Carbohydrate Research, 240 (1993) 313–322
Elsevier Science Publishers B.V., Amsterdam

Note

Synthesis of cyclophosphamide analogs from aminotrideoxy sugars

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(Received March 20th, 1992; accepted in final form September 29th, 1992)

Cyclophosphamide **1a** is a highly effective and extensively used agent for the treatment of human cancers[1]. The metabolism, pharmacokinetics, and mechanism of action of **1a** have been the subject of several recent reviews[2–5]. The activation process of **1a** involves initial hydroxylation by the hepatic cytochrome P450 system to produce one or both isomers[6,7] of 4-hydroxycyclophosphamide **1b**. Subsequent formation of aldophosphamide **2** leads to acrolein **3** and phosphoramide mustard[5] **4** generally believed to be the ultimate cytotoxic agent that cross-links interstrand DNA[8–10]. Detoxification involves enzymic reactions at C-4 with formation of 4-ketocyclophosphamide **5** or carboxyphosphamide **6** by aldehyde dehydrogenase-mediated oxidation. Thus, both formation of active metabolites and detoxification require enzymic reactions at C-4 of the six-membered cyclophosphamide ring.

Recent data suggest that acrolein (**3**), produced by the decomposition of aldophosphamide, is toxic to cultured tumor cells[11], but does not play a significant role in the anticancer activity of **1**. Acrolein may be responsible for the cardiac and pulmonary toxicities of cyclophosphamide[12] and also for cystitis and renal damage[13,14].

Since **1a** functions as a prodrug, such pre-activated analogs of **1a** as benzo-annelated cyclophosphamide[15] or 4-hydroxy-(**1b**), and 4-hydroperoxy-cyclophosphamide[16] (**1c**) have been synthesized both for possible enhancement of activity and for an understanding of the pathways of cyclophosphamide activation and metabolism.

By analogy with the well-known strategy in the design of potential anticancer drugs with increased selectivity, attachment of a cyclophosphamide ring system to "carrier" molecules has been reported. This includes steroids for hormonally dependent neoplasm[17], amino acids or peptides[18], nucleosides[19–22], and amino

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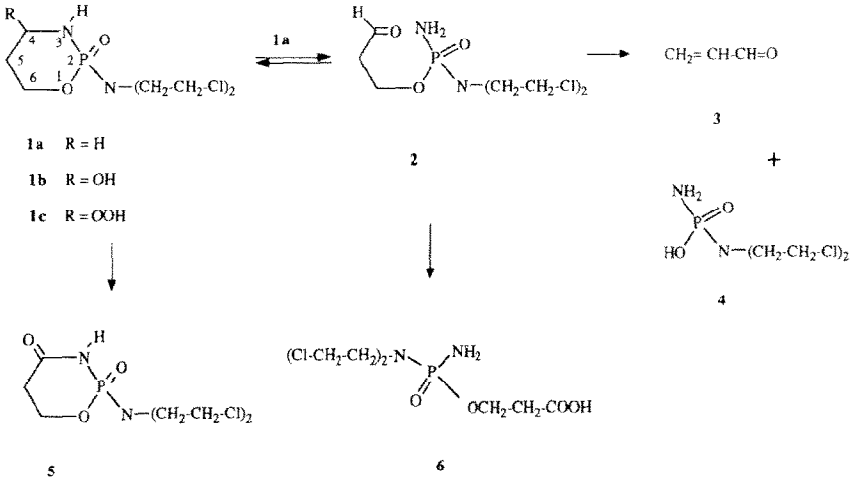


Scheme 1.

sugars for increasing transport in ascite tumor cells or across membranes and, thereby, favorably affect the pharmacological and biological properties. However, in none of these numerous analogs so far has the amino alcohol moiety been a deoxyamino sugar.

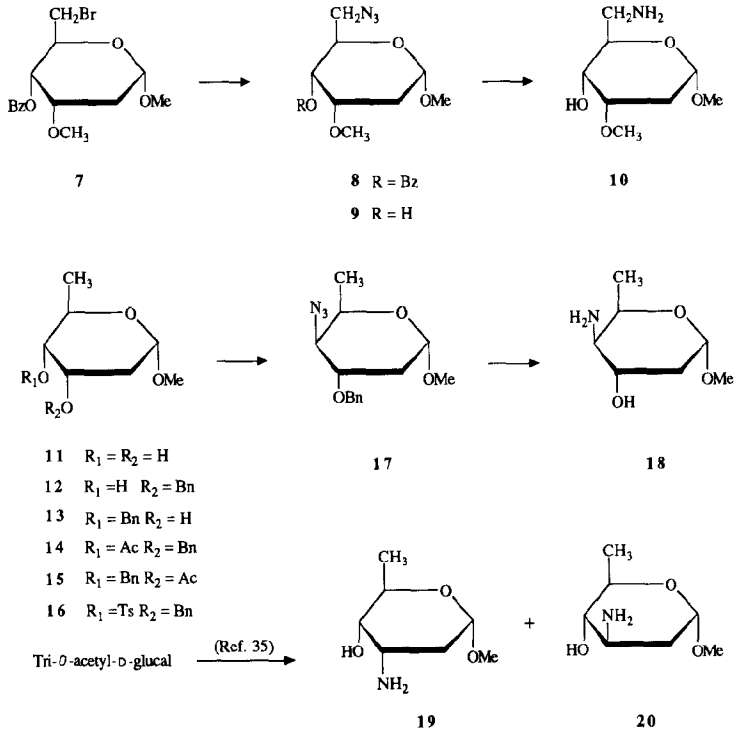
As shown with chloroethylnitrosoureas, the attachment of a sugar carrier to the cytotoxic nitrosoureido moiety reduces bone marrow toxicity without altering its antitumor activity[23]. Many nitrosoureido derivatives of amino sugars have been prepared[24–26].

For our part, we have found[27] that such derivatives of amino di- or tri-deoxy sugars exhibit significant activity in vivo against L 1210 leukemia, B 16 melanoma, and Lewis lung carcinoma. Moreover, the most active compound in this series, CY 233, was found to be very effective against advanced colon 38 adenocarcinoma[28] and was recently introduced in clinical trials.

Based upon these considerations, a series of new compounds has been synthesized, that includes 6- and also 5-membered cyclic phosphorodiamidates. Although the latter obviate the possibility of a cyclophosphamide-like mechanism of action, such nucleoside analogs have been reported[20] to be highly effective against KB cells in vitro.

The synthesis of the amino didcoxy and amino trideoxysugars **10**, **18**, **19**, and **20** is outlined in Scheme 2.

Azidolysis of methyl 4-*O*-benzoyl-6-bromo-2,6-dideoxy-3-*O*-methyl-α-D-*ribo*-hexopyranoside **7**, prepared[29] from methyl α-D-glucopyranoside, in *N*,*N*-dimethylformamide at 80°C gave **8** in 80% yield. *O*-Deacylation of **8** by catalytic transesterification afforded **9** in 89% yield and hydrogenation of this azido sugar in the presence of 10% Pd–C gave methyl 6-amino-2,6-dideoxy-3-*O*-methyl-α-D-*ribo*-hexopyranoside (**10**) in 60% yield after chromatography.

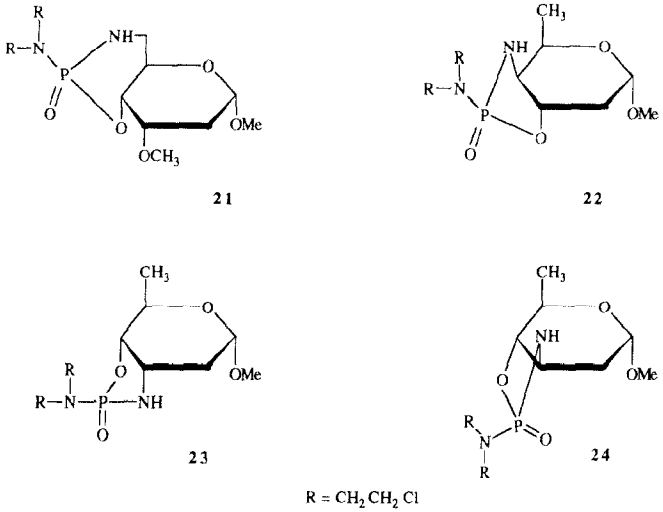


Scheme 2.

The 3-*O*-benzyl derivative **12** of methyl 2,6-dideoxy-α-D-*ribo*-hexopyranoside[30] (**11**) was obtained in 60% yield, along with its 4-*O*-benzyl isomer **13** (40% yield), via the 3,4-*O*-stannylene acetal, by treatment with benzyl bromide in benzene solution under reflux for 48 h. Compounds **12** and **13** were acetylated in pyridine solution to afford **14** and **15**, respectively. The structures of compounds **12–15** were unambiguously deduced from their ^{1}H NMR spectra. Thus, the signal at δ 3.23 for H-4 (ddd, $J_{4,5} = J_{4,OH} = 10$, $J_{3,4} = 4$ Hz) in the 3-benzyl ether **12** was found to be a dd after deuteriation ($J_{4,5}$ 10, $J_{3,H}$ 4 Hz) which shifted downfield after acetylation. A similar shift from δ 3.26 to δ 5.36 was observed for the multiplet (H-3) as present in **13**.

The surprising stereoselectivity * in favor of the formation of a (quasi)axial ether was already observed, for example in the methyl 4,6-*O*-benzyl-D-mannoside series[31]. This should be explained on the basis of stereoelectronic effects associated with the complex nature of the stannylene group[32], by 1,3-interactions, in the transition state, between the C-1–OCH_3 and the C-3–O bonds, and/or also by steric (2-deoxy sugar) factors[33].

* Highly stereoselective formation of a (quasi) axial ether was also observed during *p*-toluenesulfonylation of **11** via its stannylene derivative, since the corresponding 3-*O*-tosyl analog was isolated in 83% yield[34].



Scheme 3.

p-Toluenesulfonylation of the 4-OH of **12** led to **16** in 80% yield and, from it, the azido-sugar **17** was obtained quantitatively by azidolysis in dimethylformamide (90°C). Catalytic hydrogenation of **17** in ethanol solution in the presence of acetic acid and 10% Pd–C led to methyl 4-amino-2,4,6-trideoxy-α-D-*xylo*-hexopyranoside (**18**) whose constants were in agreement with the literature[35].

Methyl 3-amino-2,3,6-trideoxy-α-D-*ribo*-hexopyranoside (**19**) and its corresponding *arabino* isomer (**20**) were prepared from tri-*O*-acetyl-D-glucal as previously described.[36].

Compounds **10**, **18**, **19**, and **20** were converted into the corresponding amino-deoxy sugar cyclophosphamide analogs **21–24**, respectively (Scheme 3), by reaction with bis(2-chloroethyl)phosphoramidic dichloride[37] in dichloromethane in the presence of Et_3N. They were purified by chromatography on silica gel and both isomers * on the phosphorus atom could be analysed in the case of **23**, whereas only the major isomers of the other derivatives, **21**, **22**, and **24**, herein described, were isolated as pure products.

To evaluate the potential therapeutic effectiveness of these cyclophosphamide analogs, compounds **21** and **23a** were tested against advanced L1210 leukemia cells in vivo using cyclophosphamide as a reference compound[38]. To compare the different drugs at equitoxic dose level, each drug was tested in a dose range starting at 1/3 LD_{10} up to LD_{50}. The drug was administered intraperitoneally either once or, in the case of the highly insoluble compound **21**, 3 times (days 1, 4, and 7). The drugs were injected 24 h after the ip application of 1×10^6 L1210 leukemia cells. As the data in Table I show, neither compound **21** nor **23a** were

* The first eluted isomer is arbitrarily designed **a**.

TABLE I

Effects of aminodeoxy sugar cyclophosphamide analogs on the replication of L1210 cells in vivo

Compound	Schedule	T/C	Optimum dose (mg/kg)	LTS [a]
Cyclophosphamide **1a**	1×ip/ip	153	100	
		248	200	
		139	300	toxic
21	3×ip/ip	110	56.2	
	(days 1, 4, and 7)	94	74.9	
		105	100	
23a	1×ip/ip	100	300	
		95	400	
		90	500	

[a] LTS: animals surviving on day 60 are regarded as long-time survivors.

effective in this model, whereas cyclophosphamide demonstrated the well-known high therapeutic efficacy. Because of their low solubility, compounds **21** and **23a** had to be injected as suspensions in 4% starch solution. Due to the viscosity of the resulting suspension, higher dosages than those given in Table I could not be injected. Therefore, for these compounds, the maximum dose level was still below the LD_{50}, which indicates that both drugs could not be tested in this experiment on an equitoxic dose level, due to the formulation problems. In order to overcome this limitation with **21** and **23a**, but also with **22** and **24**, it might be worthwile to synthesize more highly soluble derivatives such as the corresponding 6-hydroxy or 6-phosphonate analogs.

EXPERIMENTAL

General methods.—Melting points (Kofler hot stage microscope) are uncorrected. IR spectra were recorded on a Perkin–Elmer Model 257 spectrophotometer, calibrated against polystyrene film, and are expressed in cm^{-1}. ^1H NMR spectra at 270 MHz were obtained on a Bruker HX 270 instrument in $CDCl_3$, except when otherwise stated. Chemical shifts are given in ppm downfield from internal Me_4Si. The coupling constants are reported as J values in Hz. Column chromatography was performed with E. Merck Silica Gel H 60 No 7736 and analytical thin-layer chromatography on E. Merck Silica Gel 60 F_{254}. THF and ether were dried over sodium benzophenone and distilled. Microanalyses were performed by the Laboratoire de Microanalyse du CNRS, at Gif-sur-Yvette and Lyon.

*Methyl 6-azido-4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl-α-*D*-*ribo*-hexopyranoside* (**8**). —To a solution of **7** (3.5 g, 9.7 mmol) (Ref. 29) in anhyd DMF (50 mL), NaN_3 (3 g, 46 mmol) was added, and the mixture was heated overnight at 80°C under Ar. It was then poured into water (20 mL) and extracted with ether (250 mL). The organic layer was washed with water (6 × 20 mL), dried (Na_2SO_4), and evaporated to dryness under diminished pressure to give **8** (2.5 g, 80%) as a syrup; $[\alpha]_D^{20}$ + 155°

(*c* 1, $CHCl_3$); ν_{max}^{film}: 2100 (azide) and 1720 cm^{-1} (C=O); ^1H NMR δ 7.99–7.33 (m, 5 H arom), 5.01 (dd, $J_{4,5}$ 10, $J_{4,3}$ 3 Hz, H-4), 4.75 (dd, $J_{1,2a}$ 4, $J_{1,2e} < 1$ Hz, H-1), 4.45 (m, H-5), 3.89 (m, H-3), 3.50–3.30 (m, H-6), 3.39 (s, OCH_3), 3.37 (s, OCH_3), 2.53 (m, H-2*e*), and 1.95 (m, H-2*a*). Anal. Calcd for $C_{15}H_{19}N_3O_5$ (321.37): C, 56.06; H, 5.97. Found: C, 56.39; H, 6.13.

*Methyl 6-azido-2,6-dideoxy-3-*O*-methyl-α-*D*-*ribo*-hexopyranoside* (**9**).—A solution of **8** (2.5 g, 7.8 mmol) in MeOH (20 mL) and 10% aq NaOH (20 mL) was stirred for 3 h at room temperature. After dilution of the mixture with water (40 mL) and extraction with CH_2Cl_2 (250 mL), the organic layers were washed with water, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. This gave **9** (1.8 g, 89%) as a colourless syrup; $[\alpha]_D^{20}$ + 104° (*c* 1.1 $CHCl_3$); ν_{max}^{film}: 3400 (OH), 2100 (azide) and 1290 cm^{-1} (C–O); ^1H NMR δ 4.66 (dd, $J_{1,2a}$ 4, $J_{1,2e} < 1$ Hz, H-1), 4.00 (m, H-5), 3.61 (m, H-3), 3.48 (m, H-6), 3.45 (dd, $J_{4,5}$ 10, $J_{4,3}$ 6 Hz, H-4), 3.41 (s, OCH_3), 3.34 (s, OCH_3), 2.28 (m, H-2*e*), and 1.74 (m, H-2*a*). Anal. Calcd for $C_8H_{15}N_3O_4$ (217.27): C, 44.22; H, 6.97. Found: C, 44.22; H, 7.05.

*Methyl 6-amino-2,6-dideoxy-3-*O*-methyl-α-*D*-ribo-hexopyranoside* (**10**).—A solution of **9** (3 g, 13.8 mmol) in EtOH (20 mL) was stirred under H_2 (1 atm) for 6 h in the presence of Et_3N (1 mL), and 10% Pd–C. The catalyst was removed by filtration and the filtrate was evaporated to dryness under diminished pressure. The syrup (2.5 g) obtained was purified by column chromatography on silica gel (4:1 CH_2Cl_2–MeOH) to afford 1.55 g of pure **10** as a syrup; $[\alpha]_D^{20}$ + 152° (*c* 1, MeOH); ν_{max}^{film}: 3500–3000 cm^{-1} (OH and NH_2); ^1H NMR δ 4.63 (dd, $J_{1,2a}$ 4, $J_{1,2e} < 1$ Hz, H-1), 3.70 (m, H-5), 3.59 (m, H-3), 3.41 (s, OCH_3), 3.32 (s, OCH_3), 3.48–3.40 (m, H-4 and CH_2-6), 2.75 (m, H-2*e*), and 1.71 (m, H-2*a*). Anal. Calcd for $C_8H_{17}NO_4$ (191.27): C, 50.23; H, 8.98, N, 7.32. Found: C, 50.32; H, 9.05; N, 7.50.

*Methyl 3-*O*-benzyl-2,6-dideoxy-α-*D*-ribo-hexopyranoside* (**12**) *and methyl 4-*O*-benzyl-2,6-dideoxy-α-*D*-ribo-hexopyranoside* (**13**).—Dibutylin oxide (3.1 g) was added to a solution of methyl 2,6-dideoxy-α-D-*ribo*-hexopyranoside[30] **11** (2 g, 12.3 mmol) in anhyd benzene (250 mL). The mixture was stirred and heated under reflux with azeotropic removal of water, and concentrated to half its volume. To the resulting clear solution were added Bu_4NI[39] (4.6 g, 12.4 mmol) and benzyl bromide (2.2 mL, 18.6 mmol). After 48 h reflux, the mixture was concentrated under diminished pressure and chromatographed on silica gel with 4:1 hexane–EtOAc. Elution afforded successively **12** (2.4 g, 60%) and methyl 4-*O*-benzyl-2,6-dideoxy-α-D-*ribo*-hexopyranoside **13** (1.5 g, 40%).

Compound **12** was a syrup; $[\alpha]_D^{20}$ + 164° (*c* 1.1 $CHCl_3$); ν_{max}^{film}: 3520–3400 cm^{-1} (OH); ^1H NMR δ 4.73 and 4.33 (2d, *J* 11 Hz, CH_2-benzyl), 4.62 (dd, $J_{1,2a}$ 4, $J_{1,2e} < 1$ Hz, H-1), 3.91 (dd, $J_{4,5}$ 10, $J_{5,6}$ 7 Hz, H-5), 3.77 (m, $J_{2a,3} =$, $J_{2e,3} =$, $J_{3,4} = 4$ Hz, H-3), 3.32 (s, OCH_3), 3.23 (ddd, $J_{3,4}$, 4, $J_{4,5} = J_{4\text{-}OH} = 10$ Hz, H-4), 2.49 (d, *J* 10 Hz, D_2O exch., OH), 2.29 (ddd, $J_{2a,2e}$ 14, $J_{2e,1} < 1$, $J_{2e,3}$ 4Hz, H-2*e*), 1.74 (ddd, $J_{2a,2e}$ 14, $J_{2a,1}$ 4, $J_{2a,3}$ 4Hz, H-2*a*), and 1.25 (d, *J* 7 Hz, CH_3-6). Anal. Calcd for $C_{14}H_{20}O_4$ (252.31): C, 66.64; H, 7.99; O, 25.37. Found: C, 66.83; H, 8.11; O, 25.25.

Compound **13** was a syrup; $[\alpha]_D^{20}$ + 169° (*c* 1,15 $CHCl_3$); ν_{max}^{film} 3500 (OH), 1500 cm^{-1} (Ar); ^1H NMR δ 4.69 (dd, $J_{1,2a}$ 4, $J_{1,2e}$ < 1 Hz, H-1), 4.67 and 4.48 (2d, *J* 11 Hz, CH_2-benzyl), 4.15 (bs, OH), 3.99 (dd, $J_{5,4}$ 10, $J_{5,6}$ 7 Hz, H-5), 3.33 (s, OCH_3), 3.26 (m, H-3), 3.05 (dd, $J_{3,4}$ 5, $J_{4,5}$ 10 Hz, H-4), 2.13 (ddd, J_{2a2e} 14, $J_{2e,1}$ < 1, $J_{2e,3}$ 4 Hz, H-2e), 1.82 (ddd, $J_{2a,2e}$ 14, $J_{2a,1} = J_{2a,3} = 4$ Hz, H-2*a*), and 1.29 (d, *J* 7 Hz, CH_3-6). Anal. Calcd for $C_{14}H_{20}O_4$ (252.31): C, 66.64; H, 7.99; O, 25.37. Found: C, 66.90; H, 7.80; O, 25.40.

*Methyl 4-O-acetyl-3-O-benzyl-2,6-dideoxy-α-*D-ribo-*hexopyranoside* (**14**).—Acetic anhydride (1 mL) was added to a solution of **12** (100 mg) in anhyd pyridine (5 mL). After stirring for 4 h at room temperature, the mixture was extracted with ether and usual work-up afforded **14** (85 mg, 80%) as a syrup; $[\alpha]_D^{20}$ + 203° (*c* 1, $CHCl_3$); ν_{max}^{film} 1735 (C=O), 1500 (Ar), 1240, 1051 cm^{-1} (C=O); ^1H NMR δ 4.65 and 4.44 (2d, *J* 11 Hz, CH_2-benzyl), 4.62 (m, H-1 and H-4), 4.27 (dd, $J_{4,5}$ 10, $J_{5,6}$ 7 Hz, H-5), 3.34 (s, OCH_3), 2.17 (ddd, $J_{2a,2e}$ 14, $J_{2e,3}$ 4, $J_{2e,1}$ < 1Hz, H-2e), 2.04 (s, OAc), 1.83 (ddd, $J_{2a,2e}$ 14, $J_{2a,3} = J_{2a,1} = 4$ Hz, H-2*a*), and 1.19 (d, J 7 Hz, CH_3-6). Anal. Calcd for $C_{16}H_{22}O_5$ (294.34): C, 65.28; H, 7.53. Found: C, 65.32; H, 7.47.

*Methyl 3-O-acetyl-4-O-benzyl-2,6-dideoxy-α-*D-ribo-*hexopyranoside* (**15**).—Treatment of monoether **13** as described above afforded **15** (80%) as a syrup; $[\alpha]_D^{20}$ + 242° (*c* 1, $CHCl_3$); ν_{max}^{film} 1730 (C=O), 1500 (Ar), 1245, 1050 (C=O) cm^{-1}; ^1H NMR δ 5.36 (m, H-3), 4.62 (dd, $J_{1,2a}$ 4, $J_{1,2e}$ 1 Hz, H-1), 4.60 and 4.38 (2d, *J* 11 Hz, CH_2-benzyl), 4.09 (qd, $J_{4,5}$ 9, $J_{5,6}$ 7 Hz, H-5), 3.30 (s, OCH_3), 3.15 (dd, $J_{4,5}$ 9, $J_{4,3}$ 4 Hz, H-4), 2.13 (m, H-2e), 2.08 (s, OAc), 1.87 (ddd, $J_{2a,2e}$ 14, $J_{2a,1} = J_{2a,3} = 4$ Hz, H-2*a*), and 1.24 (d, *J* 7 Hz, CH_3-6). Anal. Calcd for $C_{16}H_{22}O_5$ (294.35) C, 65.28; H, 7.53. Found: C, 65.45; H, 7.57.

*Methyl 3-O-benzyl-2,6-dideoxy-4-O-tosyl-α-*D-ribo-*hexopyranoside* (**16**).—*p*-Toluenesulfonyl chloride (1.9 g, 10 mmol) was added to a solution of **12** (1.25 g, 5 mmol) in pyridine (15 mL). After stirring for 12 h at room temperature, extraction with CH_2Cl_2 led to **13** isolated as a syrup (1.6 g, 80%); $[\alpha]_D^{20}$ + 134° (c 1.1 $CHCl_3$); ^1H NMR δ 7.72 (d) and 7.28–7.21 (m, 9H, arom), 4.58 (d) and 4.42 (d) (*J* 12 Hz, CH_2-benzyl), 4.58 (dd, $J_{1,2a}$ 4 $J_{1,2e}$ < 1 Hz, H-1), 4.32–4.21 (m, H-4 and H-5), 3.83 (ddd, $J_{3,4} = J_{2a,3} = J_{2e,3} = 4$ Hz, H-3), 3.31 (s, OCH_3), 2.42 (s, CH_3Ph), 2.14 (m, $J_{2a,2e}$ 14, $J_{2e,1}$ < 1, $J_{2e,3}$ 4 Hz, H-2*e*), 1.76 (ddd, $J_{2a,1}$ 14, $J_{2a,1} = J_{2a,3} = 4$ Hz, H-2*a*), and 1.03 (d, *J* 6.5 Hz, CH_3-6). Anal. Calcd for $C_{21}H_{26}O_6S$ (406.50): C, 62.05; H, 6.45, O, 23.62. Found: C, 62.12; H, 6.38; O, 23.50.

*Methyl 4-azido-3-O-benzyl-2,4,6-trideoxy-α-*D-xylo-*hexopyranoside* (**17**).—A mixture of sodium azide (0.26 g, 4 mmol) and compound **13** (0.4 g, 1 mmol) in DMF (25 mL) was heated at 90°C for 12 h under an Ar atmosphere. Extraction with ether and conventional processing afforded **17** in almost quantitative yield (0.25 g); $[\alpha]_D^{20}$ + 133° (*c* 0.7, $CHCl_3$); ν_{max}^{film} 2.100 cm^{-1} (azide); ^1H NMR δ 7.34–7.24 (m, 1 H, arom), 4.69 (d) and 4.49 (d) (*J* 12 Hz, CH_2-benzyl), 4.68 (dd, $J_{1,2a}$ 4, $J_{1,2e}$ < 1 Hz, H-1), 4.33 (qd, $J_{4,5}$ 2, $J_{5,6}$ 6.5 Hz, H-5), 3.74 (ddd, $J_{2a,3} = J_{2e,3} = J_{3,4} = 4$ Hz, H-3), 3.36 (s, OCH_3), 3.29 (dd, $J_{3,4}$ 4, $J_{4,5}$ 2 Hz, H-4), 2.00–1.94 (m, H-2*a* and

H-2*e*), and 1.25 (d, $J_{5,6}$ 6.5 Hz, CH_3-6). Anal. Calcd for $C_{14}H_{19}N_3O_3$ (277.32): C, 60.63; H, 6.91, N, 15.15. Found: C, 60.58; H, 6.80; N, 15.36.

*Methyl 4-amino-2,4,6-trideoxy-α-*D*-*xylo*-hexopyranoside* (**18**).—A solution of **17** (0.84 g, 3.03 mmol) in a mixture of EtOH (70 mL) and AcOH (10 mL) was stirred for 48 h under H_2 in the presence of 10% Pd–C. The catalyst was removed by filtration and the filtrate concentrated under diminished pressure to ~30 mL. Extraction with CH_2Cl_2 and washing with satd $NaHCO_3$ gave a crude product (850 mg). Chromatography on silica gel afforded 0.30 g of crystalline **18** (66%); mp 78°C; $[\alpha]_D^{20}$ + 118° (*c* 1.6, $CHCl_3$), lit.[32] (enantiomer) $[\alpha]_D^{20}$ −117° (*c* 1, $CHCl_3$); ν_{max}^{film} (Nujol): 3400–3200 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$) (see Ref. 32) (Me_2SO-d_6) δ 4.50 (dd, $J_{1,2a}$ 4, $J_{1,2e}$ 1 Hz, H-1), 4.03 (qd, $J_{5,6}$ 6.5, $J_{5,4}$ 2 Hz, H-5), 3.48 (ddd, $J_{3,4}$ 14, $J_{2a,3} = J_{2e,3} = 4$ Hz, H-3), 3.14 (s, OCH_3), 2.44 (m, H-4), 1.88 (ddd, $J_{2a,2e}$ 14, $J_{2a,3} = J_{2e,3} = 4$ Hz, H-2), 1.39 (m, H-2*a*), and 1.01 (d, *J* 6.5 Hz, CH_3-6). Anal. Calcd for $C_7H_{15}NO_3$ (161.21): C, 52.16; H, 9.38, O, 29.77. Found: C, 52.18; H, 9.25; O, 29.58.

General synthesis of the cyclophosphamide analogs (**21–24**).—To a solution of methyl hexopyranoside (1 mmol) in anhyd CH_2Cl_2 (2 mL) and Et_3N (0.3 mL, 5 mmol) at 0°C was added dropwise a solution of bis-(2-chloroethyl)phosphoramidic dichloride[37] (0.26 g, 1 mmol) in CH_2Cl_2 (2 mL). The mixture was allowed to reach room temperature and stirred for 3 h (except for **19**, 12 h). After evaporation to dryness, the residue was dissolved in hot benzene and filtered. The filtrate was concentrated under diminished pressure. The same operation was repeated three times. Extraction of the residue with CH_2Cl_2 in the usual work-up was followed by chromatography on silica gel.

Compound **21** was eluted with 47:2 CH_2Cl_2–MeOH to give 0.10 g (30%) of the major isomer; mp 130°C; $[\alpha]_D^{20}$ + 48° (*c* 1.1, $CHCl_3$); ^1H NMR δ 4.66 (d, *J* 5 Hz, H-1′), 4.29–4.15 (m, H-3′ and H-5′), 3.67–3.56 (m, 2 CH_2-5), 3.54 (dd, $J_{4',5'}$ 10, $J_{4',3'}$ 4 Hz, H-4′), 3.50–3,28 (m, 2 CH_2-4 and CH_2-6′), 3.45 (s, OCH_3-3′), 3.36 (s, OCH_3-1′), 2.62 (s, D_2O, exch., NH), 2.22 (dd, $J_{2'a,2'e}$ 14, $J_{2'e,3'}$ 3, $J_{2'e,1'} < 1$ Hz, H-2′*e*), 1.78 (m, $J_{2'a,2'e}$ 14, $J_{2'a,\ 3'}$ 4, $J_{2'a,1}$ 5 Hz, H-2′*a*). Anal. Calcd for $C_{12}H_{23}Cl_2N_2O_5P$ (377.25): C, 38.20; H, 6.16; N, 7.43; P, 8.21. Found: C, 38.66; H, 6.43; N, 7.10; P, 8.11.

Compound **22** was eluted with 49:1 CH_2Cl_2–MeOH to give 0.14 g (42%) of the major isomer; mp 145°C; $[\alpha]_D^{20}$ + 77° (*c* 1.2, $CHCl_3$); ^1H NMR δ 4.74. (dd, $J_{1',2'a} = J_{1',2'e} = 6$ Hz, H-1′), 4.29 (m, H-4′ and H-5′), 3.72 (ddd, $J_{3',4'} < 1, J_{3',2'e}$ 4, $J_{3',2'a}$ 2 Hz, H-3′), 3.63 (m, CH_2-5), 3.54-3.27 (m, CH_2-4 and NH), 3.43 (s, OCH_3), 2.51 (ddd, $J_{2'a,2'e}$ 12, $J_{2'a,3'}$ 4, $J_{2'a,1'}$ 6 Hz, H-2′*a*), and 1.28 (d, *J* 7 Hz, CH_3-6′). Anal. Calcd for $C_{11}H_{21}Cl_2N_2O_4P$ (347.21): C, 38.05; H, 6.11; N, 8.07; P, 8.92. Found: C, 38.65; H, 5.97; N, 7.55; P, 8.50.

Compound **23** was eluted with 2:1 hexane–acetone to give successively isomer **23a** (0.17 g, 50%) and isomer **23b** (0.07 g, 21%).

Isomer **23a** had: mp 129°C; $[\alpha]_D^{20}$ + 80° (*c* 1, $CHCl_3$); ^1H NMR δ 4.64. (dd, $J_{1,2'a} = J_{1,2'e} = 4$ Hz, H-1′), 4.11 (qd, $J_{4',5'}$ 10, $J_{5',6'}$ 6 Hz, H-5′), 3.94 (ddd, $J_{4',3'}$ 8,

$J_{4',5'}$ 10, $J_{4',P}$ 14 Hz, H-4′), 3.70–3.60 (m, 2 CH_2-5), 3.45–3.35 (m, 2 CH_2-4), 3.32 (s, OCH_3), 2.07–2.02 (m, H-2′*a* and 2′*e*), and 1.32 (d, *J* 6 Hz, CH_3-6′). Anal. Calcd for $C_{11}H_{21}Cl_2N_2O_4P$ (347.21): C, 38.05; H, 6.11; N, 8.07; P, 8.92. Found: C, 38.28; H, 6.11; N, 8.19; P, 8.80.

Isomer **23b** had: mp 162°C; $[\alpha]_D^{20}$ + 84° (*c* 1.1, $CHCl_3$); ^1H NMR δ 4.62. (dd, $J_{1',2'a} = J_{1',2'e}$ = 6 Hz, H-1′), 4.18 (dd, $J_{4',3} = J_{4',5'}$ = 8, Hz, H-4′), 3.84–3.74 (m, H-3′ and H-5′), 3.64–3.54 (m, 2 CH_2-5), 3.50–3.36 (m, 2 CH_2-4), 3.34 (d, OCH_3), 2.99 (m, D_2O exch., NH), and 2.11 (m, $J_{2'a,2'e}$ 14, $J_{2'a,3'}$ 10, $J_{2'a,1'}$ 6 Hz, H-2′*a*). Anal. Calcd for $C_{11}H_{21}Cl_2N_2O_4P$ (347.21): C, 38.05; H, 6.11; N, 8.07; P, 8.92. Found: C, 38.58; H, 6.33; N, 7.89; P, 8.88.

Compound **24** was eluted with 97:3 CH_2Cl_2–MeOH to give 0.15 g (43%) of the major isomer as a syrup; $[\alpha]_D^{20}$ + 78° (*c* 1.1, $CHCl_3$); ^1H NMR δ 4.73. (d, *J* 4 Hz, H-1′), 3.98–3.87 (m, H-3′ and H-5′), 3.70–3.34 (m, H-4′,2 CH_2-4 and 2 CH_2-5), 3.30 (s, OCH_3), 2.88 (m, D_2O exch., NH), 2.16 (ddd, $J_{2'a,2'e}$ 12, $J_{2'e,3'} = J_{2'e,1'}$ = 4 Hz, H-2′*e*), 1.76 (ddd, $J_{2'a,2'e}$ 14, $J_{2'a,3'}$ 10, $J_{2'a,1}$ 4 Hz, H-2′*a*), and 1.28 (d, *J* 6.5 Hz, CH_3-6′). Anal. Calcd for $C_{11}H_{21}Cl_2N_2O_4P$ (347.21): C, 38.05; H, 6.11; N, 8.07; P, 8.92. Found: C, 38.20; H, 6.15; N, 8.12; P, 8.78.

ACKNOWLEDGMENTS

We thank Dr. H.P. Kraemer and Dr. H.H. Sedlacek, from the Research Laboratories of Behringswerke AG, D-3550 Marburg, Germany for the biological results. Thanks are also due to the "Ministère de l'Industrie et de la Recherche" (France) for a grant (R.G.).

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