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Original article

Synthesis and antitumour activity of a new series of nitrosoureido sugars

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Abstract – New nitrosoureido derivatives of di- or tri-deoxy-sugars have been synthesized. Very potent antitumour activity against L1210 leukaemia was exhibited by the compounds derived from methyl 3-amino-3,4-dideoxy-β- and α- and 4-amino-2,4-dideoxy-β- and α-D-arabino-hexopyranosides, **24**, **26**, **28** and **29**, respectively. In further evaluation against B16 melanocarcinoma bearing mice, only compounds **24** and **26** displayed significant activity. Owing to its lower acute toxicity, methyl 3-[3-(2-chloroethyl)-3-nitrosoureido]-3,4-dideoxy-β-D-arabino-hexopyranoside **24** appeared as the best candidate for preclinical studies. © 2000 Éditions scientifiques et médicales Elsevier SAS

amino-deoxy-sugars / nitrosourea / antitumour

1. Introduction

N-(2-haloethyl)-*N*-nitrosoureas CCNU, MeCCNU and BCNU are representatives of one of the principle classes of anticancer agents, displaying both a wide range of activity in human cancers and being widely used to treat brain tumours, melanomas and various leukaemias [1]. However, as these drugs produce delayed and cumulative bone marrow toxicity, their clinical application was somewhat limited. On the other hand, streptozocin (SZT), a natural *N*-nitroso-*N*-methyl urea of 2-deoxy-D-glucose retains antitumour activity with less bone marrow toxicity than the non-carbohydrate nitrosourea [2]. SZT has been used clinically to treat islet cell carcinoma of the pancreas and carcinoid carcinoma, but its clinical use was limited because of the damage to the pancreas and kidneys, this being the consequence of its diabetogenic activity.

Since the discovery of SZT, many other chloroethyl-nitrosoureido derivatives of amino-sugars have been prepared and evaluated. All these compounds were recently reviewed [3] in terms of their synthetic and mechanistic aspects. Antitumour data and structure–activity relationships were also included in this review. In particular, structural variations of C-1, C-2, C-3, C-5 and C-6 nitrosoureas, as well as structural variations at two monosaccharide positions (bis-*N*-nitrosourea analogues) and disaccharide *N*-nitrosourea analogues were discussed.

For our part, several years ago, we described a series of new nitrosoureido derivatives of di- and trideoxy-sugars [4]. All these compounds were deoxygenated at the C-2 position and derived from 3-amino-2,3-dideoxy, 3-amino-2,3,6-trideoxy, 6-amino-2,6-dideoxy and 3,6-diamino-2,3,6-trideoxy hexopyranoses of α- or β-D or L-configurations. The influence of the hydroxyl substitution pattern, as well as the configuration at the anomeric centre, and of the absolute configuration of the sugar moiety on the antitumour activity of this series were studied. All these compounds displayed significant activity in vivo against L1210, B16 melanocarcinoma, and Lewis lung carcinoma.

Among the compounds which were endowed with a moderate lipophilicity, i.e. those situated between the lipophilic nitrosoureas of the first generation and the hydrophilic sugar derivatives, methyl 3-[3-(2-chloroethyl)-3-nitrosoureido]-2,3-dideoxy-α-D-arabino-hexopyranoside (ecomustine, CY 233, NCS-609224) was found to be very potent against L1210 leukaemia [5], as well as

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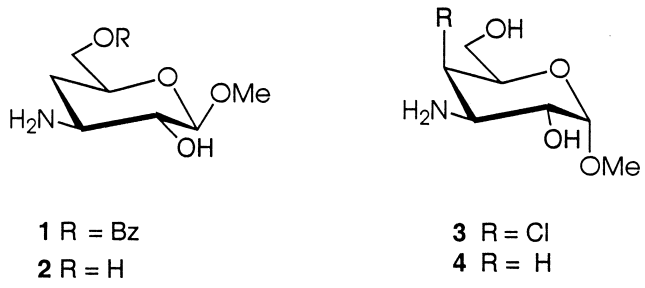
against solid tumours such as the established B16 melanoma and colon 38 adenocarcinoma [6]. It was also highly active via the i.v. route against xenograft human tumours [7]. Moreover it had limited and reversible haematological toxicity, failing to cross the blood-brain barrier or enter the bone marrow [8].

In the present paper we report the synthesis and the preliminary antitumour evaluation of two new and closely related series of nitrosoureido compounds derived either from 3-amino-3,4-dideoxy- or 4-amino-2,4-dideoxy-sugars of the D-series. In addition, a third series, including 4-nitrosoureido sugars dideoxygenated simultaneously at the C-2 and C-3 positions, was also synthesized and evaluated. As for the previous derivatives, their preparation was undertaken with the aim of attaining a better therapeutic index, and a lack of cross-resistance. Furthermore, an increased stability was expected for the same reason mentioned above.



1 R = Bz
2 R = H

3 R = Cl
4 R = H

Figure 1. Preparation of 3-amino-3,4-dideoxysugars **1–4**.

2. Chemistry

2.1. Synthesis of amino-deoxy-sugars

The preparation of the 3-amino-3,4-dideoxysugars **1–4** (*figure 1*) has already been reported [9, 10]. The regioselective synthesis of the 4-amino-2,4-dideoxy-β- and α-D-arabino-hexopyranosides **10** and **12** was achieved from D-glucal (*figure 2*). Oxidative cleavage of D-glucal, after activation of hydroxy groups by *O*-tributyl-stannylation as reported by Veyrières et al. [11], afforded **5** in 80% yield. As expected, regioselective *p*-toluenesulfonylation of **5** in pyridine led to **6a** in high yield (90%) and **6a** was subsequently treated with sodium methoxide in methanol to give 1,6:3,4-dianhydro-2-iodo-2-deoxy-β-D-galactopyranose **7a** in 94% yield. Cleavage of the 3,4-oxirane ring of 2-substituted dianhydrohexopyranoses with a wide range of nucleophiles is known to give the 4-substituted derivatives [12]. Indeed, the 4-azido component **8a** was the only isolable product, in 80% yield, by addition of sodium azide to a solution of **7a** in EtOH in the presence of NH_4Cl. It remained therefore to cleave the 1,6-anhydro bond. This was achieved by addition of Amberlyst® ion-exchange resin to a hot methanolic solution of **8a**. This led mainly to the β-methyl glycoside **9** which was easily separated (84%) and characterized. In contrast, the corresponding α-anomer, also present in small amount (25/75 ratio), could not be isolated as pure compound. Amino-sugar **10** was finally obtained by radical reduction (Bu_3SnH, AlBN, 65%) of **9**.

As we postulated that the presence of the bulky iodine at C-2 may be responsible for the selective formation of the β-anomer **9** during the cleavage of the anhydro-bond, it was expected that removal of this atom prior to this cleavage would lead to the α-anomer. Therefore, hydrogenolysis of compound **6a** was undertaken (H_2, Pd/C, Et_3N, EtOAc, 95%) to prepare the 2-deoxy-derivative **6b**, which was subsequently treated by sodium methoxide in MeOH, leading to **7b** (85% yield). Indeed, the target amino-sugar **12** was successfully obtained in ≈ 38% overall yield in three steps (**7b** → **8b** → **11** → **12**) which included azidolysis (NaN_3, NH_4Cl, EtOH, 90 °C), acidic hydrolysis (Amberlyst® 15 ion-exchange resin, MeOH, 80 °C), and catalytic hydrogenation (Pd/C 10%, Et_3N, EtOAc, 80%). On the other hand, 4-amino-1,6-anhydro-2,4-dideoxy-β-D-arabino-hexopyranose **13** was obtained by catalytic hydrogenation of **8b**.

In order to ascertain for both series of derivatives whether optimum antitumour activity is, as for 3-amino-2-3-dideoxy nitrosoureido derivatives [4], connected or not to the presence of two free OH groups in the sugar moiety, the synthesis of ethyl 4-amino-2,3,4-trideoxy-α-D-threo and α-D-erythro-hexopyranosides **17** and **22** was also investigated. They were prepared from a common intermediate, ethyl 6-*O*-benzyl-2,3-dideoxy-4-*O*-methanesulfonyl-α-D-erythro-hexopyranoside **14** (*figure 3*). Azidolysis of **14** was achieved as previously described [13] and the azido-derivative **15** was converted to **16**. Under more drastic hydrogenation conditions (Pd/C, 10%, Et_3N, EtOAc), **16** was subsequently debenzylated to afford the amino-sugar of threo configuration **17**. The corresponding erythro amino-sugar **22** was obtained from **14** in 5 steps in 51% overall yield. This involved inversion of configuration at C-4 leading to **18** (CsCOOEt, 80%), quantitative transesterification of **18** (NaOMe, MeOH) methanesulfonylation of **19** (MsCl, CH_2Cl_2, Et_3N, 85%), and azidolysis of **20** to give **21**. Finally, catalytic hydrogenation of **21** afforded the target amino-sugar **22** in 60% yield.

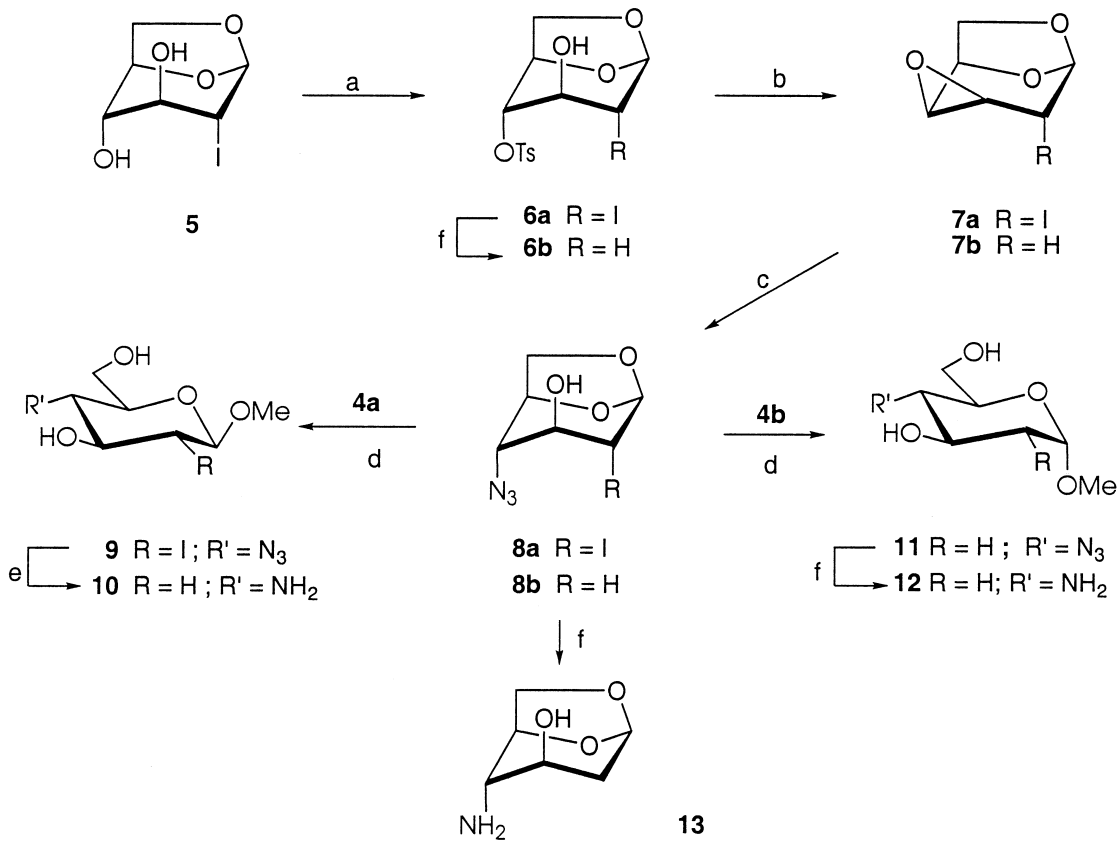


Figure 2. Regioselective synthesis of the 4-amino-2,4-dideoxy-β- and α-D-arabino-hexopyranosides **10** and **12**.

2.2. Synthesis of the 3-(2-chloroethyl)-4-nitrosoureidos **23–26** *and of the 4-(2-chloroethyl)-3-nitrosoureido derivatives* **27–32**

Amino-sugars **1**–**4** and **10**, **12**, **13**, **16**, **17** and **22** were treated with 2,4,5-trichlorophenyl-*N*-(2-chloroethyl)-*N*-nitrosocarbamate [14] to afford, in a one-step reaction, the corresponding nitrosourea derivatives **23–32** (*figure 4*, *table I*).

3. Pharmacology

The acute toxicity of compounds **23**–**32** administered intraperitoneally was determined in mice (DBA2) before the evaluation of the antineoplastic activity of those compounds. The LD_{50} values for all compounds were between 40 and 50 mg/kg (i.p.), except for compounds **25** and **26** which had LD_{50} values of 25–30 mg/kg, close to the LD_{50} values of BCNU (25 mg/kg).

Antineoplastic activity of compounds **23–32** was first evaluated using an oily suspension at 5 mg/kg/day on days 1, 5 and 9, after intraperitoneal inoculation of mice with 10^5 L1210 leukemia cells. Higher activity was observed (*table II*) with compounds **24**, **26**, **28** and **29**. The median survival time of mice treated with these compounds was greatly prolonged (T/C > 600) and 8 of the 10 animals treated survived for more than 60 days with compounds **24**, **28** and **29**.

The most active compounds **24**, **26**, **28** and **29** were next evaluated against B16 melanocarcinoma (*table III*). Compounds were administered intraperitoneally at 10 mg/kg/day at days 1, 5 and 9 after intraperitoneal

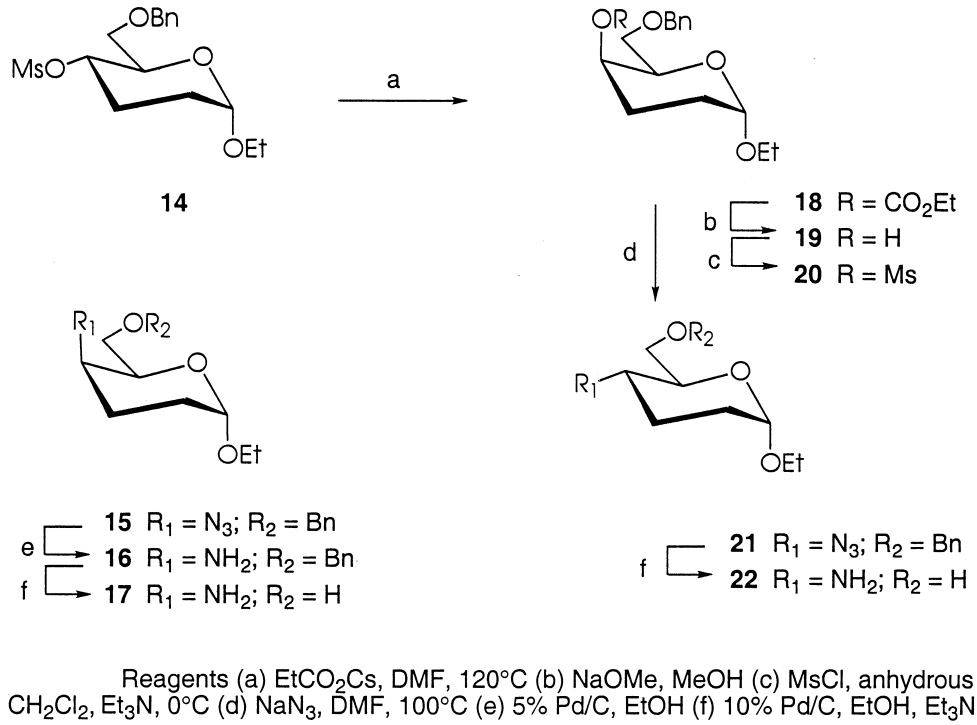


Figure 3. Synthesis of ethyl 4-amino-2,3,4-trideoxy-α-D-threo and α-D-erythro-hexopyranosides **17** and **22.**

inoculation of mice with 2×10^6 B16 melanocarcinoma. Nitrosourea derivatives **28** and **29** did not display any significant antiproliferative activity and none of the 10 animals treated survived. In contrast, compounds **24** and **26** displayed high antitumour activity (T/C 257) as 7–8 of the 10 animals treated survived for more than 60 days with those derivatives.

Further experiments were conducted with the nitrosoureido derivative **24**. Indeed, this compound displays similar in vivo activity against L1210 leukaemia cells and B16 melanocarcinoma to that of the corresponding α anomer **26**, but was less toxic as indicated (*vide supra*) by the LD_{50}. Thus, the effect of the i.v. administration of **24** on s.c. established B16 melanoma was

Table I. Analytical data of nitrosoureido sugars **23–32**.

Compound	M.p. (°C)[a]	$[\alpha]_D^{20b}$	MS (DCI/NH_3)[b] m/z = $(M + NH_4)^+$	Analytical data	M.W.
23	108	+7.5 (*c* 0.3, MeOH)	433	$C_{17}H_{22}ClN_3O_7$	415.83
24	109–111	+11 (*c* 0.75, $CHCl_3$)	329	$C_{10}H_{18}ClN_3O_6$	311.72
25	129–130	+139 (*c* 0.5, MeOH)	364	$C_{10}H_{17}Cl_2N_3O_6$	346.17
26	109–110	+121 (*c* 0.5, MeOH)	329	$C_{10}H_{18}ClN_3O_6$	311.72
27	syrup	–78 (*c* 0.4, $CHCl_3$)	297	$C_9H_{14}ClN_3O_5$	279.5
28	140	–47 (*c* 0.3, $CHCl_3$)	329	$C_{10}H_{18}ClN_3O_6$	311.72
29	145	+49 (*c* 0.5, $CHCl_3$)	329	$C_{10}H_{18}ClN_3O_6$	311.72
30	syrup	+4 (*c* 0.8, $CHCl_3$)	417	$C_{18}H_{26}ClN_3O_5$	399.87
31	syrup	+62 (*c* 0.8, $CHCl_3$)	327	$C_{11}H_{20}ClN_3O_5$	309.75
32	syrup	+81.5 (*c* 0.8, $CHCl_3$)	327	$C_{11}H_{20}ClN_3O_5$	309.75

[a]Solvent: isopropylether. [b]See experimental protocols.

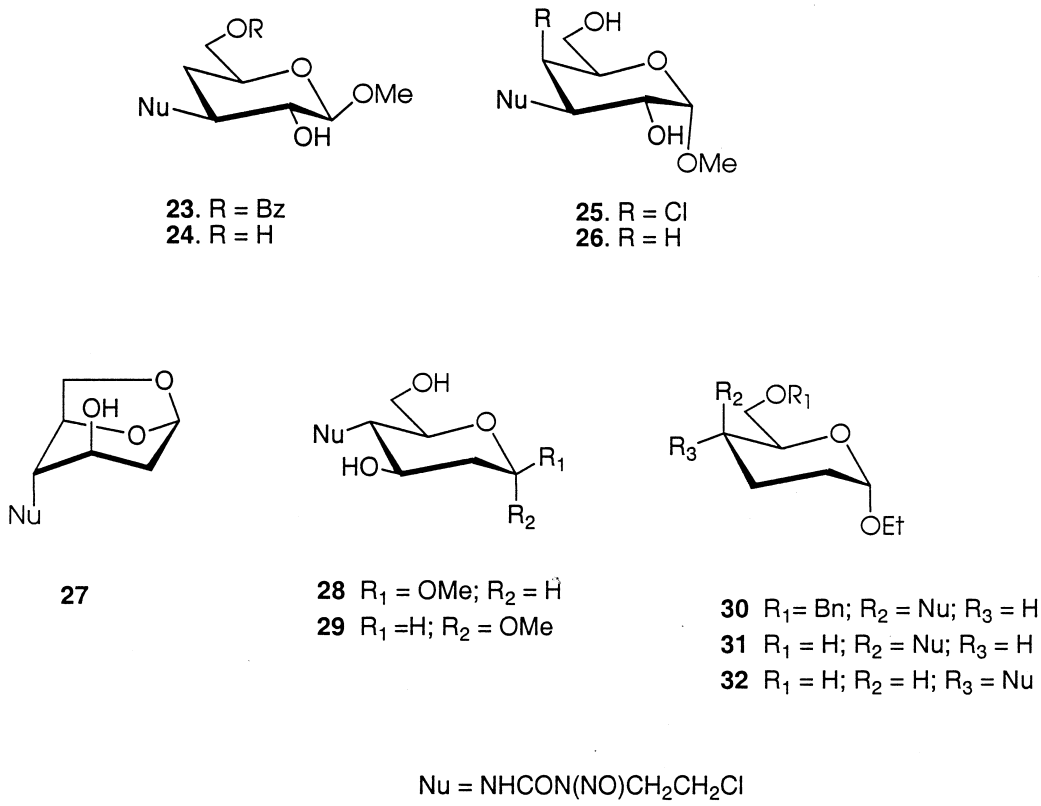


Figure 4. 3-(2-chloroethyl)-4-nitrosoureidos **23–26** and the 4-(2-chloroethyl)-3-nitrosoureido derivatives **27–32**.

investigated. Compound **24** was injected on days 3, 7 and 11 post implantation at daily doses of 10 and 20 mg/kg.

Table II. Antitumour activity of nitrosoureido sugars **23–32** versus L1210 leukaemia in vivo[a].

Compound[b]	T/C (%)[c]	LTS[d] (on day 60/total)
23	180	0
24	> 600	8/10
25	188	0/10
26	> 600	6/10
27	53	toxic
28	> 600	8/10
29	> 600	8/10
30	100	0
31	128	0
32	137	0

[a]Number of control mice: 20. Number of treated mice for each compound: 10. Weight of the mice 20 ± 1 g. [b]Administration of 5 mg/kg i.p. at days 1, 5 and 9. [c]T: median survival time in the treated mice/C: median survival time in the control group × 100. [d]Long time survival.

For comparison, BCNU was injected at 20 mg/kg by the same i.v. route and schedule. After randomization of the mice, the median tumour weight in treated and control groups was almost the same at day 3 (36 mg for **24** versus 40 mg for BCNU and untreated mice). However, as indicated in *table IV*, a large difference was observed at day 30 between treated mice with **24** at 20 mg/kg and

Table III. Antitumour activity of nitrosoureido sugars **24**, **26**, **28** and **29** against B16 melanocarcinoma[a].

Compound[b]	T/C (%)[c]	LTS[d] (on day 60/total)
24	258	8/10
26	257	7/10
28	143	0/10
29	130	0/10

[a]Number of control mice: 20. Number of treated mice for each compound: 10. Weight of the mice 20 ± 1 g. [b]Administration of 10 mg/kg i.p. at days 1, 5 and 9. [c]T: median survival time in the treated mice/C: median survival time in the control group × 100. [d]Long time survival.

Table IV. Antitumour activity of **24** against established B16 melanocarcinoma[a].

Compound[a]	Dose[b] (mg/kg i.v.)	Median tumour weight (mg) on day		T/I %[c] (on day 30)
		3	30	
24	20	36	688	91.2
	10	32	2 025	75.0
BCNU	20	40	2 581	67.0
Controls	0	40	7 812	0

[a]The tumour was implanted s.c. on day 0 in female B6C3F1 mice (weight ≈ 20 g; [b]drugs were given i.v. on days 3, 7 and 11. [c]T/I was calculated as 100 – [(median tumour weight in the treated mice/median tumour weight in the control) × 100].

untreated mice, since the values of the median tumour weight were 688 mg and 7 812, respectively. For comparison, the value observed for animals treated with BCNU was 2 581. For the tumour-growth inhibition, antitumour activity was assessed on the basis of the percentage of tumour inhibition (% TI), calculated from median tumour weight in treated and control mice on the day of evaluation, as indicated in *table IV*. The % TI was calculated as 100 – [(median tumour weight in treated mice/median tumour weight in control) × 100. At 20 mg/kg, the best inhibition was found (TI = 91%) for **24**, versus 67% for BCNU. Even at 10 mg/kg compound **24** exhibited a better activity than BCNU with a TI = 75%.

From these preliminary experiments, it appears that the nitrosoureido sugar derivative **24** is a serious candidate for further development.

4. Experimental protocols

The melting points were measured on a Köfler hot stage apparatus and are uncorrected. Mass spectra were obtained with a Nermag-Ribermag R10-10C spectrometer using either the electron impact method (EI) at 70 eV or applying a desorption chemical ionization technique (CI) using ammonia as the reagent gas. Infrared spectra were registered with a Perkin-Elmer 1710 spectrophotometer, either as chloroform solutions or KBr discs. The ^{1}H-NMR spectra (250 or 300 MHz) were recorded on a Bruker AC 250 or a Bruker AC 300 spectrometer. Chemical shifts are expressed as parts per million downfield from tetramethylsilane. Splitting patterns have been designated as follows: s (singlet), d (doublet), t (triplet), q (quadruplet), dd (doublet of doublet), m (multiplet), bs (broad singlet). Coupling constants (*J* values) are listed in hertz (Hz). Microanalyses were carried out by the 'Service Central d'Analyse du C.N.R.S., Vernaison' and analytical values are within ± 0.3% of the calculated compositions. Reactions were monitored by analytical thin layer chromatography performed on Merck 60F254 precoated plates. Silica gel Merck (230–400 Mesh ASTM) was used for column chromatography (*table V*).

4.1. 1,6-Anhydro-2-deoxy-2-iodo-4-O-(p-toluenesulfonyl)- β-D-glucopyranose **6a**

To a solution of 1,6-anhydro-2-deoxy-2-iodo-β-D-glucopyranose **5** [11] (14.7 g, 54 mmol) in pyridine (150 mL), *p*-toluenesulfonyl chloride (11.59 g, 61 mmol) was added and the mixture was stirred for 48 h at 20 °C. The reaction mixture was then diluted with water (200 mL) and extracted with ethyl acetate (3 × 250 mL). The combined extracts were washed with a 5% aqueous solution of hydrochloric acid (v/v), with water and with a saturated aqueous solution of sodium hydrogencarbonate. The organic fraction was dried over anhydrous magnesium sulfate, filtered and the solvent was removed in vacuo to give a syrup (17.85 g). This was purified by flash chromatography using dichloromethane and a mixture of dichloromethane/methanol (99:1, v/v) as eluent. This afforded a crystalline residue (14.7 g, 62%) of **9a** and a sample was recrystallized from dichloromethane: m.p. 141 °C; $[\alpha]_D^{20}$ + 20° (*c* 1, $CHCl_3$); IR ($CHCl_3$): 3 602 (OH) and 1 600 (Ar) cm^{-1}; Anal. Calcd. for $C_{13}H_{15}O_6SI$: C, 36.63; H, 3.55; I, 29.77; S, 7.52. Found: C, 36.83; H, 3.60; I, 29.38; S, 7.70.

4.2. 1,6:3,4-Di-anhydro-2-deoxy-2-iodo-β-D-galactopyranose **7a**

A 2 M solution of sodium methoxide in methanol (100 mL) was added to a solution of **6a** (19.63 g, 46 mmol) in dry chloroform (800 mL). The mixture was stirred for 2.5 h at 20 °C and diluted with water (100 mL). The organic layer was separated, washed with water (50 mL), dried over anhydrous magnesium sulfate and evaporated in vacuo to give 12.86 g of a crude syrup. Flash chromatography with dichloromethane as eluent gave 11.10 g (94%) of pure **7a:** syrup; $[\alpha]_D^{20}$ – 68° (*c* 1, $CHCl_3$); MS (DCI/NH_3): *m/z* 272 $(M + NH_4)^+$, 255 $(M + H)^+$; Anal. Calcd. for $C_6H_7O_3I$: C, 28.37; H, 2.78; I, 49.96. Found: C, 28.53; H, 2.56; I, 50.02.

4.3. 1,6-Anhydro-4-azido-2,4-dideoxy-2-iodo-β-D-arabino-hexopyranose **8a**

A solution of **7a** (11.1 g, 43.7 mmol) in ethanol (130 mL) was heated at 90 °C for 24 h in the presence of NaN_3 (11.6 g, 178.4 mmol) and NH_4Cl (46.5 g), dissolved in water (26 mL). After cooling to room tempera-

Table V. ^{1}H-NMR data of compounds **6–13** and **17–22** in $CDCl_3$, unless otherwise indicated.

Compound	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	Others
6a	5.73 bs	3.88 bd (3.1)	4.19 bs	4.48 t (2.5, 2.5)	4.65 dd (2.5, 5)	4.18 dd (8.2)	3.72 dd (8.5, 5.5.)	2.46 (CH_3), 7.37 and 7.86 (2d, CH_2Ph)
7a	5.72 bs	4.17 t (1.5, 1.5)	3.70 dd (4.1)	3.76 t (4.5, 4.5)	4.96 t (4.5, 4.5)	4.17 d (6)	3.61 dd (6.5)	
8a	5.76 bs	3.95 bd (2.5, < 1)	4.21 bs	3.60 ≈ t (2.2)	4.62 dd (5.2)	4.23 d (6.5)	3.81 dd (7.5, 5)	2.74 (OH)
6b	5.68 bs	1.87 bd (15, < 1) 2.19 dd (15.5)	3.99 m	3.54 s	4.70 d (5)	4.36 d (6.5)	3.85 dd (7.5, 5)	
7b	5.46 d (3.5)	1.96 m 2.10 d (15)	3.19 m	3.62 dd (5, 4.5)	4.87 t (5.5)	4.08 d (6.5)	3.57 dd (6.5, 5)	
8b	5.73 s	1.98 bd (15, < 1) 2.27 m	3.99 m	3.54 s	4.70 d (5)	4.36 d (6.5)	3.85 dd (65.5)	
9	4.47 d (8, 5)	← 3.85–3.82 → m		3.54 t (9)	3.60 m (9, 4, 2)	3.94 m (12, 5.5, 2)	3.85 m	3.54 s (OCH_3)
10*	4.31 dd (10, 2)	1.93 (m, 12, 5, 2) 1.24 (m, 12, 10, 10)	3.23 (m)	2.23 t (9.9)	2.95 m (9.5, 5, 3)	3.63 dd (12, 5)	3.51 dd (12, 3)	3.30 s (OCH_3)
11	4.82 d (3)	2.15 m (13, 5, 1) 1.70 m (13, 10, 3)	4.01 m (11, 9, 5)	3.36 t	3.50 m	3.86 dd (12, 2.5)	3.79 dd (12, 4)	3.30 s (OCH_3)
12*	4.70 d (3)	1.86 m (12, 5, 1) 1.43 m (12, 10, 10)	3.32 m	2.32 t (9.5, 9.5)	3.22 m	3.60 dd (12, 3)	3.48 dd (12, 5)	3.34 s (OCH_3)
13*	5.40 s	1.93 m (14, 8, 2) 1.53 d (14)	3.52 bs	2.64 bs	4.27 d (5.5)	4.20 d (5.5)	3.52 s	
17*	4.74 bs (1)	← 1.80–1.65 → m			← 3.72–3.45 → m			1.15 t (7, CH_3) 3.25 q (CH_2)
18	4.99 bs (1)	← 2.09–1.78 → m		5.00 s	4.12 m	3.79–3.71 m	3.54–3.44 m	4.52 dd (9, 4, CH_2Ph) 2.30 and 2.28 2q (CH_2) 1.13 and 1.22 2t (7, CH_3)
19	4.90 d (3)	← 2.12–1.19 → m		← 3.93–3.78 → m		378–3.68 m	3.54–3.49 m	1.25 t (7, CH_3) 3.48 q (CH_2) 4.60 dd (9, 4, CH_2Ph)
20	4.92 d (3)	← 2.25–1.25 → m		3.70 m	← 3.85–3.75 → m			1.28 t (7, CH_3) 3.45 q (CH_2) 4.55 dd (9, 4, CH_2Ph)
21	4.86 dd (2, 1)	← 1.95–1.83 → m		m	← 3.73–3.64 → m			1.25 t (7, CH_3) 3.48 q (CH_2) 4.60 dd (9, 4, CH_2Ph)
22*	4.72 bs (1)	← 1.63–1.57 → m		m	← 3.68–3.33 → m			1.14 t (7, CH_3) 3.20 q (CH_2)

* in DMSO.

ture, the reaction mixture was filtered and the filtrate was diluted with dichloromethane (500 mL). The organic layer was separated, washed with water (2 × 25 mL) and dried over anhydrous magnesium sulfate. Evaporation in vacuo gave a crude product which was purified by flash chromatography with pentane/acetone (5:1, v/v). This afforded **8a** (10.47 g, 80%) as a crystalline compound: m.p. 67 °C; $[\alpha]_D^{20}$ + 24.5° (*c* 0.8, $CHCl_3$); IR ($CHCl_3$): 2 107 cm^{-1} (N_3); MS (DCI/NH_3): *m/z* 315 $(M + NH_4)^+$. Anal. Calcd. for $C_6H_8N_3O_3I$: C, 24.26; H, 2.71; N, 14.15. Found: C, 24.53; H, 2.56; N, 14.66.

4.4. Methyl 4-azido-2,4-dideoxy-2-iodo-β-D-arabino-hexopyranoside **9**

A methanolic solution of **8a** (5.1g in 500 mL) was heated under reflux for 48 h in the presence of Amberlite R15 $(H)^+$. The solution was then filtered and the filtrate evaporated under reduced pressure. The residue (7.1 g) was chromatographed using pentane/acetone (5/1, v/v) as eluent. This afforded successively unreacted starting material **8a** (0.69 g, 13%) and **9** (4.79 g, 84%) as a crystalline residue: m.p. 118 °C; $[\alpha]_D^{20}$ + 187° (*c* 1.06,

$CHCl_3$); IR ($CHCl_3$) 3 600 (OH), 2 116 (N_3) cm^{-1}; MS (DCI/NH_3): *m/z* 347 $(M + NH_4)^+$. Anal. Calcd. for $C_7H_{12}N_3O_4I$: C, 25.55; H, 3.68; N, 12.77. Found: C, 25.96; H, 3.77; N, 12.45.

4.5. Methyl 4-amino-2,4-dideoxy-β-D-arabino-hexopyranoside **10**

To a solution of **9** (1.2 g, 3.8 mmol) in toluene (60 mL) kept under argon atmosphere, tributyltin hydride (4 mL, 14.9 mmol) and AIBN (0.3 g) were added. The reaction mixture was stirred under reflux for 3 h, by which time the reaction has gone to completion. The toluene was subsequently removed under vacuum and flash chromatography of the residue (1.18 g) with dichloromethane and dichloromethane/saturated NH_3 methanol (98:2, v/v) afforded **10** (441 mg, 65%) as crystals: m.p. 96 °C; $[\alpha]_D^{20}$ – 65° (*c* 1, MeOH); IR ($CHCl_3$): 3 336 cm^{-1} (OH, NH_2); MS (DCI/NH_3): *m/z* 178 $(M + H)^+$. Anal. Calcd. for $C_7H_{15}NO_4$: C, 47.45; H, 8.53; N,: 7.90. Found: C: 47.18; H, 8.31; N, 7.82.

4.6. 1,6-Anhydro-2,4-dideoxy-4-O-(p-toluenesulfonyl)-β-D-glucopyranose **6b**

A solution of **6a** (10.24 g, 24 mmol) in ethyl acetate (250 mL) was stirred under hydrogen atmosphere (1 atm.) for 18 h in the presence of 10% palladium-on-charcoal (2 g) and Et_3N (5 mL). The catalyst was removed by filtration and the filtrate was evaporated under reduced pressure to afford 6.87 g (95%) of **6b** as a crystalline residue pure enough for the next step. A sample was recrystallized from ethyl acetate for analysis: m.p. 101 °C; $[\alpha]_D^{20}$– 69° (*c* 0.8, $CHCl_3$); IR 3 558 cm^{-1} (OH); MS (DCI/NH_3): *m/z* 318 $(M + NH_4)^+$. Anal. Calcd. for $C_{13}H_{16}O_6S$: C, 51.99; H, 5.37; S, 10.68. Found: C, 51.90; H, 5.50; S, 10.69.

4.7. 1,6:3,4-Dianhydro-2-deoxy-β-D-galactopyranose **7b**

Following the procedure for the preparation of **7a**, the title compound was prepared from the tosyl derivative **6b** and isolated as a syrup (0.38 g, 85%): $[\alpha]_D^{20}$ – 108° (*c* 1, $CHCl_3$); MS (DCI/NH_3): *m/z* 146 $(M + NH_4)^+$. Anal. Calcd. for $C_6H_8O_3$: C, 56.25; H, 6.29. Found: C, 56.29; H, 6.20.

4.8. 1,6-Anhydro-4-azido-2,4-dideoxy-β-D-arabino-hexopyranoside **8b**

Following the procedure for the preparation of **8a**, the title compound was prepared by treatment of the anhydro derivative **7b** (0.35 g, 2.7 mmol) in solution, in methanol (8 mL), by sodium azide (0.72 g, 11 mmol) and NH_4Cl (2.88 g, 53.8 mmol) dissolved in water (1.6 mL). After refluxing for 24 h, the reaction mixture was concentrated in vacuo and the residue was taken up in dichloromethane. Usual work-up of the organic solution followed by chromatography on silica gel with a mixture of cyclohexane/EtOAc as eluent (2:1, v/v) afforded successively the 3-azido (0.118 g, 25%) and the 4-azido **8b** (0.276 g, 55%) sugar derivatives. Compound **8b**: m.p. 85 °C; $[\alpha]_D^{20}$ – 154° (*c* 1, $CHCl_3$); IR ($CHCl_3$): 3 563 (OH), 2 104 (N_3) cm^{-1}; MS (DCI/NH_3): *m/z* 189 $(M + NH_4)^+$; 171 $(M + NH_4 - H_2O)^+$. Anal. Calcd. for $C_6H_9N_3O_3$: C, 42.11; H, 5.30; N, 24.55. Found: C, 42.37; H, 5.10; N, 24.50.

4.9. Methyl 4-azido-2,4-dideoxy-α-D-arabino-hexopyranoside **11**

The title compound was prepared from **8b** following the procedure already described for **9**. Purification was carried out by flash chromatography with a mixture of cyclohexane/EtOAc (1:1, v/v) as eluent, giving 86% of pure **11** as a crystalline residue: m.p. 85 °C; $[\alpha]_D^{20}$ + 142° (*c* 0.8, $CHCl_3$); IR ($CHCl_3$) 3 600 (OH) 2 109 cm^{-1} (N_3); MS (DCI/NH_3): *m/z* 221 $(M + NH_4)^+$, 204 $(M + H)^+$. Anal. Calcd. for $C_7H_{13}N_3O_4$: C, 41.38; H, 6.45; N, 20.68. Found C, 41.48; H, 6.61; N, 20.74.

4.10. Methyl 4-amino-2,4-dideoxy-α-D-arabino-hexopyranoside **12**

A solution of **11** (0.9 g, 4.43 mmol) in ethyl acetate (50 mL) was stirred for 24 h under H_2 atmosphere (1 atm.) in the presence of 10% Pd-on-charcoal (0.5g) and Et_3N (0.9 mL). The catalyst was then removed by filtration and the filtrate concentrated in vacuo. This afforded the amino-sugar **12** (0.62g, 80%) as amorphous crystals: $[\alpha]_D^{20}$ + 101° (*c* 0.8, MeOH); IR ($CHCl_3$) 3 347 (OH) cm^{-1}; MS (DCI/NH_3): *m/z* 178 $(M + H)^+$. Anal. Calcd. for $C_7H_{15}NO_4$: C, 47.45; H, 8.53; N, 7.90. Found: C, 46.88; H, 8.44; N, 7.72.

4.11. 4-Amino-1,6-anhydro-2,4-dideoxy-β-D-arabino-hexopyranoside **13**

Following the procedure already described for the preparation of **12**, reduction of **8b** readily afforded **13** in 81% yield: crystal; m.p. 139 °C; $[\alpha]_D^{20}$ – 106° (*c* 0.8, MeOH); IR ($CHCl_3$): 3 360 cm^{-1} (OH); MS (DCI/NH_3): *m/z* 146 $(M + H)^+$. Anal. Calcd. for $C_6H_8O_3$: C, 49.65; H, 6.29; N, 9.65. Found: C, 49.29; H, 6.64; N, 9.45.

4.12. Ethyl 4-amino-6-O-benzyl-2,3,4-trideoxy-α-D-threo-hexopyranoside **16**

To an ethanolic solution of **15** (1.62g, 5.6 mmol in 50 mL) were added Et_3N (900 mL) and 5% Pd-on-charcoal (140 mg). The suspension was stirred for 6 h under hydrogen atmosphere (1 atm.), filtered and the filtrate was concentrated in vacuo to give **16** (1.5g, 95%), pure enough for the next step: syrup; MS (DCI/NH_3): *m/z* 266 $(M + H)^+$.

4.13. Ethyl 4-amino-2,3,4-trideoxy-α-D-threo-hexopyranoside **17**

Following the previous procedure used for **16** but with 10% Pd-on-charcoal, compound **16** was converted into **17**: syrup; MS (DCI/NH_3): *m/z* 176 $(M + H)^+$. Anal. Calcd. for $C_8H_{17}NO_3$: C, 54.84; H, 9.78; N, 7.99. Found: C, 55.01; H, 9.82; N, 7.62.

4.14. Ethyl 6-O-benzyl-2,3-dideoxy-4-O-propionyl-α-D-threo-hexopyranoside **18**

Caesium propionate (6.55 g) was added to a solution of ethyl 6-O-benzyl-2,3-dideoxy-4-O-methanesulfonyl-α-D-erythro-hexopyranoside [13] **14** (5.8 g, 16.86 mmol) in DMF (80mL) and the reaction mixture was heated with stirring for 30 h at 120 °C. After cooling to room temperature and addition of water (80 mL), the mixture was extracted with ether (1 L, then 0.5 L). The combined organic layers were washed several times with water, dried over $MgSO_4$ and concentrated in vacuo. The crude residue (4.85 g) was purified by flash chromatography using cyclohexane, then a mixture of cyclohexane/EtOAc (90:10, v/v) as eluent. This led to isolation of 4.33 g (80%) of **18**. A sample was recrystallized from cyclohexane; m.p. ≈ 50 °C: $[\alpha]_D^{20}$ + 30° (*c* 0.8, $CHCl_3$); IR ($CHCl_3$): 1 731 cm^{-1} (CO); MS (DCI/NH_3): *m/z* 340 $(M + NH_4)^+$, 321 $(M + H)^+$, 294 $(M + NH_4 - 46)^+$, 277 $(M + H - 46)^+$. Anal. Calcd. for $C_{18}H_{24}O_5$: C, 67.48; H, 7.55. Found: C, 67.83; H, 7.38.

4.15. Ethyl 6-O-benzyl-2,3-dideoxy-α-D-threo-hexopyranoside **19**

To a solution of **18** (2 g, 6.2 mmol) in methanol (40 mL), 1 M sodium methoxide in methanol (4 mL) was added. After stirring for 5 h at room temperature and neutralization by IRC 50S ion-exchange resin, the solution was concentrated in vacuo. Flash chromatography using a mixture of cyclohexane/EtOAc (90:10, v/v) as eluent afforded **19** (1.71 g, ≈ 100%) as a syrup: $[\alpha]_D^{20}$ + 82° (*c* 1, $CHCl_3$); IR ($CHCl_3$): 3 497 cm^{-1} (OH); MS (DCI/NH_3): *m/z* 284 $(M + NH_4)^+$, 267 $(M + H)^+$. Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.65; H, 8.33. Found: C, 67.84; H, 8.22.

4.16. Ethyl 6-O-benzyl-2,3-dideoxy-4-O-methanesulfonyl-α-D-threo-hexopyranoside **20**

A solution of **19** (1.55 g, 5.82 mmol) in anhydrous dichloromethane (60 mL) containing triethylamine (1.23 mL) was cooled to 0 °C and then methanesulfonyl chloride (0.65 mL, 8.43 mmol) was added. The solution was stirred at 0 °C for 5 h and then poured into 30 mL of ice-water. Extraction with dichloromethane (2 × 150 mL) and usual work-up afforded 2.49 g of residue. This was purified by flash chromatography with cyclohexane-EtOAc (4:1, v/v), giving 2.14 g (85%) of pure **20** as a syrup: $[\alpha]_D^{20}$ + 31° (*c* 1, $CHCl_3$); MS (DCI/NH_3): *m/z* 362 $(M + NH_4)^+$. Anal. Calcd. for $C_{16}H_{24}O_6S$: C, 67.65; H, 8.33. Found: C, 67.84; H, 8.22.

4.17. Ethyl 4-azido-6-O-benzyl-2,3,4-trideoxy-α-D-erythro-hexopyranoside **21**

Sodium azide (0.46 g, 7.07 mmol) was added to a solution of **20** (1.6 g, 4.65 mmol) in DMF (25 mL) and the mixture was stirred at 100 °C for 24 h. After cooling to room temperature, extraction was carried out with ether (85 mL) after addition of H_2O (15 mL). Usual work-up afforded a crude residue (1.24 g, 91%), pure enough for the next step; $[\alpha]_D^{20}$ + 134.5° (*c* 1, $CHCl_3$); IR ($CHCl_3$): 2 103 cm^{-1} (N_3); MS (DCI/NH_3): *m/z* 309 $(M + NH_4)^+$. Anal. Calcd. for $C_{15}H_{21}N_3O_3$: C, 61.84; H, 7.27; N, 14.42. Found: C, 61.92; H, 7.42; N, 14.12.

4.18. Ethyl 4-amino-2,3,4-trideoxy-α-D-erythro-hexopyranoside **22**

Following the procedure already described for **12** azido-sugar **21** was reduced in ethanol solution to afford compound **22** which was isolated as a pure compound, after flash chromatography with dichloromethane-NH_3-saturated methanol (95:5, v/v), in 60% yield as a syrup: $[\alpha]_D^{20}$ + 157° (*c* 0.8, MeOH); MS (DCI/NH_3): *m/z* 176 $(M + H)^+$, 130 $(M + H - C_2H_5OH)^+$. Anal. Calcd. for $C_8H_{17}NO_3$. C, 54.84; H, 9.78; N, 7.99. Found: C, 54.76; H, 9.77; N, 8.04.

4.19. Typical procedure for the synthesis of nitrosoureido derivatives **23**–**32**

To a cooled solution (≈ 0 °C) of the amino-sugar (1 mmol) in anhydrous DMF (5 mL), N-(2-chloroethyl)-N-nitrosocarbamate of p-nitrophenyl [14] (1.2 mmol)

was added and the mixture was stirred at the same temperature for 3–5 h. The DMF was removed under reduced pressure and the residue was dissolved in EtOAc (100 mL). The organic solvent was washed several times with water, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by flash chromatography (cyclohexane-EtOAc) and the pure product crystallized from isopropyl ether.

5. Antineoplastic evaluation

5.1. In vivo tests

The experimental procedures, the type of mice used (B6D2F1 or C57BL/6), and the calculation of the median survival times of the groups of control and treated animals were in accordance with the protocols of the National Cancer Institute (Instr. 271F, November 1983).

*5.1.1. Test against L1210 leukaemia in oily suspension (*table II*)*

On day 0, mice (B6D2F1) were inoculated intraperitoneally with 10^5 L1210 leukaemia cells. On days 1, 5 and 9, the mice (10 for each group treated) received intraperitoneally the dose of 5 mg/kg of compounds **23–32** in 0.2 mL of neutralized and sterilized olive oil suspension. The control group (20 mice) received the same volume of solvent only. The mortality of the mice was monitored daily and autopsies were performed to find out whether deaths were due to leukaemia or to a toxic action of the drug. The observation period lasted at least 60 days.

*5.1.2. Test against B16 melanocarcinoma in oily suspension (*table III*)*

On day 0, mice (C57BL/6) were inoculated intraperitoneally with 2×10^6 B16 melanocarcinoma cells. The treatment protocol and the observation period are similar to the above procedure, except the dose which was 10 mg/kg/day in 0.5 mL. Ten mice for each treated group and 20 mice for control groups were used.

*5.1.3. Activity against established B16 melanocarcinoma (*table IV*)*

B16 melanoma was maintained in syngenetic adult C57BL/6 mice. For evaluation, the B16 tumour was subcutaneously (s.c.) implanted on day 0 in B6CFF1 male mice. After 1 g of tumour was mixed with 9 mL of cold balanced salt, the preparation was homogenized and each mouse was inoculated with 0.5 mL tumour homogenate. Compounds **24** and BCNU were given i.v. on days 3, 7 and 11 at the doses indicated. BCNU was kindly donated by the Drug Research and Development (DRD), Division of Cancer Treatment (DCI, NCI, Bethesda, Md, USA).

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