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Carbohydrate Research 308 (1998) 57–62

Alkylating agents from sugars. Cyclophosphamides derived from 2-amino-2-deoxy-D-allose

Fernando Iglesias-Guerra *, Isidora Romero, Felipe Alcudia, José M. Vega-Pérez *

Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, E-41071 Sevilla, Spain

Received 30 October 1997; accepted 22 February 1998

Abstract

Cyclophosphamides derived from alkyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranosides have been synthesized with good yield by treatment of the corresponding 2-amino-2-deoxy-D-allose derivatives with bis(2-chloroethyl)phosphoramide dichloride. The ring-forming reaction took place with very high diastereoselectivity. Subsequent hydrogenolysis gave excellent yields of cyclophosphamides derived from alkyl 2-amino-2-deoxy-β-D-allopyranosides, with hydrophilicity greater than that of the precursors. The starting material was easily available from 2-acetamido-2-deoxy-D-glucose.

Keywords: Alkylating agents; Cyclophosphamides from sugars ; 2-amino-2-deoxy-D-allose derivatives

1. Introduction

Alkylating agents have been pioneering drugs in the treatment of malignant metastases. Among them, cyclophosphamide, still has a broad spectrum of action against a great variety of neoplasias, and is commonly used to this end, both alone and in combined treatments [1]. Despite the advantages it offers, it is not free of drawbacks, partly because of the possible inducement of resistance, and also because of its low selectivity. The synthesis of new, potentially more selective drugs could help to solve these two problems.

One way of improving the targeting of the drug is to couple it with primary metabolites that act as selective carriers, since the cancerous cells—undergoing rapid growth—have a greater demand for them than do healthy cells.

In the present report, the synthesis of cyclophosphamides derived from sugars, of which there is scarce mention in the scientific literature [2–4] is presented. Moreover, the described compounds contain different lipophilic moieties in order to modulate the hydrophilic-lipophilic balance (HLB) of these compounds.

2. Results and discussion

The synthesis of cyclophosphamides derived from 2-acetamido-2-deoxy-D-allose (**17**–**22**) described in this paper was carried out using commercial

* Corresponding authors.

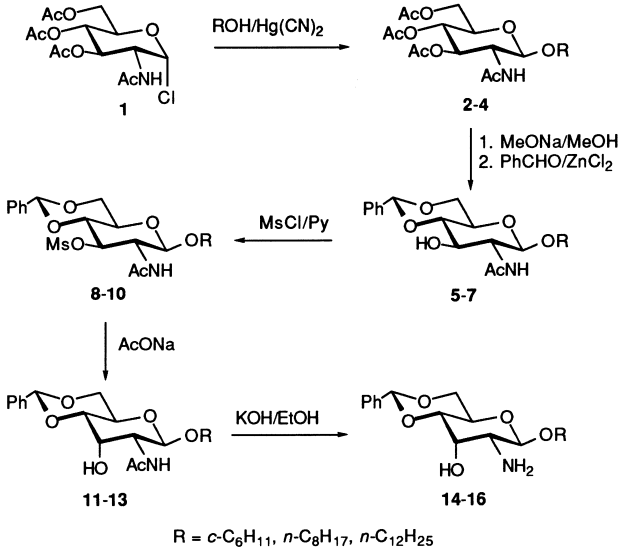
0008-6215/98/$19.00
PII S0008-6215(98)00056-1

2-acetamido-2-deoxy-D-glucose as starting sugar. The latter was transformed into the corresponding alkyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranosides (**14**–**16**) by a sequence of reactions described in the literature for the corresponding benzyl glycoside [5–7] (Scheme 1).

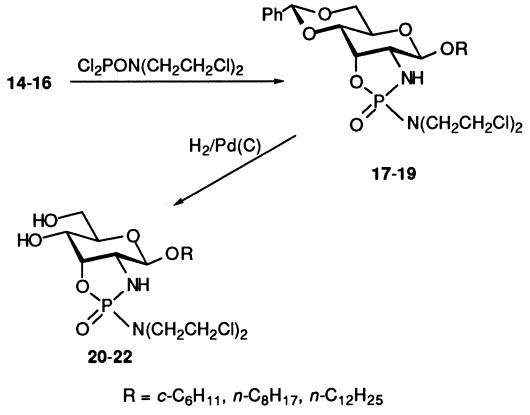
The most characteristic ^{1}H NMR signals of **14**–**16** were those corresponding to H-1 at 4.6–4.5 ppm as a doublet ($J_{1,2}$ 8.1 Hz), H-2 at 2.7 ppm as a double doublet ($J_{1,2}$ 8.1, $J_{2,3}$ 2.6 Hz) and H-3 at 4.2 ppm as a triplet ($J_{2,3} = J_{3,4}$ 2.6 Hz). These assignments were confirmed by double resonance experiments on **16**.

The cyclophosphamides **17**–**19** were obtained by reaction of **14**–**16** with bis(2-chloroethyl)phosphoramide dichloride in dichloromethane at 0 °C under an inert atmosphere (Scheme 2). Compounds **17**–**19** were purified on a silica gel column, and obtained as perfectly stable, white solids in yields of 78–87%. Owing to the *cis* fusion of the five-membered ring to the pyranose ring, these molecules are free of angular strain. The NMR data confirmed the proposed structures. In the ^{1}H NMR spectrum of **19**, for example, the signals for H-1 (a doublet with $J_{1,2}$ 8.1 Hz at 4.86 ppm), H-2 (part of the multiplet at 4.1–3.2 ppm), and H-3 (a triplet with $J_{2,3} = J_{3,4}$ 3.5 Hz at 4.73 ppm) were observed at low-field as compared with the spectrum of the precursor **16**.

It is also noteworthy that the ring-forming reaction took place with high diastereoselectivity with regard to the substituents on the chiral phosphorus atom, as shown by the absence of signal duplicity



Scheme 1.



Scheme 2.

in the ^{1}H NMR spectra, and, above all, because the ^{31}P NMR spectra presented a single signal for each of compounds **17**–**19**, at δ 25.96, 26.10 and 26.08 ppm, respectively.

Compounds **17**–**19** were very soluble in dichloromethane, moderately soluble in methanol and ethanol, and totally insoluble in water. In order to modulate the HLB of these substances and increase their hydrosolubility, we prepared the cyclophosphamides **20**–**22**, obtained readily in excellent yield (> 85%) by hydrogenolysis in the presence of palladium catalyst. The ^{1}H NMR spectrum of **21**, for example, showed the signals for OH-4 at 5.16 ppm as a doublet ($J_{4,OH}$ 7.1 Hz) and for OH-6 at 4.54 ppm as a triplet ($J_{6,OH}$ 5.8 Hz). The ^{31}P NMR spectra indicated a single diastereomer. These compounds showed moderate solubility in both dichloromethane and water, varying gradually depending on the nature of the glycoside moiety. COSY and CHCORR experiments for **21** confirmed the predicted structure of these cyclophosphamides.

The cyclophosphamides synthesized will be tested for cytotoxicity and inhibition of cell growth (cytostatic activity) using the appropriate cell lines in *in vitro* experiments.

3. Experimental

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. Mass spectra were

recorded on a Kratos MS-80-RFA mass spectrometer 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 ^{1}H, 50 MHz for ^{13}C, and 81 MHz for ^{31}P, and on a Bruker AMX-500 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C. The chemical shifts are reported in ppm on the δ scale relative to Me_4Si and H_3PO_4. Compounds **4**, **7**, **10** and **13** were synthesized in collaboration with other colleagues in relation with other works and will be published elsewhere [8]; their elemental analyses and physical data are in agreement with the proposed structures.

Alkyl 2-acetamido-3,4,6-tri-O-*acetyl-2-deoxy*-β-D-*glucopyranosides* (**2–4**).—A soln of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride [9] (**1**) (18.3 g, 50 mmol) in dry $MeNO_2$ (40 mL) was added dropwise, with exclusion of moisture, to a stirred mixture of alcohol (cyclohexanol, 1-octanol, 1-dodecanol; 30 mL in each case) in anhyd toluene (40 mL) containing 4 Å molecular sieves (30 g) and Hg(CN) (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 H_2O, then dried (Na_2SO_4) and concentrated. The residue was suspended in hexane with stirring for 1 h. The solid obtained was filtered and washed with hexane.

Cyclohexyl glycoside (**2**).—Recrystallized from EtOH; 17.8 g (83%); mp 176–178 °C; $[\alpha]_D$ −37.5° (*c* 0.8, CH_2Cl_2); CIMS: *m*/*z* 430 (25%) [MH^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.00 (d, 1-H, $J_{2,NH}$ 8.5 Hz, N–H), 5.34 (dd, 1-H, $J_{2,3}$ 10.5, $J_{3,4}$ 9.5 Hz, H-3), 4.97 (t, 1-H, $J_{3,4}=J_{4,5}$ 9.5 Hz, H-4), 4.81 (d, 1-H, $J_{1,2}$ 8.3 Hz, H-1), 4.21 (dd, 1-H, $J_{5,6}$ 5.0, $J_{6,6'}$ 12.1 Hz, H-6), 4.04 (dd, 1-H, $J_{5,6'}$ 2.5, $J_{6,6'}$ 12.1 Hz, H-6′), 3.6 (m, 3. H, H-2, H-5, OCH), 2.00, 1.96, 1.95, 1.87 (4s, 4 CH_3), 2.0–1.0 ($(CH_2)_5$); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.6, 170.5, 170.3, 169.4 (4 C=O), 98.8 (C-1), 77.6 (OCH), 72.1 (C-4), 71.3 (C-3), 68.9 (C-5), 62.3 (C-6), 55.2 (C-2), 33.1, 31.6, 25.4, 23.7, 23.6 [$(CH_2)_5$], 23.2, 20.6, 20.5 (4 CH_3). Anal. Calcd for $C_{20}H_{31}NO_9$: C, 55.93; H, 7.28; N, 3.26. Found: C, 55.87; H, 7.04; N, 3.52.

1-Octyl glycoside (**3**).—Recrystallized from Et_2O–hexane.—18.4 g (80%); mp 126–127 °C; $[\alpha]_D$ −36.4° (*c* 0.6, CH_2Cl_2); CIMS: *m*/*z* 460 (48%) [MH^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.42 (d, 1-H, $J_{2,NH}$ 8.6 Hz, N–H), 5.29 (dd, 1-H, $J_{2,3}$ 10.5, $J_{3,4}$ 9.3 Hz, H-3), 5.04 (t, 1-H, $J_{3,4}=J_{4,5}$ 9.5 Hz, H-4), 4.66 (d, 1-H, $J_{1,2}$ 8.3 Hz, H-1), 4.25 (dd, 1-H, $J_{5,6}$ 4.7, $J_{6,6'}$ 12.2 Hz, H-6), 4.10 (dd, 1-H, $J_{5,6'}$ 2.6, $J_{6,6'}$ 12.2 Hz, H-6′), 3.9–3.6 (m, 3-H, H-2, H-5, OC*H*HR), 3.45 (m, 1-H, OCH*H*R), 2.06, 2.01, 2.00, 1.92 (4-s, 4CH_3), 1.7–1.1 [$(CH_2)_6$], 0.85 (t, 3-H, *J* 6.4 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.9, 170.8, 170.1, 169.4 (4C=O), 100.7 (C-1), 72.3 (C-4), 71.8 (C-3), 70.0 (OCH_2R), 68.7 (C-5), 62.2 (C-6), 55.0 (C-2), 31.8, 29.4, 29.3, 29.2, 25.9, 22.6 [$(CH_2)_6$], 23.3 (CH_3CON), 20.8, 20.7, 20.6 (4-CH_3COO), 14.1 (CH_3). Anal. Calcd for $C_{22}H_{37}NO_9$: C, 57.50; H, 8.12; N, 3.05. Found: C, 57.54; H, 8.18; N, 3.00.

Alkyl 2-acetamido-4,6-O-*benzylidene-2-deoxy*-β-D-*glucopyranosides* (**5–7**).—To a soln of alkyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**2–4**) (20 mmol) in MeOH (200 mL) was added a soln of NaOMe (4 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–H_2O (500 mL) with stirring. The precipitate was filtered, washed with H_2O and with hexane, and then recrystallized from EtOH.

Cyclohexyl derivative (**5**).—6.0 g (77%); mp 259–261 °C; $[\alpha]_D$ −122.4° (*c* 0.7, DMF); CIMS: *m*/*z* 392 (31%) [MH^+]; ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 7.80 (d, 1-H, $J_{2,NH}$ 8.9 Hz, N–H), 7.5–7.3 (m, 5-H, Ph), 5.56 (s, 1-H, PhC*H*), 5.25 (d, 1-H, $J_{3,OH}$ 5.5 Hz, O–H), 4.56 (d, 1-H, $J_{1,2}$ 8.3 Hz, H-1), 4.16 (dd, 1-H, $J_{5,6e}$ 4.5, $J_{6e,6a}$ 10.1 Hz, H-6_e), 3.8–3.2 (m, 6-H, H-2, H-3, H-4, H-5, H-6_a, OCH), 1.80 (s, CH_3), 2.0–1.0 [$(CH_2)_5$]; ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 169.4 (C=O), 137.9, 129.0, 128.2, 126.5 (Ph), 100.7 (Ph*C*H), 100.1 (C-1), 81.4 (C-4), 76.1 (OCH), 70.5 (C-3), 68.1 (C-6), 66.0 (C-5), 56.7 (C-2), 33.0, 31.1, 25.3, 23.2, 22.9 [$(CH_2)_5$], 23.1 (CH_3). Anal. Calcd for $C_{21}H_{29}NO_6$: C, 64.43; H, 7.47; N, 3.58. Found: C, 64.22; H, 7.39; N, 3.57.

1-Octyl derivative (**6**).—6.4 g (76%); mp 191–193 °C; $[\alpha]_D$ −84.7° (*c* 1.0, CH_2Cl_2); CIMS: *m*/*z* 422 (45%) [MH^+]; ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 7.81 (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*), 5.24 (d, 1-H, $J_{3,OH}$ 5.2 Hz, O–H), 4.44 (d, 1-H, $J_{1,2}$ 8.0 Hz, H-1), 4.18 (dd, 1-H, $J_{5,6e}$ 4.2, $J_{6e,6a}$ 9.9 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), 1.5–1.1 [$(CH_2)_6$], 0.85 (t, 3-H, *J* 6.4 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 168.9 (C=O), 137.7, 128.8, 128.0, 126.3 (Ph), 101.6 (PhCH), 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), 31.2, 29.0, 28.7, 25.3, 22.1 [$(CH_2)_6$], 23.0 (CH_3CON), 13.9 (CH_3). Anal. Calcd for $C_{23}H_{35}NO_6$: C, 65.54; H, 8.37; N, 3.32. Found: C, 65.49; H, 8.53; N, 3.30.

Alkyl 2-acetamido-4,6-O-*benzylidene-2-deoxy-3*-O-*methylsulfonyl*-β-D-*glucopyranosides* (**8–10**).—Alkyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**5–7**) (15 mmol) was dissolved in pyridine (50 mL), and $MeSO_2Cl$ (3 mL) was added with cooling to 0 °C. The mixture was stored at 0 °C for 24 h and poured into ice-water. The solid was collected by filtration, washed with H_2O, dried (Na_2SO_4), and was recrystallized from EtOH.

Cyclohexyl derivative (**8**).—5.3 g (76%); mp 170–172 °C; [α]D −45.2° (c 1.7, DMF); FABMS: *m/z* 492 (100%) [MNa^+]; ^1H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5H, Ph), 5.80 (d, 1-H, $J_{2,NH}$ 7.2 Hz, N-H), 5.50 (s, 1-H, PhC*H*), 5.33 (m, 2-H, H-1, H-3), 4.36 (dd, 1-H, $J_{5,6e}$ 4.7, $J_{6e,6a}$ 10.3 Hz, H-6_e), 3.9–3.5 (m, 4-H, H-4, H-5, H-6_a, OCH), 3.24 (m, 1-H, H-2), 2.90 (s, 3-H, CH_3SO_3), 1.99 (s, CH_3CON), 1.95–1.15 [m, 10-H, $(CH_2)_5$]; ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.3 (C=O), 136.6, 129.4, 128.4, 126.0 (Ph), 100.8 (PhCH), 98.3 (C-1), 79.2 (C-3), 78.4 (C-4), 78.2 (OCH), 68.7 (C-6), 65.4 (C-5), 58.0 (C-2), 38.5 (CH_3SO_3), 33.3, 31.7, 25.4, 23.9, 23.5 [$(CH_2)_5$], 23.7 (CH_3CON). Anal. Calcd for $C_{22}H_{31}NO_8S$: C, 56.27; H, 6.67; N, 2.98. Found: C, 56.26; H, 6.63; N, 2.89.

1-Octyl derivative (**9**).—5.3 g (71%); mp 192–194 °C; [α]D −375.9° (c 1.0, CH_2Cl_2); CIMS: *m/z* 500 (5%) (MH^+); ^1H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5H, Ph), 5.89 (d, 1-H, $J_{2,NH}$ 7.7 Hz, N–H), 5.50 (s, 1-H, PhC*H*), 5.33 (t, 1-H, $J_{2,3}=J_{3,4}$ 10.0 Hz, H-3), 5.09 (d, 1-H, $J_{1,2}$ 8.2 Hz, H-1), 4.36 (dd, 1-H, $J_{5,6e}$ 4.6, $J_{6e,6a}$ 10.2 Hz, H-6_e), 3.9–3.3 (m, 6-H, H-2, H-4, H-5, H-6_a, OCH_2R), 2.91 (s, 3-H, CH_3SO_3), 2.00 (s, 3-H, CH_3CON), 1.7–1.1 [m, 12H, $(CH_2)_6$], 0.86 (t, 3-H, *J* 6.4 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.3 (C=O), 136.6, 129.4, 128.4, 126.0 (Ph), 101.7 (PhCH), 100.3 (C-1), 79.2 (C-3), 78.6 (C-4), 70.6 (OCH_2R), 68.7 (C-6), 65.5 (C-5), 57.2 (C-2), 38.5 (CH_3SO_3), 31.8, 29.5, 29.3, 25.8, 22.6 [$(CH_2)_6$], 23.4 (CH_3CON), 14.1 (CH_3). Anal. Calcd for $C_{24}H_{37}NO_8S$: C, 57.71; H, 7.41; N, 2.80. Found: C, 57.53; H, 7.20; N, 2.79.

Alkyl 2-acetamido-4,6-O-*benzylidene-2-deoxy*-β-D-*allopyranosides* (**11–13**).—A soln of alkyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methylsulfonyl-β-D-glucopyranoside (**8–10**) (7.5 mmol) and anhyd NaAcO (3 g) in 96:4 2-methoxyethanol–H_2O (50 mL) was heated at the reflux temperature for 12 h. After cooling, the mixture was poured into H_2O, and the precipitate was collected by filtration and recrystallized from EtOH.

Cyclohexyl derivative (**11**).—2.1 g (72%); mp 214–216 °C; [α]D −209.8° (c 0.6, CH_2Cl_2); CIMS: *m/z* 392 (23%) [MH^+]; ^1H NMR (200 MHz, Me_2SO-d_6): δ 7.85 (d, 1-H, $J_{2,NH}$ 9.1 Hz, N–H), 7.5–7.3 (m, 5-H, Ph), 5.62 (s, 1-H, PhC*H*), 5.42 (d, 1-H, $J_{3,OH}$ 3.7 Hz, O–H), 4.69 (d, 1-H, $J_{1,2}$ 8.6 Hz, H-1), 4.21 (dd, 1-H, $J_{5,6e}$ 4.8, $J_{6e,6a}$ 9.6 Hz, H-6_e), 3.91 (m, 1-H, H-3), 3.85–3.45 (m, 5-H, H-2, H-4, H-5, H-6_a, OCH), 1.82 (s, CH_3), 1.8–1.1 [$(CH_2)_5$]; ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 168.6 (C=O), 137.8, 128.8, 127.9, 126.4 (Ph), 100.6 (PhCH), 98.2 (C-1), 78.6 (C-4), 76.2 (OCH), 68.4 (C-6), 67.5 (C-3), 62.6 (C-5), 52.8 (C-2), 33.1, 31.2, 25.2, 23.3, 22.6 [$(CH_2)_5$], 23.0 (CH_3). Anal. Calcd for $C_{21}H_{29}NO_6$: C, 64.43; H, 7.47; N, 3.58. Found: C, 64.11; H, 7.33; N, 3.52.

1-Octyl derivative (**12**).—2.5 g (78%); mp 221–223 °C; [α]D −97.6° (c 2.1, CH_2Cl_2); CIMS: *m/z* 422 (22%) [MH^+]; ^1H NMR (200 MHz, Me_2SO-d_6): δ 7.92 (d, 1-H, $J_{2,NH}$ 8.8 Hz, N–H), 7.5–7.3 (m, 5-H, Ph), 5.62 (s, 1-H, PhC*H*), 5.43 (d, 1-H, $J_{3,OH}$ 4.3 Hz, O–H), 4.61 (d, 1-H, $J_{1,2}$ 8.7 Hz, H-1), 4.21 (dd, 1-H, $J_{5,6e}$ 4.6, $J_{6e,6a}$ 9.5 Hz, H-6_e), 3.94 (m, 1-H, H-3), 3.8–3.4 (m, 6-H, H-2, H-4, H-5, H-6_a, OCH_2R), 1.82 (s, 3H, CH_3CON), 1.5–1.2 [$(CH_2)_6$], 0.85 (t, 3-H, *J* 6.4 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 168.6 (C=O), 137.8, 128.6, 127.9, 126.4 (Ph), 100.6 (PhCH), 99.5 (C-1), 78.6 (C-4), 68.8 (OCH_2R), 68.3 (C-6), 67.4 (C-3), 62.7 (C-5), 52.7 (C-2), 31.2, 29.1, 28.7, 25.4, 22.1 [$(CH_2)_6$], 22.6 (CH_3CON), 13.9 (CH_3). Anal. Calcd for $C_{23}H_{35}NO_6$: C, 65.54; H, 8.37; N, 3.32. Found: C, 65.57; H, 8.21; N, 3.26.

Alkyl 2-amino-4,6-O-*benzylidene-2-deoxy*-β-D-*allopyranosides* (**14–16**).—A soln of alkyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside (**11–13**) (8 mmol) in a hot mixture of KOH (15 g) and 96% EtOH (50 mL) was heated under reflux for 5–6 h. The hot soln was poured carefully into hot H_2O (400 mL). After being cooled to room temperature, the precipitate was kept overnight at −10 °C. The solid was filtered off, washed with H_2O, dried, and then crystallized from EtOH.

Cyclohexyl derivative (**14**).—2.6 g (94%); mp 186–188 °C; [α]D −219.1° (c 1.6, CH_2Cl_2); CIMS:

m/z 350 (13%) [MH^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5H, Ph), 5.54 (s, 1-H, PhC*H*), 4.62 (d, 1-H, $J_{1,2}$ 8.1 Hz, H-1), 4.33 (dd, 1-H, $J_{5,6e}$ 4.8, $J_{6e,6a}$ 10.0 Hz, H-6$_e$), 4.23 (t, 1-H, $J_{2,3}=J_{3,4}$ 2.6 Hz, H-3), 3.96 (td, 1-H, $J_{4,5}=J_{5,6a}$ 9.8, $J_{5,6e}$ 4.8 Hz, H-5), 3.74 (t, 1-H, $J_{5,6a}=J_{6e,6a}$ 10.1 Hz, H-6$_a$), 3.6 (m, 2-H, H-4, OCH), 2.68 (dd, 1-H, $J_{1,2}$ 8.1, $J_{2,3}$ 2.8 Hz, H-2), 2.0-1.1 [$(CH_2)_5$]; ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.2, 129.2, 128.3, 126.2 (Ph), 101.8 (C-1), 101.0 (Ph*C*H), 79.6 (C-4), 77.5 (OCH), 69.3 (C-6), 69.1 (C-3), 63.5 (C-5), 55.1 (C-2), 33.7, 31.9, 25.5, 24.2, 24.0 [$(CH_2)_5$]. Anal. Calcd for $C_{19}H_{27}NO_5$: C, 65.33; H, 7.73; N, 4.01. Found: C, 65.05; H, 7.60; N, 4.05.

1-Octyl derivative (**15**).—2.7 g (88%); mp 153–155 °C; [α]D −59.2° (*c* 1.0, CH_2Cl_2); CIMS: *m/z* 380 (9%) [MH^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5H, Ph), 5.53 (s, 1-H, PhC*H*), 4.49 (d, 1-H, $J_{1,2}$ 8.1 Hz, H-1), 4.34 (dd, 1-H, $J_{5,6e}$ 4.7, $J_{6e,6a}$ 10.0 Hz, H-6$_e$), 4.22 (m, 1-H, H-3), 4.0–3.3 (m, 5-H, H-4, H-5, H-6$_a$, OCH_2R), 2.69 (dd, 1-H, $J_{1,2}$ 8.1, $J_{2,3}$ 2.5 Hz, H-2), 1.7–1.1 [$(CH_2)_6$], 0.86 (t, 3H, *J* 6.4 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.2, 129.2, 128.3, 126.2 (Ph), 102.9 (C-1), 101.9 (Ph*C*H), 79.7 (C-4), 70.4 (OCH_2R), 69.2 (C-6), 68.9 (C-3), 63.4 (C-5), 55.2 (C-2), 31.8, 29.6, 29.4, 29.2, 26.0, 22.6 [$(CH_2)_6$], 14.1 (CH_3). Anal. Calcd for $C_{21}H_{33}NO_5$: C, 66.46; H, 8.76; N, 3.69. Found: C, 66.49; H, 8.55; N, 3.60.

1-Dodecyl derivative (**16**).—2.6 g (75%); mp 117–119 °C; [α]D −103.7° (*c* 0.5, CH_2Cl_2); FABMS: *m/z* 458 (100%) [MNa^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5–7.3 (m, 5-H, Ph), 5.54 (s, 1-H, PhC*H*), 4.50 (d, 1-H, $J_{1,2}$ 8.1 Hz, H-1), 4.34 (dd, 1-H, $J_{5,6e}$ 4.7, $J_{6e,6a}$ 10.1 Hz, H-6$_e$), 4.22 (t, 1-H, $J_{2,3}=J_{3,4}$ 2.5 Hz, H-3), 4.0–3.4 (m, 5-H, H-4, H-5, H-6$_a$, OCH_2R), (2.69 dd, 1-H, $J_{1,2}$ 8.1, $J_{2,3}$ 2.6 Hz, H-2), 1.7–1.1 [$(CH_2)_{10}$], 0.84 (t, 3-H, *J* 6.6 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.2, 129.2, 128.3, 126.2 (Ph), 102.9 (C-1), 101.8 (Ph*C*H), 79.7 (C-4), 70.4 (OCH_2R), 69.2 (C-6), 69.0 (C-3), 63.4 (C-5), 55.2 (C-2), 31.9, 29.6, 29.5, 29.4, 29.3, 26.0, 22.7 [$(CH_2)_{10}$], 14.1 (CH_3). Anal. Calcd for $C_{25}H_{41}NO_5$: C, 68.93; H, 9.49; N, 3.22. Found: C, 69.00; H, 9.28; N, 3.11.

Alkyl 2-amino-4,6-O-*benzylidene-2*-N-*3*-O-*[bis (2-chloroethyl)amino]phosphoryl-2-deoxy*-β-D-*allopyranosides* (**17–19**).—A soln of alkyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside (**14–16**) (5 mmol) and Et_3N (20 mL) in distilled and dried CH_2Cl_2 (200 mL) under argon atmosphere was cooled to 0 °C. Then bis(2-chloroethyl)phosphoramide dichloride (5.5 mmol) was added quickly. The reaction mixture was stirred overnight at room temperature. At the end of the reaction (TLC) the mixture was diluted with CH_2Cl_2, and washed successively, with aq 1 N HCl, aq satd soln of $NaHCO_3$, and H_2O, then dried (Na_2SO_4) and concentrated to give a solid product. The product obtained was purified by flash chromatography on silica gel using CH_2Cl_2–2-PrOH (120:1) as eluent. A small fraction of pure compound was recrystallized from EtOH.

Cyclohexyl derivative (**17**).—2.35 g (87%); mp 220–222 °C; [α]D −46.2° (*c* 0.8, MeOH); FABMS: *m/z* 557 (100%) [MNa^+]; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.6–7.3 (m, 5-H, Ph), 5.56 (s, 1-H, PhC*H*), 5.04 (d, 1-H, $J_{1,2}$ 7.6 Hz, H-1), 4.78 (t, 1-H, $J_{2,3}=J_{3,4}$ 3.4 Hz, H-3), 4.44 (dd, 1-H, $J_{5,6e}$ 5.3, $J_{6e,6a}$ 10.5 Hz, H-6$_e$), 4.01 (td, 1-H, $J_{4,5}=J_{5,6a}$ 9.9, $J_{5,6e}$ 5.6 Hz, H-5), 3.8–3.1 [m, 13-H, H-2, H-4, H-6$_a$, N-H, OCH, $N(CH_2CH_2Cl)_2$], 2.1–1.1 [$(CH_2)_5$]; ^{13}C NMR (125 MHz, $CDCl_3$): δ 137.2, 129.3, 128.3, 126.4 (Ph), 102.7 (C-1), 101.6 (Ph*C*H), 78.0, 77.0, 76.7 (OCH, C-3, C-4), 68.9 (C-6), 63.1 (C-5), 55.2 (C-2), 49.2 (NCH_2), 42.0 (CH_2Cl), 33.6, 31.9, 25.4, 24.2, 24.0 [$(CH_2)_5$]. ^{31}P NMR (81 MHz, $CDCl_3$): δ 25.96. Anal. Calcd for $C_{23}H_{33}Cl_2N_2O_6P$: C, 51.59; H, 6.21; N, 5.23. Found: C, 51.64; H, 6.12; N, 5.13.

1-Octyl derivative (**18**).—2.40 g (85%); mp 122–124 °C; [α]D −51.0° (*c* 1.0, CH_2Cl_2); FABMS: *m/z* 587 (100%) [MNa^+]; ^{1}H NMR (500 MHz, Me_2SO-d_6): δ 7.5–7.3 (m, 5-H, Ph), 5.76 (dd, 1-H, $J_{2,NH}$ 2.5, $^2J_{H,P}$ 17.6 Hz, N–H), 5.66 (s, 1-H, PhC*H*), 4.71 (dd, 1-H, $J_{2,3}$ 3.5, $J_{3,4}$ 5.0 Hz, H-3), 4.58 (d, 1-H, $J_{1,2}$ 6.9 Hz, H-1), 4.29 (dd, 1-H, $J_{5,6e}$ 5.6, $J_{6e,6a}$ 9.8 Hz, H-6$_e$), 3.9–3.3 [m, 14-H, H-2, H-4, H-5, H-6$_a$, OCH_2R, $N(CH_2CH_2Cl)_2$], 1.5–1.2 [$(CH_2)_6$], 0.85 (t, 3H, *J* 6.9 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 136.8, 129.3, 128.3, 126.4 (Ph), 103.6 (C-1), 102.7 (Ph*C*H), 77.2, 76.7 (C-3, C-4), 70.7 (OCH_2R), 68.9 (C-6), 62.8 (C-5), 57.2 (C-2), 49.2 (NCH_2), 42.0 (CH_2Cl), 31.8, 29.6, 29.3, 29.2, 25.9, 22.6 [$(CH_2)_6$]. ^{31}P NMR (81 MHz, $CDCl_3$): δ 26.10. Anal. Calcd for $C_{25}H_{39}Cl_2N_2O_6P$: C, 53.10; H, 6.95; N, 4.95. Found: C, 53.13; H, 6.86; N, 4.80.

1-Dodecyl derivative (**19**).—2.45 g (78%); mp 133–135 °C; [α]D −40.7° (*c* 0.9, CH_2Cl_2); FABMS: *m/z* 643 (100%) [MNa^+]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.6–7.3 (m, 5-H, Ph), 5.54 (s, 1-H, PhC*H*), 4.86 (d, 1-H, $J_{1,2}$ 8.1 Hz, H-1), 4.73 (t, 1-H, $J_{2,3}=J_{3,4}$ 3.5 Hz, H-3), 4.39 (dd, 1-H, $J_{5,6e}$ 4.8, $J_{6e,6a}$ 10.2 Hz, H-6$_e$), 4.1–3.2 [m, 15-H, H-2, H-4,

H-5, H-6$_a$, N-H, OCH_2R, $N(CH_2CH_2Cl)_2$], 1.7–1.1 [$(CH_2)_{10}$], 0.86 (t, 3-H, J 6.6 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 136.8, 129.4, 128.4, 126.4 (Ph), 103.6 (C-1), 102.7 (Ph*C*H), 77.1, 76.7 (C-3, C-4), 70.7 (OCH_2R), 68.9 (C-6), 62.8 (C-5), 57.1 (C-2), 49.3, 49.2 (NCH_2), 42.1 (CH_2Cl), 31.9, 29.6, 29.3, 25.9, 22.7 [$(CH_2)_{10}$]. ^{31}P NMR (81 MHz, $CDCl_3$): δ 26.08. Anal. Calcd for $C_{29}H_{47}Cl_2N_2O_6P$: C, 56.04; H, 7.62; N, 4.51. Found: C, 56.15; H, 7.51; N, 4.30.

Alkyl 2-amino-2-N-*3*-O-*[bis(2-chloroethyl) amino] phosphoryl-2-deoxy*-β-D-*allopyranosides* (**20–22**).—A soln of alkyl 2-amino-4,6-*O*-benzylidene-2-*N*-3-*O*-[bis(2-chloroethyl)amino]phosphoryl-2-deoxy-*β*-D-allopyranoside (**17–19**) (0.3 mmol) in MeOH (30 mL) was hydrogenolyzed over 10% Pd(C) (30 mg) at room temperature and a pressure of 50 PSI in a Parr hydrogenation apparatus. TLC indicated complete reaction after 12–20 h. The mixture was diluted with MeOH, the catalyst was filtered off and washed with MeOH, and the filtrate was concentrated under reduced pressure.

Cyclohexyl derivative (**20**).—0.12 g (88%); mp 82–84 °C; $[\alpha]_D$ −309.1° (c 0.4, CH_2Cl_2); FABMS: m/z 469 (100%) [MNa^+]; ^1H NMR (200 MHz, Me_2SO-d_6): δ 5.42 (dd, 1-H, $J_{2,NH}$ 2.1, $^2J_{H,P}$ 16.8 Hz, N-H), 5.16 (d, 1-H, $J_{4,OH}$ 6.3 Hz, OH-4), 4.52 (m, 3-H, H-1, H-3, OH-6), 3.8–3.1 (m), 2.0–1.1 [m, $(CH_2)_5$]; ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 100.9 (C-1), 80.1 (C-3), 75.5 (OCH), 74.2 (C-5), 65.2, 65.1 (C-4), 60.5 (C-6), 55.9, 55.7 (C-2), 48.4, 48.3 (NCH_2), 42.0 (CH_2Cl), 32.9, 30.9, 25.0, 23.4, 23.1 [$(CH_2)_5$]; ^{31}P NMR (81 MHz, Me_2SO-d_6): δ 30.17. Anal. Calcd for $C_{16}H_{29}Cl_2N_2O_6P$: C, 42.96; H, 6.54; N, 6.26. Found: C, 42.84; H, 6.41; N, 6.19.

1-Octyl derivative (**21**).—0.12 g (85%); mp 99–101 °C; $[\alpha]_D$ −147.6° (c 1.0, CH_3OH); FABMS: m/z 499 (100%) [MNa^+]; ^1H NMR (500 MHz, Me_2SO-d_6): δ 5.53 (dd, 1-H, $J_{2,NH}$ 2.4, $^2J_{H,P}$ 16.8 Hz, N–H), 5.16 (d, 1-H, $J_{4,OH}$ 7.1 Hz, OH-4), 4.54 (t, 1-H, $J_{6,OH}$ 5.8 Hz, OH-6), 4.50 (t, 1-H, $J_{2,3} = J_{3,4}$ 4.3 Hz, H-3), 4.43 (d, 1-H, $J_{1,2}$ 7.2 Hz, H-1), 3.78 (m, 1-H, OC*H*HR), 3.65 [m, 5-H, H-6, $(CH_2Cl)_2$], 3.5–3.3 [m, H-4, H-5, H-6′, OCH*H*R, $N(CH_2)_2$], 3.27 (m, 1-H, H-2), 1.5–1.1 [m, $(CH_2)_6$], 0.85 (t, 3H, J 6.8 Hz, CH_3); ^{13}C NMR (125 MHz, Me_2SO-d_6): δ 103.2 (C-1), 79.9 (C-3), 74.3 (C-5), 68.8 (OCH_2R), 65.4 (C-4), 60.9 (C-6), 56.0 (C-2), 48.6 (NCH_2), 42.3 (CH_2Cl), 31.2, 29.1, 28.9, 28.6, 25.5, 22.0 [$(CH_2)_6$], 13.9 (CH_3); ^{31}P NMR (81 MHz, Me_2SO-d_6) δ 30.21. Anal. Calcd for $C_{18}H_{35}Cl_2N_2O_6P$: C, 45.29; H, 7.39; N, 5.87. Found: C, 45.37; H, 7.36; N, 5.75.

1-Dodecyl derivative (**22**).—0.15 g (90%); mp 103–105 °C; $[\alpha]_D$ −68.0° (c 1.5, CH_2Cl_2); FABMS: m/z 555 (100%) [MNa^+]; ^1H NMR (500 MHz, Me_2SO-d_6): δ 5.53 (dd, 1-H, $J_{2,NH}$ 2.2, $^2J_{H,P}$ 16.8 Hz, N-H), 5.16 (d, 1-H, $J_{4,OH}$ 7.1 Hz, OH-4), 4.54 (t, 1-H, $J_{6,OH}$ 5.6 Hz, OH-6), 4.50 (t, 1-H, $J_{2,3} = J_{3,4}$ 4.3 Hz, H-3), 4.42 (d, 1-H, $J_{1,2}$ 7.2 Hz, H-1), 3.9-3.1 [m, H-2, H-4, H-5, H-6, H-6′, $N(CH_2CH_2Cl)_2$, OCH_2R], 1.6–1.1 [m, $(CH_2)_{10}$], 0.84 (t, 3H, J 6.8 Hz, CH_3); ^{13}C NMR (50 MHz, Me_2SO-d_6): δ 103.2 (C-1), 80.0 (C-3), 74.4 (C-5), 68.8 (OCH_2R), 65.4, 65.3 (C-4), 60.8 (C-6), 56.0, 55.9 (C-2), 48.6, 48.5 (NCH_2), 42.3 (CH_2Cl), 31.3, 29.2, 29.0, 28.7, 25.5, 22.1 [$(CH_2)_{10}$], 14.0 (CH_3). ^{31}P NMR (81 MHz, Me_2SO-d_6): δ 30.22. Anal. Calcd for $C_{22}H_{43}Cl_2N_2O_6P$: C, 49.53; H, 8.12; N, 5.25. Found: C, 49.58; H, 7.86; N, 5.07.

Acknowledgement

We thank the DGICYT (Spain) for financial support (grant PB94-1431).

References

[1] I. Niculescu-Duvaz, I. Baracu, and T. Balaban, Alkylating Agents, in D.E.V. Wilman (Ed.), *The Chemistry of Antitumor Agents*, Blackie & Son, Glasgow and London, 1990, pp 63–130, and refs. therein.
[2] A. Okruzek and J.G. Verkade, *J. Med. Chem.*, 22 (1979) 882–885.
[3] T.S. Lin, P.H. Fischer, and W.H. Prusoff, *J. Med. Chem.*, 23 (1980) 1235–1237.
[4] M. Yamashita, C. Takahashi, and K. Seo, *Heterocycles*, 36 (1993) 651–654.
[5] D. Charon, R. Chaby, A. Malinvaud, M. Mondange, and L. Szabó, *Biochemistry*, 24 (1985) 2736–2742.
[6] P.H. Gross and R.W. Jeanloz, *J. Org. Chem.*, 32 (1967) 2759–2763.
[7] W. Meyer zu Reckendorf, *Methods Carbohydr. Chem.*, 6 (1972) 266–269.
[8] J.G. Fernández-Bolaños and J.M. Vega-Pérez, unpublished results.
[9] D. Horton, *Org. Synth.*, 5 (1973) 1–5.