugs highly selective against carcinoma cells in rapid growth, which have a great demand for the primary metabolites amino acids and carbohydrates.[5–8] Our aim is to correct the low selectivity of alkylating agents for neoplastic tissues,[9] the prime challenge being the synthesis of new potential anticancer drugs with the greatest activity against neoplastic tissues and the lowest possible toxicity for healthy tissues. We have previously described the synthesis of cyclophosphamides bonded to a sugar moiety of 2-aminoallose derivatives[10] and the synthesis of chlorambucil derived from 2-aminosugar derivatives with *gluco*, *allo* and *altro* configuration,[1] since both cyclophosphamide and chlorambucil are widely used clinically in the treatment of different types of cancer.[11,12]

☆ Potential anticancer drugs, part 3. For part 2, see reference 1

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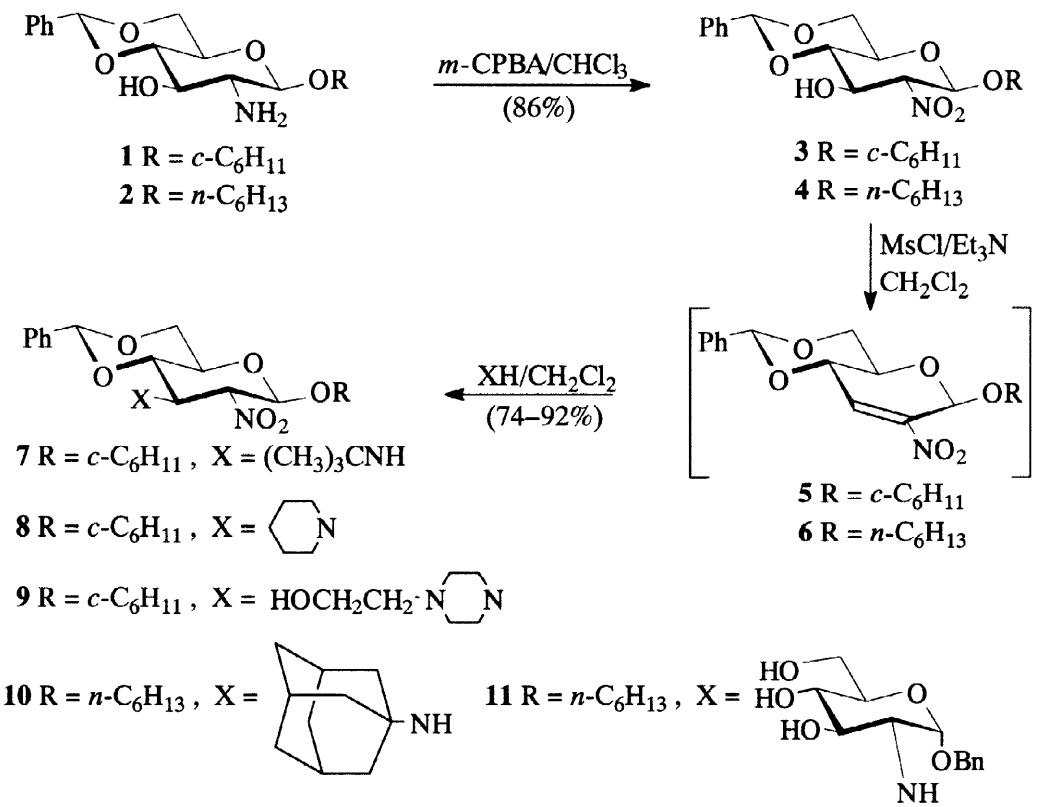
In this paper, we describe the synthesis of esters of chlorambucil with alkyl 2,3-diamino-D-glucopyranoside derivatives. The introduction of a spacer arm between the sugar and the chlorambucil is a strategy widely used to separate the active moiety from the carrier;[13] at the same time, the spacer helps to regulate the hydrophilic-lipophilic balance (HLB) of these compounds.

RESULTS AND DISCUSSION

The saturated 2-nitrosugar **4** was synthesized from the corresponding 2-aminosugar **2**[1] by oxidation of the amino group with *m*-CPBA, as previously described for **3**.[2] Transformation of **3** and **4** into the corresponding 3-amino-2-nitro derivatives **7–11** takes place *via* the 2-nitroalkene intermediates (Scheme 1).

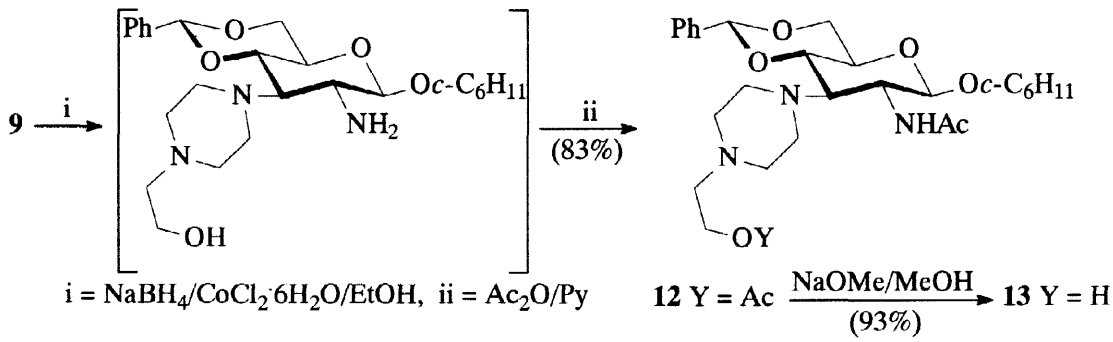


Scheme 1

The reaction of **3** and **4** with $MsCl/Et_3N/CH_2Cl_2$[14] gives the 2-nitroalkenes **5** and **6**, which react *in situ* with a primary or secondary amine to obtain the 3-alkyl(dialkyl)amino-2-nitro derivatives **7–11**. The intermediate **5** was prepared in two steps from **3** by acetylation with Ac_2O/Py and subsequent treatment with $NaHCO_3$/benzene[4] of the previously isolated and characterized intermediate cyclohexyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-nitro-β-D-glucopyranoside.[2] Compounds **7–11** were isolated in good yield. Elemental analyses and MS and NMR data confirmed the proposed structures. In some cases, double resonance, DEPT,

COSY, and CHCORR experiments were performed to assign the different signals in the spectra. The analysis of the chemical shift of ^1H and ^{13}C NMR spectrum signals indicated only one diastereomer in every case. The coupling constant values ($J_{1,2}$ ~8 Hz, $J_{2,3}$ ~10.5 Hz and/or $J_{3,4}$ ~10 Hz) in the ^1H spectra of compounds **7–11** indicate a *gluco* configuration in β-D-hexopyranoside rings.

We also describe the transformation of the 3-amino-2-nitrosugar derivative into 2,3-diaminosugar derivative (Scheme 2). Thus, reduction of the 2-nitro group of **9** with the $NaBH_4/CoCl_2{\cdot}6H_2O$/EtOH system[15] gives the corresponding 2-amino derivative, which was acetylated *in situ* to obtain compound **12**. The NMR spectra showed the signals corresponding to the N-H of the acetamido group at 5.37 ppm as a doublet, the two acetyl groups introduced at 1.98 and 1.95 ppm as two singlets (^1H), and at 171.0, 170.1, 23.5 and 21.0 ppm (^{13}C). The upfield chemical shift of the signal corresponding to H-2 in ~1 ppm and C-2 in 34.2 ppm, and the downfield chemical shift of the signal corresponding to CH_2O of the spacer in ~0.6 ppm with respect to compound **9** as the most characteristic signals.

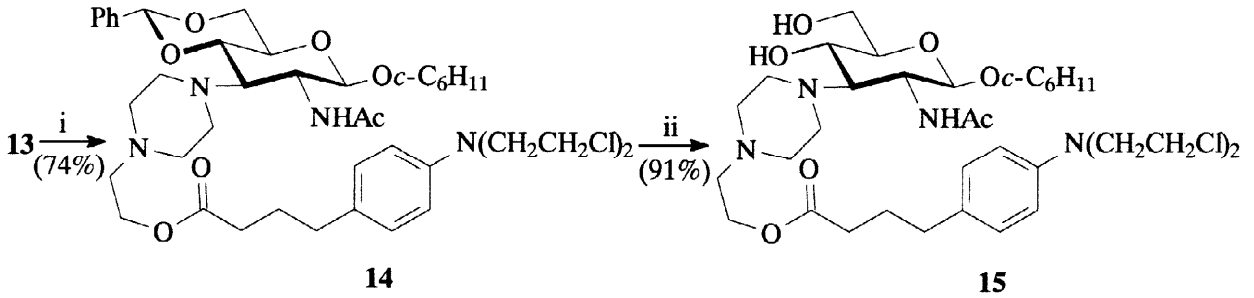


Scheme 2

The reaction of **12** with NaOMe/MeOH gave compound **13**. The *O*-deacetylation was confirmed by the presence in its ^1H NMR spectrum of the signal corresponding to a free hydroxyl group at 4.32 ppm as a triplet.

Compound **13** contains the sugar carrier and the spacer appropriately functionalized to bind the active molecule by the formation of an ester, one of the most-used bonds between a drug and the labile modulating group when preparing drug carriers.[16,17] Acylation of the hydroxyl of **13** with activated chlorambucil (DMAP/DCC) led to compound **14** (Scheme 3). The NMR spectra showed the characteristic signals corresponding to the introduced chlorambucil moiety (7.01 and 6.58 ppm in ^1H spectrum, and 173.4 and 40.5 ppm in ^{13}C spectrum).

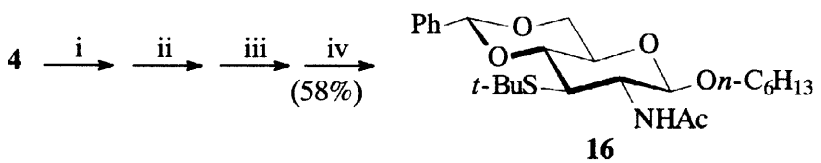
Removal of the benzylidene group in **14** gave diol **15** in almost quantitative yield, the structure of which was confirmed by elemental analysis and MS and NMR data.



i = chlorambucil/DMAP/DCC/THF, ii = $HCl/CH_2Cl_2/MeOH$

Scheme 3

We also studied the addition of thiols to 2-nitroalkene **6**, using *tert*-butanethiol as nucleophile. The product obtained was transformed, after isolation, into the 2-amino derivative by reduction of the nitro group using the $NaBH_4/CoCl_2{\cdot}6H_2O/EtOH$ system, isolated, and characterized as the 2-acetamido derivative **16** (1-Hexyl 2-acetamido-4,6-*O*-benzylidene-3-*t*-butylthio-2,3-dideoxy-β-D-glucopyranoside) (Scheme 4). The NMR data confirm the *gluco* configuration for this compound.



i = $MsCl/Et_3N/CH_2Cl_2$, ii = t-BuSH/CH_2Cl_2

iii = $NaBH_4/CoCl_2{\cdot}6H_2O/EtOH$, iv = Ac_2O/Py

Scheme 4

In conclusion, we present an easy procedure with good yield and very high stereoselectivity to transform 2-amino-D-glucopyranoside derivatives into 2,3-diamino and 2-amino-3-thio-D-glucopyranoside derivatives *via* 2-nitroalkenes. This methodology allows the synthesis of *N*-acetyl-D-glucosamines modified at position 3 which can be used as drug carriers.

EXPERIMENTAL SECTION

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 + Stanley Ltd P-20 polarimeter at 25 °C. Infrared (IR) spectra were obtained on a Jasco FT/IR-410 spectrophotometer. EI and CI mass spectra were recorded on a Micromass AUTOSPECQ mass spectrometer at 70 eV for EI and 150 eV for CI. FAB mass spectra were recorded on a Kratos MS-80-RFA using a thioglycerol matrix. NMR spectra were recorded at 25

°C on a Bruker AC-200 spectrometer at 200 MHz for ^{1}H and 50 MHz for ^{13}C. The chemical shifts are reported in ppm on the δ scale relative to TMS.

1-Hexyl 4,6-*O*-benzylidene-2-deoxy-2-nitro-β-D-glucopyranoside (4).

A solution of 1-hexyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[1] (**2**) (1.40 g, 4.0 mmol) in chloroform (100 mL) and solid magnesium sulphate (8.0 g) was heated under reflux with stirring, and *m*-chloroperbenzoic acid (Aldrich 57-86%) (8.0 g) was added. The suspension was stirred until completion of the reaction (2 hour, TLC). It was then left to cool to room temperature, diluted with dichloromethane, and filtered off. The organic phase was washed successively with 0.1 N aqueous solution of sodium hydroxide and water, then dried ($MgSO_4$) and evaporated to dryness. The solid obtained was recrystallized from ethanol as white crystals; yield 1.30 g (85%); mp 98–100 °C; $[\alpha]_D$ –46.2 (c 0.8, $CHCl_3$); IR (KBr) 3440, 1558, 1375, 1090 cm^{-1}; MS (CI) *m/z* 382 (100%) (MH^+); ^1H NMR (200 MHz, DMSO-d_6) δ 7.5–7.3 (m, 5H, Ph), 6.28 (d, 1H, $J_{2,OH}$ 6.1 Hz, OH-3), 5.62 (s, 1H, PhC<u>H</u>), 5.07 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.42 (dd, 1H, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 9.9 Hz, H-2), 4.3–4.1 (m, 2H, H-3, H-6_e), 3.8–3.4 (m, 5H, H-4, H-5, H-6_a, OCH_2R), 1.6–1.1 [m, 8H, $(CH_2)_4$], 0.83 (t, 3H, *J* 6.4 Hz, CH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 137.4, 129.0, 128.1, 126.4 (Ph), 100.9 (Ph<u>C</u>H), 99.2 (C-1), 91.6 (C-2), 79.8 (C-4), 70.4 (C-3), 69.4 (OCH_2R), 67.4 (C-6), 66.0 (C-5), 30.8, 28.7, 24.8, 22.0 [$(CH_2)_4$], 13.9 (CH_3). Anal. Calcd for $C_{19}H_{27}NO_7$: C, 59.83; H, 7.14; N, 3.67. Found: C, 59.61; H, 7.18; N, 3.59.

Reaction of alkyl 2-nitrosugar compounds (3 and 4) with primary or secondary amines.

To a solution of alkyl 4,6-*O*-benzylidene-2-deoxy-2-nitro-β-D-glucopyranoside (**3**,[2] **4**) (3.0 mmol) in dichloromethane (30 mL) cooled to 0 °C, methanesulphonyl chloride (0.5 mL, 6.5 mmol), was added. Then a solution of triethylamine (2 mL) in dichloromethane (5 mL) was added dropwise and stirred for 30 min at 0 °C. An aliquot was washed with water, dried, and evaporated. For **5**: ^1H NMR (200 MHz, $CDCl_3$) δ 7.5–7.2 (m, 6H, Ph, H-3), 5.92 (d, 1H, $^5J_{1,4}$ 2.5 Hz, H-1), 5.58 (s, 1H, PhC<u>H</u>), 4.49 (dt, 1H, $^5J_{1,4} = J_{3,4}$ 2.0 Hz, $J_{4,5}$ 8.5 Hz, H-4), 4.33 (dd, 1H, $J_{5,6e}$ 4.5 Hz, $J_{6e,6a}$ 10.2 Hz, H-6_e), 3.86 (t, 1H, $J_{5,6a} = J_{6e,6a}$ 10.2 Hz, H-6_a), 3.70 (m, 2H, H-5, OCH), 2.0–1.2 [m, 10H, $(CH_2)_5$]; ^{13}C NMR (50 MHz, $CDCl_3$) δ 147.6 (C-2), 134.6 (C-3), 136.4, 129.2, 128.2, 126.0 (Ph), 102.1 (Ph<u>C</u>H), 94.9 (C-1), 78.9 (OCH), 73.2 (C-4), 69.7 (C-5), 68.7 (C-6), 33.3, 31.4, 25.2, 23.8, 23.7 [$(CH_2)_5$]. The nitro-olefin obtained (**5**, **6**) was reacted *in situ* with a solution of the corresponding amine [*tert*-butylamine, piperidine, *N*-(2-hydroxyethyl)piperazine, 1-adamantylamine, benzyl 2-amino-2-deoxy-α-D-glucopyranoside] (3.3 mmol) in dichloromethane (5 mL) added dropwise. The reaction mixture was kept for 30 min at room temperature, then washed several times with water, dried (Na_2SO_4) and evaporated to dryness giving a solid residue. The product obtained was purified by recrystallization or by flash chromatography on a silica gel column.

Cyclohexyl 4,6-*O*-benzylidene-3-*t*-butylamino-2,3-dideoxy-2-nitro-β-D-glucopyranoside (7).

Recrystallized from ethanol as white needles; yield 1.20 g (92%); mp 172–174 °C; $[\alpha]_D$ –65.2 (c 0.8, $CHCl_3$);

IR (KBr) 3430, 1556, 1375, 1092 cm^{-1}; MS (CI) *m/z* 435 (21%) (MH^+); ^1H NMR (200 MHz, $CDCl_3$) δ 7.5–7.3 (m, 5H, Ph), 5.47 (s, 1H, PhC<u>H</u>), 5.05 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 4.31 (dd, 1H, $J_{5,6e}$ 4.6 Hz, $J_{6e,6a}$ 10.2 Hz, H-6_e), 4.20 (dd, 1H, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 10.2 Hz, H-2), 3.8–3.4 (m, 4H, H-3, H-5, H-6_a, OCH), 3,21 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{4,5}$ 9.0 Hz, H-4), 1.9–1.1 [m, 10H, $(CH_2)_5$], 0.95 [s, 9H, $(CH_3)_3$]; ^{13}C NMR (50 MHz, $CDCl_3$) δ 136.8, 129.0, 128.2, 126.0 (Ph), 102.1 (Ph<u>C</u>H), 98.9 (C-1), 93.4 (C-2), 80.9 (C-4), 78.0 (OCH), 68.4 (C-6), 68.1 (C-5), 56.1 (C-3), 50.2 [$(CH_3)_3\underline{C}$], 33.1, 30.8, 25.3, 23.7, 23.4 [$CH_2)_5$], 30.1 [$(\underline{C}H_3)_3C$]. Anal. Calcd for $C_{23}H_{34}N_2O_6$: C, 63.57; H, 7.89; N, 6.45. Found: C, 63.50; H, 7.91; N, 6.45.

Cyclohexyl 4,6-*O*-benzylidene-2,3-dideoxy-2-nitro-3-(1-piperidyl)-β-D-glucopyranoside (8). Recrystallized from ethanol as a white solid; yield 1.15 g (86%); mp 143–145 °C; $[\alpha]_D$ –69.1 (c 0.6, $CHCl_3$); IR (KBr) 1556, 1375, 1093 cm^{-1}; MS (CI) *m/z* 447 (62%) (MH^+); ^1H NMR (200 MHz, $CDCl_3$) δ 7.5–7.3 (m, 5H, Ph), 5.53 (s, 1H, PhC<u>H</u>), 4.98 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.47 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 11.2 Hz, H-2), 4.34 (dd, 1H, $J_{5,6e}$ 4.8 Hz, $J_{6e,6a}$ 10.6 Hz, H-6_e), 3.9–3.3 (m, 5H, H-3, H-4, H-5, H-6_a, OCH), 2.92 (m, 2H, $C\underline{H}_AH_BNC\underline{H}_AH_B$), 2.56 (m, 2H, $CH_A\underline{H}_BNCH_A\underline{H}_B$), 1.9–1.1 [m, 16H, 8($CH_2$)]; ^{13}C NMR (50 MHz, $CDCl_3$) δ 137.0, 129.0, 128.3, 125.9 (Ph), 101.0 (Ph<u>C</u>H), 99.3 (C-1), 87.8 (C-2), 78.0 (OCH), 77.2 (C-4), 68.7 (C-6), 67.8 (C-5), 66.5 (C-3), 51.0 (CH_2NCH_2), 33.1, 31.0, 26.7,26.5, 25.3, 24.6, 23.7, 23.4 [8(CH_2)]. Anal. Calcd for $C_{24}H_{34}N_2O_6$: C, 64.55; H, 7.67; N, 6.27. Found: C, 64.41; H, 7.89; N, 6.29.

Cyclohexyl 4,6-*O*-benzylidene-2,3-dideoxy-3-{1-[4-(2-hydroxyethyl)piperazyl]}-2-nitro-β-D-glucopyranoside (9). Chromatography column, dichloromethane-methanol (75:1) as a white solid; yield 1.30 g (88%); mp 180–182 °C; $[\alpha]_D$ –45.98 (c 0.4, CH_2Cl_2); IR (KBr) 3440, 1557, 1373, 1092 cm^{-1}; MS (FAB) *m/z* 514 (100%) (MNa^+); ^1H NMR (200 MHz, $CDCl_3$) δ 7.5–7.3 (m, 5H, Ph), 5.52 (s, 1H, PhC<u>H</u>), 5.00 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 4.47 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 11.2 Hz, H-2), 4.33 (dd, 1H, $J_{5,6e}$ 4.7 Hz, $J_{6e,6a}$ 10.4 Hz, H-6_e), 3.9–3.4 (m, 7H, H-3, H-4, H-5, H-6_a, OCH, C<u>H</u>$_2$OH), 2.96, 2.65, 2.45 [3m, 10H, 5(CH_2N)], 1.8–1.1 [m, 10H, $(CH_2)_5$]; ^{13}C NMR (50 MHz, $CDCl_3$) δ 136.8, 129.2, 128.4, 125.9 (Ph), 101.2 (Ph<u>C</u>H), 99.2 (C-1), 87.6 (C-2), 78.1 (OCH), 77.0 (C-4), 68.7 (C-6), 67.8 (C-5), 65.5 (C-3), 59.1 (CH_2OH), 57.6, 53.3, 49.4 (CH_2N), 33.1, 31.0, 25.3, 23.7, 23.4 [$(CH_2)_5$]. Anal. Calcd for $C_{25}H_{37}N_3O_7$: C, 61.08; H, 7.59; N, 8.55. Found: C, 60.94; H, 7.55; N, 8.44.

1-Hexyl 3-(1-adamantylamino)-4,6-*O*-benzylidene-2,3-dideoxy-2-nitro-β-D-glucopyranoside (10). Recrystallized from ethanol as a white solid; yield 1,25 g (81%); mp 109–111 °C; $[\alpha]_D$ –72.9 (c 0.9, $CHCl_3$); IR (KBr) 3433, 1556, 1376, 1095 cm^{-1}; MS (CI) *m/z* 515 (100%) (MH^+); ^1H NMR (200 MHz, $CDCl_3$) δ 7.5–7.3 (m, 5H, Ph), 5.50 (s, 1H, PhC<u>H</u>), 4.94 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 4.33 (dd, 1H, $J_{5,6e}$ 4.7 Hz, $J_{6e,6a}$ 10.3 Hz, H-6_e), 4.18 (dd, 1H, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 10.3 Hz, H-2), 3.9–3.4 (m, 5H, H-3, H-5, H-6_a, OCH_2R), 3,21 (t, 1H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4), 1.96 [m, 3H, 3(CH)], 1.6–1.1 [m, 20H, 10(CH_2)], 0.86 (t, 3H, *J* 6.6 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 136.9, 129.1, 128.2, 126.1 (Ph), 102.1 (Ph<u>C</u>H), 100.8 (C-1), 93.3 (C-2), 81.1 (C-4), 70.8 (OCH_2R), 68.5 (C-6), 68.3 (C-5), 54.3 (C-3), 50.1 (C adamantyl), 43.7, 36.3 (CH_2 adamantyl), 29.6 (CH

adamantyl), 31.4, 29.1, 25.3, 22.5 [$(CH_2)_4$], 14.0 (CH_3). Anal. Calcd for $C_{29}H_{42}N_2O_6$: C, 67.68; H, 8.23; N, 5.44. Found: C, 67.47; H, 8.37; N, 5.33.

1-Hexyl 3-[2-(benzyl 2-deoxy-α-D-glucopyranosyl)amino]-4,6-*O*-benzylidene-2,3-dideoxy-2-nitro-β-D-glucopyranoside (11). Chromatography column, dichloromethane-methanol (30:1) as a white solid; yield 1.40 g (74%); mp 99–101 °C; $[\alpha]_D$ +163.3 (c 0.6, $CHCl_3$); IR (KBr) 3454, 1556, 1380, 1093 cm^{-1}; MS (FAB) *m/z* 655 (100%) (MNa^+); ^1H NMR (200 MHz, DMSO-d_6, D_2O) δ 7.5–7.2 (m, 10H, 2Ph), 5.64 (s, 1H, PhC<u>H</u>), 5.02 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1 β-sugar), 4.73 (d, 1H, $J_{1,2}$ 4.1 Hz, H-1 α-sugar), 4.55 (m, 2H, H-6_e, PhC<u>H_A</u>H_B), 4.24 (d, 1H, ^2J 11.2 Hz, PhCH_A<u>H_B</u>), 3,06 (t, 1H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4 β-sugar), 2.90 (dd, 1H, $J_{1,2}$ 4.2 Hz, $J_{2,3}$ 9.6 Hz, H-2 α-sugar), 1.5–1.1 [m, 8H, $(CH_2)_4$], 0.87 (t, 3H, *J* 6.5 Hz, CH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 137.8, 137.2, 128.8, 128.2, 128.0, 127.7, 127.4, 126.0 (2Ph), 101.2 (Ph<u>C</u>H), 100.4 (C-1 β-sugar), 96.5 (C-1 α-sugar), 90.2 (C-2 β-sugar), 80.4 (C-4 β-sugar), 73.1 (C-4 α-sugar), 72.2 (C-3 α-sugar), 70.5 (C-5 α-sugar), 69.3 (OCH_2R), 68.4 (C-6 β-sugar), 67.6 (O<u>C</u>H_2Ph), 67.0 (C-5 β-sugar), 60.9 (C-6 α-sugar), 60.4 (C-3 β-sugar), 57.4 (C-2 α-sugar), 30.8, 28.7, 24.8, 22.0 [$(CH_2)_4$], 13.8 (CH_3). Anal. Calcd for $C_{32}H_{44}N_2O_{11}$: C, 60.75; H, 7.01; N, 4.43. Found: C, 60.68; H, 6.92; N, 4.41.

Cyclohexyl 2-acetamido-3-{1-[4-(2-acetoxyethyl)piperazyl]}-4,6-*O*-benzylidene-2,3-dideoxy-β-D-glucopyranoside (12).

To a solution of 2-nitro-β-D-glucopyranoside derivative (**9**) (1.00 g, 2.0 mmol) and cobalt (II) chloride hexahydrate (1.00 g, 4.2 mmol) in ethanol (50 mL) heated at 120 °C, sodium borohydride (0.76 g, 20.1 mmol) was added in small portions and the mixture was stirred for 2 hours at 120 °C. The solvent was evaporated, and the residue suspended in dichloromethane (120 mL) with stirring for 30 min. The solid was removed by filtration, and the filtrate was evaporated to dryness: MS (CI) *m/z* 462 (100%) (MH^+); HRMS (EI): M^+, found 461.2901. $C_{25}H_{39}N_3O_5$ requires 461.2890. Acetic anhydride/pyridine (1:1) mixture (10 mL) was added and the resulting mixture was kept overnight at room temperature. The solution was diluted with dichloromethane, washed several times with water, dried (Na_2SO_4) and concentrated *in vacuo* to give an oil which was solidified by stirring with ethyl ether-hexane (1:2) mixture. The solid obtained was purified by flash chromatography on silica gel, using dichloromethane-methanol (50:1) as eluent, to give compound **12** as a white solid: yield 0.90 g (83%); mp 214–216 °C; $[\alpha]_D$ –110.6 (c 0.3, CH_2Cl_2); IR (KBr) 3306, 1742, 1655, 1557, 1242, 1092 cm^{-1}; MS (CI) *m/z* 546 (100%) (MH^+); ^1H NMR (200 MHz, $CDCl_3$) δ 7.5–7.3 (m, 5H, Ph), 5.47 (s, 1H, PhC<u>H</u>), 5.37 (d, 1H, $J_{2,NH}$ 8.3 Hz, NH), 4.76 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 4.28 (dd, 1H, $J_{5,6e}$ 4.8 Hz, $J_{6e,6a}$ 10.4 Hz, H-6_e), 4.13 (t 2H, *J* 6.0 Hz, NCH_2<u>C</u>H_2OAc), 3.8–3.3 (m, 5H, H-2, H-4, H-5, H-6_a, OCH), 3.15 (t, 1H, $J_{2,3} = J_{3,4}$ 10.6 Hz, H-3), 2.95, 2.65, 2.40 [3m, 8H, 4(CH_2N)], 2.52 (t, 2H, *J* 6.0 Hz, N<u>C</u>$H_2$$CH_2$OAc), 1.98, 1.95 (2s, 6H, $2CH_3CO$), 1.8–1.1 [m, 10H, $(CH_2)_5$]; ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.0, 170.1 (2C=O), 137.3, 128.9, 128.2, 126.0 (Ph), 100.9 (Ph<u>C</u>H), 100.4 (C-1), 78.2 (OCH), 77.3 (C-4), 69.0 (C-6), 67.4 (C-5), 65.1 (C-3), 61.8 (<u>C</u>H_2OAc),

56.6, 54.5, 49.3 (CH_2N), 53.4 (C-2), 33.4, 31.7, 25.5, 23.9, 23.7 [$(CH_2)_5$], 23.5, 21.0 ($2\underline{C}H_3CO$). Anal. Calcd for $C_{29}H_{43}N_3O_7$: C, 63.83; H, 7.94; N, 7.70. Found: C, 63.55; H, 7.84; N, 7.35.

Cyclohexyl 2-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-3-{1-[4-(2-hydroxyethyl)piperazyl]}-β-D-glucopyranoside (13).

To a solution of cyclohexyl 3-{1-[4-(2-acetoxyethyl)piperazyl]-β-D-glucopyranoside derivative (**12**) (0.82 g, 1.5 mmol) in methanol (30 mL) was added a solution of sodium methoxide (0.3 mmol) in methanol (10 mL). After 30 min at room temperature, the mixture was neutralized by addition of Dowex 50 resin (H^+ form), filtered, and evaporated to dryness. The solid obtained was recrystallized from ethanol-water as white solid: yield 0.70 g (93%); mp 221–223 °C; $[\alpha]_D$ –62.6 (c 0.6, DMF); IR (KBr) 3438, 3303, 1656, 1555, 1370, 1086 cm^{-1}; MS (CI) *m/z* 504 (100%) (MH^+); ^1H NMR (200 MHz, DMSO-d_6) δ 7.57 (d,1H, $J_{2,NH}$ 9.3 Hz, NH), 7.45–7.3 (m, 5H, Ph), 5.61 (s, 1H, PhC$\underline{H}$), 4.49 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 4.32 (t, 1H, *J* 5.2 Hz, OH), 4.17 (dd, 1H, $J_{5,6e}$ 4.9 Hz, $J_{6e,6a}$ 10.1 Hz, H-6_e), 3.71(t, 1H, $J_{5,6e} = J_{6e,6a}$ 10.0 Hz, H-6_a), 2.90, 2.25 [2m, 6H, $3(CH_2N)$], 2.71 (t, 1H, $J_{2,3} = J_{3,4}$ 10.5 Hz, H-3), 1.9–1.1 [m, 13H, CH_3CON, $(CH_2)_5$]; ^{13}C NMR (50 MHz, DMSO-d_6) δ 168.7 (C=O), 137.9, 128.7, 128.1, 126.0 (Ph), 100.8 (Ph$\underline{C}$H), 99.9 (C-1), 77.6 (OCH), 75.7 (C-4), 68.2 (C-6), 67.0 (C-5), 65.7 (C-3), 60.5 (CH_2OH), 58.4, 54.4, 48.8 (CH_2N), 51.1 (C-2), 32.9, 31.1, 25.2, 23.2, 23.0 [$(CH_2)_5$], 22.8 ($\underline{C}H_3CON$). Anal. Calcd for $C_{27}H_{41}N_3O_6$: C, 64.39; H, 8.21; N, 8.34. Found: C, 64.32; H, 8.30; N, 8.31.

Cyclohexyl 2-acetamido-4,6-*O*-benzylidene-3-(1-{4-[2-(4-{4-[bis(2-chloroethyl)amino]phenyl} butanoyloxy)ethyl]piperazyl})-2,3-dideoxy-β-D-glucopyranoside (14).

To a solution of cyclohexyl 3-{1-[4-(2-hydroxyethyl)piperazyl]-β-D-glucopyranoside derivative (**13**) (0.60 g, 1.2 mmol) in dichloromethane (60 mL), chlorambucil (0.55 g, 1.8 mmol), 4-(*N,N*-dimethylamino)pyridine (0.01 g, 0.08 mmol) and *N,N'*-dicyclohexylcarbodiimide (0.42 g, 2.0 mmol) were added and stirred overnight at room temperature. The solid was removed by filtration, and the filtrate was diluted with dichloromethane and washed successively with a 1N aqueous solution of acetic acid and water, dried (Na_2SO_4), and evaporated to dryness. The solid was purified by flash chromatography on silica gel, using hexane-ethyl acetate (4:1) and then dichloromethane-methanol (40:1) as eluents, to give **14** as a white solid: yield 0.75 g (74%); mp 148–150 °C; $[\alpha]_D$ –116.3 (c 0.3, CH_2Cl_2); IR (KBr) 3306, 1743, 1655, 1555, 1240, 1092 cm^{-1}; MS (FAB) *m/z* 811 (100%) (MNa^+); ^1H NMR (200 MHz, $CDCl_3$) δ 7.5–7.3 (m, 5H, Ph), 7.01, 6.58 (2d, 4H, *J* 8.6 Hz, *p*-C_6H_4), 5.46 (s, 1H, PhC$\underline{H}$), 5.41 (d,1H, $J_{2,NH}$ 8.7 Hz, NH), 4.75 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 4.28 (dd, 1H, $J_{5,6e}$ 4.8 Hz, $J_{6e,6a}$ 10.4 Hz, H-6_e), 4.14 (t, 2H, *J* 5.9 Hz, RCO_2CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.4, 170.1 (2C=O), 144.2, 137.3, 130.5, 129.6, 128.8, 128.2, 126.0, 112.1 (2Ar), 100.8 (Ph$\underline{C}$H), 100.4 (C-1), 78.1 (OCH), 77.3 (C-4), 69.0 (C-6), 67.4 (C-5), 65.2 (C-3), 61.7 ($RCO_2\underline{C}H_2$), 56.7, 54.5, 53.5, 49.3 (CH_2N), 53.3 (C-2), 40.5 (CH_2Cl), 33.8, 33.5, 33.4, 31.7, 26.7, 25.5, 23.9, 23.7 [$8(CH_2)$], 23.5 ($\underline{C}H_3CON$). Anal. Calcd for

$C_{41}H_{58}Cl_2N_4O_7$: C, 62.35; H, 7.40; N, 7.09. Found: C, 62.23; H, 7.46; N, 7.12.

Cyclohexyl 2-acetamido-3-(1-{4-[2-(4-{4-[bis(2-chloroethyl)amino]phenyl}butanoyloxy)ethyl]piperazyl}) -2,3-dideoxy-β-D-glucopyranoside (15).

To a solution of cyclohexyl 4,6-*O*-benzylidene derivative (**14**) (0.50 g, 0.6 mmol) in dichloromethane-methanol (1:1) (60 mL) was added conc. hydrochloric acid (0.5 mL), and the solution was heated under reflux for 2 hours with stirring. After being cooled to room temperature, the mixture was neutralized by addition of Amberlite IRA-400C resin (OH^- form), filtered, and evaporated. The solid was purified by column chromatography on silica gel, using dichloromethane-methanol (20:1) as eluent, to give **15** as a white solid: yield 0.40 g (91%); mp 111–113 °C; $[\alpha]_D$ –93.3 (c 0.2, CH_2Cl_2); IR (KBr) 3440, 3306, 1742, 1655, 1557, 1242, 1092 cm^{-1}; MS (FAB) *m/z* 723 (23%) (MNa^+); ^1H NMR (200 MHz, $CDCl_3$) δ 7.04, 6.60 (2d, 4H, *J* 8.7 Hz, *p*-C_6H_4), 5.49 (d,1H, $J_{2,NH}$ 6.8 Hz, NH), 4.97 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1), 4.17 (t, 2H, *J* 5.7 Hz, RCO_2CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.4, 170.6 (2C=O), 144.3, 130.5, 129.6, 112.1 (*p*-C_6H_4), 98.9 (C-1), 77.4 (OCH), 76.2 (C-4), 66.4 (C-5), 66.3 (C-3), 63.1 (C-6), 61.5 ($RCO_2\underline{C}H_2$), 56.7, 54.4, 53.5, 48.7 (CH_2N), 53.9 (C-2), 40.5 (CH_2Cl), 33.9, 33.5, 31.8, 26.7, 25.4, 24.0, 23.8, 23.5 [8(CH_2)], 23.7 ($\underline{C}H_3CON$). Anal. Calcd for $C_{34}H_{54}Cl_2N_4O_7$: C, 58.19; H, 7.76; N, 7.98. Found: C, 58.31; H, 7.81; N, 7.92.

1-Hexyl 2-acetamido-4,6-*O*-benzylidene-3-*t*-butylthio-2,3-dideoxy-β-D-glucopyranoside (16).

Compound **6** (3.0 mmol) (obtained from **4**, as described above) was treated with a solution of *tert*-butanethiol (0.4 mL, 3.5 mmol) in dichloromethane (5 mL) added dropwise. After 30 min at room temperature, the mixture was washed with water, dried (Na_2SO_4), and evaporated to dryness: MS (CI) *m/z* 454 (55%) (MH^+). The solid was reduced with the $NaBH_4/CoCl_2{\cdot}6H_2O$/EtOH system under the same conditions as for compound **12**, to give a solid: MS (CI) *m/z* 424 (100%) (MH^+); ^1H NMR (200 MHz, $CDCl_3$) δ 7.6–7.3 (m, 5H, Ph), 5.53 (s, 1H, PhC$\underline{H}$), 4.40 (d, 1H, $J_{1,2}$ 6.8 Hz, H-1), 4.20 (dd, 1H, $J_{5,6e}$ 4.7 Hz, $J_{6e,6a}$ 10.2 Hz, H-6_e), 2.63 (dd, 1H, $J_{1,2}$ 6.6 Hz, $J_{2,3}$ 8.8 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 137.3, 128.7, 127.9, 126.1 (Ph), 105.5 (C-1), 101.9 (Ph$\underline{C}$H), 80.8 (C-4), 70.2 (OCH_2R), 70.0 (C-3), 68.7 (C-6), 56.2 (C-5), 48.5 (C-2), 43.9 [$(CH_3)_3\underline{C}$], 32.0 [$(\underline{C}H_3)_3C$], 31.4, 29.5, 25.5, 22.4 [$(CH_2)_4$], 13.9 (CH_3). HRMS (EI): M^+, found 423.2441. $C_{23}H_{37}NO_4S$ requires 423.2443. The solid obtained was acetyled in the usual way and column chromatographed on silica gel, using dichloromethane-methanol (60:1) as eluent, to give **16** as a white solid: yield 0.95 g (58%); mp 203–205 °C; $[\alpha]_D$ –46.7 (c 0.6, $CHCl_3$); IR (KBr) 3312, 1656, 1550, 1373, 1094 cm^{-1}; MS (FAB) *m/z* 488 (100%) (MNa^+); ^1H NMR (200 MHz, DMSO-d_6) δ 7.89 (d, 1H, $J_{2,NH}$ 9.2 Hz, NH), 7.5–7.3 (m, 5H, Ph), 5.60 (s, 1H, PhC$\underline{H}$), 4.61 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.18 (dd, 1H, $J_{5,6e}$ 4.6 Hz, $J_{6e,6a}$ 10.0 Hz, H-6_e), 3.8–3.6 (m, 2H, H-2, H-3), 2.89 (t, 1H, $J_{3,4} = J_{4,5}$ 10.8 Hz, H-4), 1.79 (s, 3H, CH_3CON), 1.6–1.1 [m, 17H, $(CH_2)_4$, $(CH_3)_3C$], 0.85 (t, 3H, *J* 6.0 Hz, CH_3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 168.5 (C=O), 137.7, 128.7, 128.0, 126.0 (Ph), 102.0 (Ph$\underline{C}$H), 100.8 (C-1), 80.4 (C-4), 69.1 (C-3), 68.6 (C-6), 67.9 (OCH_2R), 54.4 (C-5), 46.5 (C-2), 43.1 [$(CH_3)_3\underline{C}$], 31.7

[($\underline{C}H_3)_3C$], 31.0, 29.0, 25.0, 22.1 [$(CH_2)_4$], 22.9 ($\underline{C}H_3CON$), 13.9 (CH_3). Anal. Calcd for $C_{25}H_{39}NO_5S$: C, 64.48; H, 8.44; N, 3.01. Found: C, 64.28; H, 8.29; N, 3.03.

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