reported.[10] ^{125}I-labeled gastrin binding in guinea pig gastric glands was determined according to literature procedures.[10,18] Details of these experimental procedures are described in a forthcoming paper.[13]

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Mark G. Bock,* Robert M. DiPardo, Ben E. Evans
Kenneth E. Rittle, Willie L. Whitter, Daniel F. Veber
Paul S. Anderson, Roger M. Freidinger
Merck Sharp & Dohme Research Laboratories
West Point, Pennsylvania 19486
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Articles

Rationale for the Synthesis and Preliminary Biological Evaluation of Highly Active New Antitumor Nitrosoureido Sugars

Pierre Roger,*,† Claude Monneret,‡ Jean-Paul Fournier,§ Patrick Choay,† Roselyne Gagnet,‡ Charles Gosse,‖ Yves Letourneux,⊥ Ghanem Atassi,# and Alain Gouyette‖

Département de Chimie Thérapeutique, Institut Choay, 10 rue Morel, 92120 Montrouge, France, Département de Pharmacognosie associé au CNRS 484, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France, Département de Chimie Thérapeutique, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France, Institut Gustave-Roussy, 39, rue Camille-Desmoulins, 94805 Villejuif Cedex, France, Laboratoire de Chimie Organique, UER des Sciences Pharmaceutiques, 1 rue Gaston-Veil, 44035 Nantes Cedex, France, and Institut Jules-Bordet, 1 rue Hêger-Bordet, 1000 Bruxelles, Belgique.
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Various new nitrosoureido derivatives of di- or trideoxy sugars were synthesized. The influence of the hydroxyl substitution pattern, the configuration at the anomeric center, and the absolute configuration of the sugar moiety on the antitumor activity of a series of nitrosoureido derivatives of di- and trideoxy sugars was studied. All compounds showed a very significant activity in vivo against L1210 leukemia, B16 melanocarcinoma, and Lewis lung carcinoma. Methyl 3-[3-(2-chloroethyl)-3-nitrosoureido]-2,3-dideoxy-α-D-*arabino*-hexopyranoside, **24** (NSC 609224), was found to be the most active compound. When treated with **24** (NSC 609224) at 20 mg/kg on day 1, at least 90% of the L1210 leukemia and B16 melanocarcinoma bearing mice showed a survival of over 60 days for a LD50 value for this compound of 42 mg/kg.

The (chloroethyl)nitrosoureas CCNU, MeCCNU, and BCNU represent an important class of antitumor agents which have a broad spectrum of activity in human cancers mainly against lymphomas, melanomas, gliomas, and a few solid tumors.[1,2] However, these drugs produce delayed and cumulative bone marrow toxicity which seriously limit their clinical application.[3,4] Since streptozocin (SZ),[5,6] the *N*-nitroso-*N*-methylurea of 2-deoxy-D-glucose, has antitumor activity with less bone marrow toxicity[7] than the noncarbohydrate nitrosoureas but suffers from diabetogenic activity,[8,9] it became of interest to prepare nitrosoureido derivatives of amino sugars. Among the numerous analogues of SZ which have been synthesized,[10,11] replacement of the methyl group with a 2-chloroethyl group, as present in nitrosoureas of the first generation, had markedly enhanced effectiveness against L1210 leukemia.[12] Furthermore diabetogenic activity in animals as reported for SZ and hepatic dysfunction were not observed.

Since then, many other nitrosoureido derivatives of amino sugars have been prepared as analogues of streptozocin including the water-soluble GANU,[13] MCNU,[14] and KCNU,[15] respectively C-1, C-6, and C-3 substituted glu-

* Proof and reprint requests to: Pierre Roger, Institut Choay, 10, rue Morel, 92120 Montrouge, France.
† Institut Choay.
‡ Département de Pharmacognosie associé au CNRS.
§ Département de Chimie Thérapeutique.
‖ Institut Gustave-Roussy.
⊥ U.E.R. des Sciences Pharmaceutiques.
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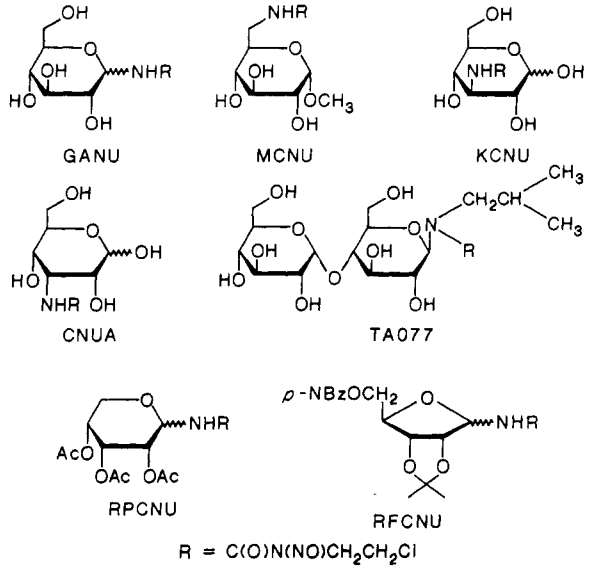
cose, CNUA,[16] a C-3 substituted mannose, and TA-077 C-1 substituted maltose derivatives.[17] Some of them (GANU, MCNU, and TA077) are under clinical trials in Japan today.[18] Water-insoluble (chloroethyl)nitrosoureido sugars have also been synthesized which include fully protected pentose derivatives such as RPCNU and RFCNU.[19-21] In the case of RFCNU, hydrolysis of the protecting groups occurs very quickly to give the unsubstituted ribosyl-(chloroethyl)nitrosourea which is much less stable.[22]



Whatever the addition of a sugar carrier to the cytotoxic nitrosoureido moiety, it reduces bone marrow toxicity without significantly altering its antitumor activity.[23]

Lipophilicity, alkylating activity, and carbamoylating activity have been considered to be possible determinants of (chloroethyl)nitrosourea antitumor effects and toxicity. For example, results obtained by Hansch[24] on the structure–activity relationships of a series of nitrosoureas suggested that activity and toxicity may be separated on the basis of lipid solubility and concluded that more potent and less toxic drugs should be produced by making less lipophilic compounds.

Although carbamoylation through isocyanate intermediates has been postulated to exert no influence on the cytotoxicity,[25] isocyanates can react in vivo with enzymes that effect DNA repair and interfere with this important cellular recovery mechanism.[26] On the basis of these findings and of previous evidence, further clinical trials of appropriate noncarbamoylating (chloroethyl)nitrosourea seemed to be justified.

Thus different approaches could be considered as fruitful, and for example Japanese authors have recently synthesized 1-(2-chloroethyl)-3,3-disubstituted-1-nitrosoureas in order to prevent the carbamoylation mechanism. After release of the chloroethyl diazodihydroxide moiety, such compounds should give an intramolecular carbamate.[27]

On our part, the synthesis of nitrosoureido derivatives of di- and trideoxy sugars seemed to be very promising since such compounds should act as intermediates between water-soluble and water-insoluble (chloroethyl)nitrosoureas. Although slightly more lipophilic than water-soluble drugs, for example, in contrast with RFCNU, the lack of labile protective groups must give much more stable compounds. Thus, with the triple aim to prepare new drugs which, compared to known nitrosoureas, could possess a better therapeutic index, a lack of cross-resistance with a broader spectrum of activity, and an increased stability around physiological pH, the (chloroethyl)nitrosoureido derivatives of methyl glycosides of 3-amino-2,3-dideoxy-, 3-amino-2,3,6-trideoxy-, 6-amino-2,6-dideoxy-, and 3,6-diamino-2,3,6-trideoxyhexopyranoses of α-D-*arabino* configuration were synthesized.[28] In order to determine the influence of the configuration at the anomeric center and the influence of absolute configuration, nitrosoureido derivatives of the methyl glycosides of 3-amino-2,3-dideoxy-3-amino-2,3,6-trideoxy-β-D-*arabino* and of 3-amino-2,3,6-trideoxy-α- and -β-L-*arabino*-hexopyranosides were also prepared. All these compounds represent a new series of nitrosoureido sugars.

Chemistry

Synthesis of Amino Deoxy Sugars. Methyl 3-azido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-*arabino*-hexopyranoside (**1**)[29] has been used as a common precursor of methyl glycosides of 3-amino-2,3,6-trideoxy-D-*arabino*-hexopyranose (D-acosamine) (**5**), 3-amino-2,3,6-trideoxy-4-*O*-methyl-D-*arabino*-hexopyranoside (D-actinosamine) (**6**), 6-hydroxy-D-acosamine (**8**), and of the diamino derivative **11**.

Thus the benzylidene ring opening of **1** with *N*-bromosuccinimide leads, as previously reported,[30-32] to the methyl 3-azido-4-*O*-benzoyl-6-bromo-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (**2**) in 75% yield. Debenzoylation of **2** with 2 N NaOH in ethanol gives in nearly quantitative yield **3**, which was then reduced with lithium aluminum hydride in THF at reflux. Methyl 3-amino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (**5**) was isolated in 73% yield as a crystalline compound.[33] The corresponding 4-*O*-methyl ether **6** was obtained when prior to catalytic hydrogenation (EtOH, Pd–C (10%), Et_3N (5%)) compound **3** was alkylated (Me_2SO_4, NaOH) to provide intermediate **4** in quantitative yield.

On the other hand, acid hydrolysis of **1** (acetyl chloride in MeOH) to remove the benzylidene acetal led quantitatively to **7**, which was hydrogenated (MeOH, Pd–C

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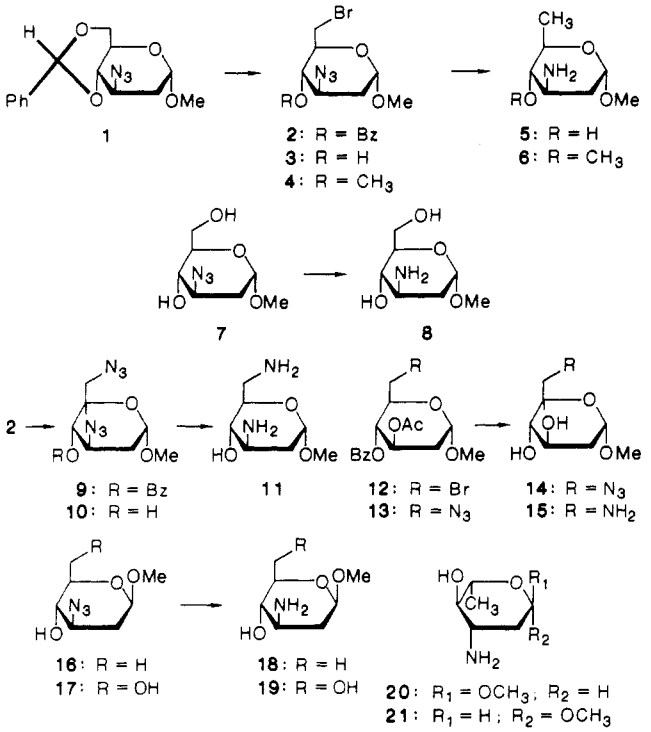
Table I. Nitrosoureido Sugars in Suspension in Olive Oil versus L1210 Leukemia in Vivo[a]

compd	dose, mg/kg ip (days 1, 5, 9)	mortality (D1–D9)	median survival time, days[d] control mice (range)[b]	median survival time, days[d] nonsurviving treated mice (range)[b]	T/C %[c,d]	treated mice 30-day survivors	treated mice 60-day survivors
22	20	0/15	8.93 (8–10)			15/15	15/15
26	20	0/15	8.93 (8–10)	23.5 (21–42)	263	4/15	3/15
24	20	0/15	8.93 (8–10)	57.5 (33–57)	644	15/15	7/15
25	20	0/15	8.93 (8–10)	*e*		12/15	10/15
23	20	0/15	8.93 (8–10)			15/15	15/15

[a] Number of control mice: 30. Number of treated mice for each drug: 15. Weight of the mice: 20 ± 1 g. [b] Range of individual animal deaths. [c] For each compound, the oncostatic effects of specified doses were expressed as (T median survival time (MST) in the treated group of mice/C median survival time (MST) in the control group) × 100. [d] Median survival time and T/C % were not calculated when all treated animals or more than 50% of treated animals were alive on day 60. In that last case, if death occurred (before D60), the days were indicated. [e] Two deaths on day 27, one death on day 29, two deaths on day 41.

Scheme I



(10%), Et_3N (5%)) to afford in 98% yield methyl 3-amino-2,3-dideoxy-α-D-*arabino*-hexopyranoside or 6-hydroxyacosaminide (**8**).

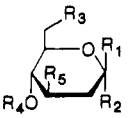
Azidolysis of **2** with sodium azide in DMF at 80 °C for 8 h gave the diazido compound **9** in 98% yield. Removal of the benzoyl ester with 2 N NaOH in ethanol (98% yield) followed by catalytic hydrogenation of **10** led to methyl 3,6-diamino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (**11**).

Treatment of methyl 3-*O*-acetyl-4-*O*-benzoyl-6-bromo-2,6-dideoxy-α-D-*arabino*-hexopyranoside (**12**)[34] with sodium azide in DMF at 80 °C for 8 h led almost quantitatively to azido compound **13**. Transesterification of **13** with sodium methanolate and further catalytic hydrogenation of **14** gave in 95% overall yield for the two steps 6-amino-2,6-dideoxy-α-D-*arabino*-hexopyranoside (**15**).

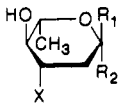
The corresponding β anomers of amino sugars **5** and **8** (i.e. **18** and **19** in Scheme I) were obtained from their respective precursors, the azido derivatives **16**[35] and **7**. Thus treatment of **7** with methanol in the presence of a catalytic amount of *p*-toluenesulfonic acid afforded a mixture of starting material with a small amount of the β anomer **17**, which could be isolated by chromatography on silica gel after acetylation. Catalytic hydrogenation of **16** and **17** (Pd–C (10%) and H_2) led to the corresponding amino compounds **18** and **19**, respectively.

Methyl 3-amino-2,3,6-trideoxy-α- and -β-L-*arabino*-hexopyranosides (**20** and **21**) (methyl α- and β-L-acosaminides) were prepared from methyl 3,4-anhydro-2,6-dideoxy-α- and -β-L-*ribo*-hexopyranosides and were isolated in 60% and 20% yields, respectively, as previously reported.[36]

Synthesis of 3-(2-Chloroethyl)-3-nitrosoureido Derivatives. Carbamoylation of the amino sugars **5**, **6**, **8**, **11**, **15**, **18**, **19**, **20**, and **21** with 2-chloroethyl isocyanate provided the corresponding carbamates, which were immediately treated by conventional nitrosation to afford the (chloroethyl)nitrosoureido derivatives **22–30**, respectively.



	R_1	R_2	R_3	R_4	R_5
22	H	OCH_3	H	H	X
23	H	OCH_3	H	CH_3	X
24	H	OCH_3	OH	H	X
25	H	OCH_3	X	H	X
26	H	OCH_3	X	H	OH
27	OCH_3	H	H	H	X
28	OCH_3	H	OH	H	X



29: $R_1 = OCH_3$; $R_2 = H$
30: $R_1 = H$; $R_2 = OCH_3$

X = $NHC(O)N(NO)CH_2CH_2Cl$

Antineoplastic Evaluation

The acute toxicity of compounds **22–29** administered intraperitoneally was determined in mice (DBA2) before the evaluation of the antineoplastic activity of those compounds. The LD50 values for all compounds were between 40 and 50 mg/kg. Only compound **29** had a LD50 value near the LD50 value of BCNU (25 mg/kg).

The first tests were realized on L1210 leukemia, B16 melanocarcinoma, and Lewis lung carcinoma with compounds **22–26** administered intraperitoneally as an oily

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Table II. Nitrosoureido Sugars in Suspension in Olive Oil versus (3LL) Lewis Lung in Vivo[a]

compd	dose, mg/kg ip (days 1, 5, 9)	mortality (D1–D9)	median survival time, days[d] control mice (range)[b]	median survival time, days[d] nonsurviving treated mice (range)[b]	T/C %[c,d]	treated mice 30-day survivors	treated mice 60-day survivors
22	20	0/20	12.45 (9–21)	*e*		20/20	15/20
26	20	0/20	12.45 (9–21)	39.0 (21–41)	313	13/20	8/20
24	20	0/20	12.45 (9–21)	*f*		20/20	18/20
25	20	0/20	12.45 (9–21)	*g*		18/20	15/20
23	20	0/20	12.45 (9–21)	35.0 (19–46)	281	14/20	6/20

[a] Number of control mice: 40. Number of treated mice for each compound: 20. Weight of the mice: 20 ± 1 g. [b] See footnote *b* in Table I. [c,d] See footnote *c,d* in Table I. [e] Five deaths: one on D43, one on D46, one on D48, one on D54, one on D57. [f] Two deaths: one on D37, one on D51. [g] Five deaths: one on D25, one on D29, one on D38, one on D41, one on D46.

Table III. Nitrosoureido Sugars in Suspension in Olive Oil versus B16 Melanocarcinoma in Vivo[a]

compd	dose, mg/kg ip (days 1, 5, 9)	mortality (D1–D9)	median survival time, days control mice (range)[b]	median survival time, days nonsurviving treated mice (range)[b]	T/C %[c]	treated mice 30-day survivors	treated mice 60-day survivors
22	20	0/20	13.92 (12–27)	36.25 (13–51)	260	14/20	1/20
26	20	0/20	13.92 (12–27)	30 (23–45)	215	9/20	1/20
24	20	0/20	13.92 (12–27)	33.33 (12–47)	239	13/20	3/20
25	20	0/20	13.92 (12–27)	31.37 (14–55)	225	13/20	2/20
23	20	0/20	13.92 (12–27)	23.62 (20–32)	170	1/20	0/20

[a] Number of control mice: 40. Number of treated mice for each compound: 20. Weight of the mice: 20 ± 1 g. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I.

Table IV. Nitrosoureido Sugars in Aqueous Solution versus L1210 Leukemia in Vivo[a]

compd	dose mg/kg ip (days 1, 5, 9)	mortality (D1–D9)	median survival time, days[d] control mice (range)[b]	median survival time, days[d] nonsurviving treated mice (range)[b]	T/C %[c,d]	treated mice 30-day survivors	treated mice 60-day survivors
22	5	0/10	9.45 (9–10)	14.2 (13–16)	150	0/10	0/10
24	5	0/10	9.45 (9–10)	*e*		6/10	6/10
29	5	0/10	9.45 (9–11)	49.0 (23–49)	519	7/10	4/10
28	5	0/10	9.45 (9–11)	*f*		6/10	6/10
27	10	1/10[g]	9.45 (9–11)	18.75 (15–25)	198	0/10	0/10
BCNU	5	0/10	9.45 (9–11)	16.75 (16–19)	177	0/10	0/10

[a] Number of control mice: 20. Number of treated mice for each compound: 10. Weight of the mice: 20 ± 1 g. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I. [e] Four deaths: one on D18, one on D20, one on D21. [f] Four deaths: one on D20, one on D21, one on D22, one on D25. [g] One death on D9.

suspension at 20 mg/kg per day on days 1, 5, 9 after intraperitoneal inoculation of mice with 10^5 L1210 leukemia cells, with 2×10^6 B16 melanocarcinoma cells and with 2×10^6 Lewis lung carcinoma cells.

Those experiments showed the high activity of this family of compounds as well on L1210 leukemia as on other experimental tumors (B16 melanocarcinoma, Lewis lung carcinoma).

The best results on L1210 leukemia (Table I) were obtained with compounds **22–25**. The median survival time of mice treated with those compounds was greatly prolonged (T/C > 600%), and with compounds **22** and **23** all treated animals (15/15) survived until the experiment was terminated after 60 days.

With the Lewis lung carcinoma model (Table II), the results were also very convincing with a median survival time of treated mice greatly prolonged (T/C > 480%) and 18 of the 20 treated animals survived for more than 60 days with compound **24**.

On B16 melanocarcinoma (Table III), all compounds showed a very significant activity and for four of them, **22**, **24**, **25**, and **26**, T/C exceeded 200%.

From these preliminary in vivo biological evaluations, the two derivatives **22** and **24** appeared to be the most active. With those two compounds as references, the influence of the configuration at the anomeric center and the influence of the absolute configuration of the sugar moiety were investigated. Therefore, the activities of β-D isomers **27** and **28** were compared with those of **22** and **24**, respectively. Moreover, the activity of the α-L derivative **29** was compared with that of **22**.

All compounds had the great advantage of being more soluble in water than the lipophilic drugs such as BCNU and sufficiently soluble to undertake the following tests with aqueous preparations of the drugs.

The comparison between those compounds and BCNU on L1210 leukemia in mice following the previously described protocol by the intraperitoneal route in aqueous solution at 5 mg/kg per day on days 1, 5, 9 (Table IV) showed that (1) compounds **24**, **27**, **28**, and **29** were much more active than BCNU; (2) compound **29** of α-L configuration was more active than its corresponding α-D isomer **22**; (3) compound **24** of α-D configuration and its corresponding β-D anomer **28** had the same level of activity.

A study of the dose–effect relationship with BCNU and compounds **24** and **29** (Table V) on L1210 leukemia was carried out in mice according to the previous protocol and allowed the determination of the significantly active dose for each compound: 2.5 mg/kg for BCNU, 1.25 mg/kg for compound **29**, and only 0.625 mg/kg for the most active compound, **24**. Moreover, at 5 and 10 mg/kg doses of compound **24**, all treated mice (8/8) survived until the experiment was terminated after 60 days.

The good activity of compound **24** was confirmed by a monodose study against L1210 leukemia (Table VI) and a monodose study against B16 melanocarcinoma (Table

Table V. Comparative Study between BCNU and Nitrosoureido Sugars versus L1210 Leukemia in Vivo[a]

compd	dose mg/kg ip (days 1, 5, 9)	mortality (D1–D9)	median survival time, days[d] control mice (range)[b]	median survival time, days[d] nonsurviving treated mice (range)[b]	T/C %[c,d]	treated mice 30-day survivors	treated mice 60-day survivors
29	0.625	0/8	9.0 (8–10)	10.6 (10–11)	118	0/8	
	1.25	0/8	9.0 (8–10)	12.9 (11–15)	144	0/8	
	2.5	0/8	9.0 (8–10)	21.9 (17–25)	244	0/8	
	5	0/8	9.0 (8–10)	48.5 (43–54)	539	8/8	4/8
BCNU	0.625	0/8	9.0 (8–10)	9.4 (9–10)	104	0/8	
	1.25	0/8	9.0 (8–10)	9.9 (9–11)	110	0/8	
	2.5	0/8	9.0 (8–10)	11.5 (11–12)	128	0/8	
	5	0/8	9.0 (8–10)	19.8 (18–24)	220	0/8	
	10	0/8	9.0 (8–10)			8/8	8/8
24	0.625	0/8	9.0 (8–10)	11.25 (10–12)	125	0/8	
	1.25	0/8	9.0 (8–10)	16.9 (16–19)	188	0/8	
	2.5	0/8	9.0 (8–10)	43.2 (40–54)	480	8/8	0/8
	5	0/8	9.0 (8–10)			8/8	8/8
	10	0/8	9.0 (8–10)			8/8	8/8

[a] Number of control mice: 16. Number of treated mice of each compound: 8. Weight of the mice: 25.5 ± 1 g. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I.

Table VI. Dose–Effect Relationship for Compound **24** versus L1210 Leukemia (Monodose Study) in Vivo[a]

compd	dose, mg/kg ip (day 1)	mortality (D1–D9)	median survival time, days[d] control mice (range)[b]	median survival time, days[d] nonsurviving treated mice (range)[b]	T/C %[c,d]	treated mice 30-day survivors	treated mice 60-day survivors
24	2.5	0/10	9.4 (9–12)	12.9 (11–14)	137	0/10	0/10
	5	0/10	9.4 (9–12)	15.4 (11–32)	164	3/10	2/10
	10	0/10	9.4 (9–12)	*e*		9/10	9/10
	20	0/10	9.4 (9–12)			10/10	10/10
	30	1/10[f]	9.4 (9–12)			9/10	9/10

[a] Number of control mice: 20. Number of treated mice for each dosage: 10. Weight of the mice: 25 ± 1 g. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I. [e] One death on D22. [f] One death on D8 (toxicity).

Table VII. Dose–Effect Relationship for Compound **24** versus B16 Melanocarcinoma (Monodose Study) in Vivo[a]

compd	dose, mg/kg ip (day 1)	mortality (D1–D9)	median survival time, days[d] control mice (range)[b]	median survival time, days[d] nonsurviving treated mice (range)[b]	T/C %[c,d]	treated mice 30-day survivors	treated mice 60-day survivors
24	5	0/10	19.7 (18–24)	40.0 (30–47)	203	9/10	1/10
	10	0/10	19.7 (18–24)	44.0 (34–47)	223	10/10	3/10
	20	0/10	19.7 (18–24)			10/10	9/10[e]
	30	0/10	19.7 (18–24)			10/10	9/10[f]

[a] Number of control mice: 20. Number of treated mice for each dosage: 10. Weight of the mice: 25 ± 1 g. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I. [e] One death on day 41. [f] One death on day 49.

VII) with a large interval of activity: 2.5 mg/kg to 30 mg/kg against L1210 leukemia model and 5 mg/kg to 30 mg/kg against B16 melanocarcinoma. In both models, at a simple dose of 20 mg/kg at least 90% of the treated mice showed a survival time of over 60 days.

Toxicological Evaluation of 24

The acute intravenous toxicity (LD10, LD50, LD90) to the mouse was determined for compound **24**. The LD10, LD50, and LD90 values were calculated from the observed mortality data by using the probit method of Finney (*Probit Analysis*, 3rd ed.; Cambridge University Press: New York, 1971) and were estimated as follows: male mice, LD10 = 31.2 mg/kg, LD50 = 43.3 mg/kg, LD90 = 60.0 mg/kg; female mice, LD10 = 29.4 mg/kg, LD50 = 40.7 mg/kg, LD90 = 56.4 mg/kg.

In dogs, the hematological toxicity was reversible and returned to within normally accepted limits in a few days; the return to acceptable limits was quick for **24** compared to other alkylating agents according to additional autoradiographic studies in mice (to be published) which indicate that compound **24** does not cross the blood–brain barrier nor distributes into bone marrow, thus confirming the hydrophilic properties of this compound. These autoradiographic results were obtained whatever the position of the labeling of **24** on the sugar component, the urea, and the chloroethyl group.

Discussion

The experimental results clearly indicated the important contribution of structural features such as, in particular, the number of hydroxyl groups, the configuration at the anomeric center, and the absolute configuration of the sugar component on the antitumoral activity of these new nitrosoureido sugars.

The presence of two hydroxyl substituents on the sugar component proved to be optimum for biological activity as compound **24** was more active than compounds **22** (one OH) and **23** (hydroxyl free). Furthermore, the presence of two OH groups in **24** brings about a 10-fold increase in water solubility compared to that of BCNU, which is of considerable importance for clinical use.

The results obtained with compounds **22–27** and **24–28** demonstrate that there is no significant difference in activity between the α and β anomers. In contrast, the L sugar derivatives appeared to be more active than the corresponding D isomers (compare **22** with **29**) but more toxic.

In conclusion, in this new family of nitrosoureido deoxy sugars, the antineoplastic and toxicological data, the water solubility, and the effectiveness of the synthesis led to the selection of compound **24** for further investigation. This compound proved to be active on a wide spectrum of experimental tumors (L1210 leukemia, B16 melanocarcinoma, Lewis lung carcinoma). It is more effective than BCNU against L1210 leukemia: the first active dose has been noticed at a dose of **24** which is 4 times lower than that of BCNU. Moreover the LD50 value for **24** (42 mg/kg iv) is much higher than the LD50 value for BCNU (19–25 mg/kg po). Both sets of data (toxicity and antitumor activity) award a higher therapeutic index for **24** than for BCNU in this model. Finally, contrary to BCNU, compound **24** does not cross the blood–brain barrier and does not reach the bone marrow, limiting the hematological side effects.

Experimental Section

General Methods. Melting temperatures were determined in capillary tubes heated in a Totoli apparatus or on a Kofler hot stage microscope and were uncorrected. IR spectra were recorded on a Perkin-Elmer Model 289 spectrophotometer, calibrated against polystyrene film, and were expressed in cm^{-1}. ^1H NMR spectra at 270 MHz were obtained on a Bruker HX 270 instrument in $CDCl_3$ except when signaled. Chemical shifts were expressed in ppm downfield from internal Me_4Si with the notations indicating the multiplicity of the signal (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet). The coupling constants are expressed as J values in units of hertz. Optical rotations were measured at 20 °C with a Perkin-Elmer Model 241 polarimeter on 1% solutions. Silica gel for column chromatography was Merck silica gel H60 7736. Analytical thin-layer chromatographies were performed on Merck silica gel 60 F_{254}. Microanalyses (C, H, N) were realized on a Perkin-Elmer Model 240 elemental analyzer.

Methyl 3-Azido-6-bromo-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (3). A suspension of **2**[30,32] (32 g) in 2 N aqueous NaOH (140 mL) and MeOH (140 mL) was stirred at room temperature until it became clear. The solution was then left at room temperature for an additional 2 h and the methanol was removed by distillation under reduced pressure. The aqueous residue was extracted with dichloromethane (2 × 250 mL) and the organic solution was dried (Na_2SO_4) and evaporated to give **3** as a syrup (22 g, 95 %): $[\alpha]^{20}_D$ +124° (*c* 1, chloroform); IR (film) 3340 (OH), 2100 cm^{-1} (azide); ^1H NMR ($CDCl_3$) δ 4.82 (d, J = 3, J' < 1, 1-H), 3.85–3.56 (m, 3-H, 5-H, 6-CH_2), 3.41 (dd, J = J' = 10, 4-H), 3.36 (s, OCH_3), 2.51 (br s, OH), 2.18 (dd, J = 13, J' = 4.5, 2e-H), and 1.74 (m, J = 13, J' = 12, J'' = 4, 2a-H). Anal. ($C_7H_{12}BrN_3O_3$) C, H, N.

Methyl 3-Azido-6-bromo-2,3,6-trideoxy-4-*O*-methyl-α-D-*arabino*-hexopyranoside (4). To a stirred solution of **3** (5.5 g, 20.6 mmol) in anhydrous THF (200 mL) in which sodium hydroxide powder (20 g) was suspended was added dimethyl sulfate (20 mL, 211 mmol). After reflux overnight and dilution with water (200 mL), extraction with ether (2 × 125 mL) affords **4** (5.75 g, 98%) as a colorless syrup: $[\alpha]^{20}_D$ +188° (*c* 1, chloroform); IR (film) 2100 cm^{-1} (azide); ^1H NMR ($CDCl_3$) δ 4.76 (d, J = 3, J' < 1, 1-H), 3.90–3.60 (m, 3-H, 5-H, and 6-CH_2), 3.61 and 3.44 (2 s, OCH_3), 3.07 (dd, J = J' = 10, 4-H), 2.10 (dd, J = 13, J' = 4.5, 2e-H), 1.66 (m, J = 13, J' = 12.5, J'' = 4, 2a-H). Anal. ($C_{18}H_{14}BrN_3O_3$) C, H, N.

Methyl 3-Amino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (5). To a suspension of LAH (15 g, 400 mmol) in 600 mL of anhydrous THF was added dropwise a solution of **3** (30 g, 80 mmol) in 200 mL of anhydrous THF. After complete addition, the suspension was refluxed for 5 h under nitrogen atmosphere. After cooling, the excess of reagent was destroyed by careful addition of water (15 mL), 15% aqueous NaOH solution (15 mL), and water (45 mL). The salts were removed by filtration and washed with a mixture of dichloromethane–methanol (8:2) several times. The combined organic solutions were evaporated under reduced pressure to give a syrup (10 g) which was chromatographed on silica gel with CH_2Cl_2–MeOH (8:2) as eluent. This afforded 9.4 g (73%) of pure **5** as a crystalline compound: mp 128–129 °C; $[\alpha]^{20}_D$ +129.0° (*c* 1, chloroform); IR (Nujol) 3320, 3295 (OH, NH_2), and 1585 cm^{-1} (lit.[33] mp 128.5–129.5 °C; $[\alpha]^{20}_D$ +142.8° (MeOH); series L, lit.[37] mp 132.0–133.0 °C; $[\alpha]^{20}_D$ −145.1° (*c* 0.61, methanol); lit.[38] mp 130.0–132.0 °C; $[\alpha]^{20}_D$ −144.0° (*c* 0.52, MeOH).

Methyl 3-Amino-2,3,6-trideoxy-4-*O*-methyl-α-D-*arabino*-hexopyranoside (6). When treated under the conditions giving **5** from **3**, compound **4** (5.15 g, 18.4 mmol) afforded 2.25 g (70%) of crystalline **6**: mp 73–74 °C; $[\alpha]^{20}_D$ +109.0° (*c* 1, chloroform); IR (Nujol) 3295 cm^{-1} (OH, NH_2); ^1H NMR ($CDCl_3$) δ 4.62 (d, J = 4, J' < 1, 1-H), 3.59 (m, 5-H), 3.52 (s, OCH_3), and 3.28, (s, OCH_3), 3.11 (m, 3-H), 2.51 (dd, J = J' = 10, 4-H), 1.97 (dd, J = 13.5, J' = 5, 2e-H), 1.57 (s, NH_2, exch D_2O), 1.54 (m, J = 13.5, J' = 10, J'' = 4, 2a-H), 1.28 (d, J = 6.5, 6-CH_3). Anal. ($C_8H_{17}NO_3$) C, H, N.

Methyl 3-Azido-2,3-dideoxy-α-D-*arabino*-hexopyranoside (7). To a stirred solution of **1** (7 g, 24 mmol) in anhydrous methanol (250 mL) was added dropwise 5 mL of acetyl chloride. After stirring overnight at room temperature, the reaction mixture was neutralized with bubbling ammonia gas and evaporated under reduced pressure to afford a residue which was dissolved in acetone under reflux. The insoluble was filtered and evaporation of the filtrate gave a colorless syrup. Trituration with hexane afforded 4.8 g (98%) of **7** as crystalline compound: mp 120–122 °C (chloroform): $[\alpha]^{20}_D$ +162.5° (*c* 1, methanol); IR (Nujol) 3340 (OH) 2100 cm^{-1} (azide); ^1H NMR ($CDCl_3$) δ 4.77 (d, J = 3, J' < 1, 1-H), 3.83 (d, 6-CH_3), 3.85–3.69 (m, 5-H), 3.59 (m, 3-H), 3.50 (dd, J = J' = 10, 4-H), 3.32 (s, OCH_3), 2.13 (dd, J = 13.5, J' = 5, 2e-H), 1.66 (m, J = 13.5, J' = 12, J'' = 4, 2a-H). Anal. ($C_7H_{13}N_3O_4$) C, H, N.

Methyl 3-Amino-2,3-dideoxy-α-D-*arabino*-hexopyranoside (8). A solution of **7** (5 g, 24.5 mmol) in ethanol (100 mL) was stirred at room temperature under hydrogen (1 atm) in the presence of Pd–charcoal (10%). The catalyst was then removed by filtration and the filtrate was evaporated under reduced pressure to give 4.3 g of **8** (98%) as a crystalline compound: mp 160 °C (CH_3CN); $[\alpha]^{20}_D$ +129° (*c* 1, H_2O); ^1H NMR ($CDCl_3$) δ 4.60 (d, J = 3, J' < 1, 1-H), 3.61 (dd) and 3.42 (dd) (6-CH_3), 3.22 (m, 5-H), 3.19 (s, OCH_3), 2.85 (dd, J = J' = 10, 4-H), 2.74 (m, 3-H), 1.79 (dd, J = 13.5, J' = 4, 2e-H), and 1.34 (m, J = 13.5, J' = 12, J'' = 4, 2a-H). Anal. ($C_7H_{15}NO_4$) C, H, N.

Methyl 3,6-Diazido-4-*O*-benzoyl-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (9). A solution of bromo benzoate **2** (6 g, 16 mmol) in *N*,*N*-dimethylformamide (50 mL) containing sodium azide (6 g, 92 mmol) was heated at 80 °C for 8 h with stirring in an atmosphere of nitrogen. The cooled mixture was poured into water (150 mL) and extracted three times with ether. The combined extracts were washed with water, dried, and evaporated under reduced pressure to give **9** (5 g, 98%) as a colorless syrup: $[\alpha]^{20}_D$ +51° (*c* 2.2, chloroform); IR (film) 2100 (azide), 1725 and 1280 cm^{-1} (ester). Anal. ($C_{14}H_{16}N_6O_4$) C, H, N.

Methyl 3,6-Diazido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (10). To a solution of **9** (14 g, 48 mmol) in methanol (65 mL) was added 65 mL of 2 N NaOH aqueous solution and the mixture was stirred until it became completely clear. After 2 h, as TLC (CH_2Cl_2–MeOH, 95:5) indicated complete conversion of **9** into a more slowly migrating product, the methanol was partially removed by evaporation under reduced pressure. The resulting syrup was extracted with dichloromethane (2 × 250 mL) and the organic layer was washed with water, dried, and evaporated. This afforded **10** as a colorless syrup (8.5 g, 89%): $[\alpha]^{20}_D$ +128° (*c* 1, chloroform); IR (film) 3450 (OH), 2100 cm^{-1} (N_3); ^1H NMR ($CDCl_3$) δ 4.79 (d, J = 3, J' < 1, 1-H), 3.78–3.65 (m, 3-H, 5-H), 3.50 (7, 6-CH_2), 3.39 (dd, J = J' = 10, 4-H), 3.34 (s, OCH_3), 2.19 (dd, J = 13.5, J' = 4.5, 2e-H), 1.74 (m, J = 13.5, J' = 12, J'' = 3, 2a-H). Anal. ($C_7H_{12}N_6O_3$) C, H, N.

Methyl 3,6-Diamino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (11). A solution of **10** (3 g, 13 mmol) in ethanol (100 mL) was shaken in an atmosphere of hydrogen in the presence of triethylamine (1 mL) and 10% palladium-on-charcoal (1 g).

(37) Gupta, S. K. *Carbohydr. Res.* **1974**, *37*, 381.
(38) Lee, W. W.; Wu, H. Y.; Christensen, J. E.; Goodman, L.; Henry, D. W. *J. Med. Chem.* **1975**, *18*, 769.

Table VIII. Analytic Data of Nitrosoureido Sugars **22–30**

compd	mp, °C	solvent	$[\alpha]_D$, deg	anal. data	MW
22	100	isopropyl ether	+92.8 (*c* 0.5, $CHCl_3$)	$C_{10}H_{18}ClN_3O_5$	295.71
23	60	isopropyl ether	+68.8 (*c* 0.5, $CHCl_3$)	$C_{11}H_{20}ClN_3O_5$	309.75
24	118	cyclohexane	+96.2 (*c* 0.5, $CHCl_3$)	$C_{10}H_{18}ClN_3O_6$	311.71
25	102	isopropyl ether	−59 (*c* 0.5, $CHCl_3$)	$C_{13}H_{22}Cl_2N_6O_7$	445.26
26	101	isopropyl ether	+26.2 (*c* 0.5, $CHCl_3$)	$C_{10}H_{18}ClN_3O_6$	311.71
27	103–105	isopropyl ether	−25 (*c* 0.5, $CHCl_3$)	$C_{10}H_{18}ClN_3O_5$	295.71
28	68–70	isopropyl ether	−38 (*c* 0.4, $CHCl_3$)	$C_{10}H_{18}ClN_3O_6$	311.71
29	100	isopropyl ether	−90 (*c* 0.4, $CHCl_3$)	$C_{10}H_{18}ClN_3O_5$	295.71
30	109–110	isopropyl ether	+22 (*c* 0.5, $CHCl_3$)	$C_{10}H_{18}ClN_3O_5$	295.71

Table IX. ^1H NMR Data of Nitrosoureido Sugars **22–30** in DMSO-d_6

compd	H-1′	H-2′	H-3′ (m)	H-4′ (t)	H-5′ (m)	H-6′	NH (d)	OCH_3 (s)	CH_2-H (t), $J = J' = 6$	CH_2Cl (t), $J = J' = 6$
22 (or **29**)	4.65 (d)	1.94–1.70 (m)	4.10	3.04	3.51	1.15 (d)	8.48	3.25	3.60	4.10
23	4.66 (d)	1.87 (m)	4.18	3.01	3.53	1.19 (d)	8.73	3.35 3.25	3.63	4.10
24	4.73 (d)	1.88–1.82 (m)	3.53–3.35	3.43	3.53–3.35	3.67–3.49 (m)	8.53	3.28	3.61	4.10
25	4.66 (d)	1.84 (m	—		3.77 (m)		8.41	3.15	3.77	4.06
26	4.75 (d)	1.85 (dd, 2e-H) 1.45 (t, 2a-H)	3.38–3.28	2.95	3.38–3.28	3.75–3.51 (m)	8.50	3.10	3.75	4.06
27 (or **30**)	4.44 (d)	1.98 (dd, 2e-H) 1.67 (m, 2a-H)	—		3.89 (m)		1.23 (d)	8.66	3.34	3.60	4.08
28	4.48 (d)	1.99 (dd, 2e-H) 1.68 (m, 2a-H)	—		3.43 (m)			8.64	3.38	3.60	4.09

After stirring overnight, filtration followed by removal of the solvent under reduced pressure gave quantitatively **11** (2.3 g) as a syrup: $[\alpha]^{20}_D$ +130° (*c* 1.13, methanol); IR (film) 3515, 3360 cm^{-1} (OH, NH_2); ^1H NMR ($CDCl_3$) δ 4.67 (d, $J = 4$, $J' < 1$, 1-H), 3.45 (m, 5-H), 3.30 (s, OCH_3), 3.19 (dd, $J = J' = 10$, 4-H), 3.10–2.90 (m, 3-H, 6-CH_2), 1.90 (dd, $J = 13.5$, $J' = 4.5$, 2e-H), 1.50 (m, 2a-H). Anal. ($C_7H_{16}N_2O_3$) C, H, N.

Methyl 3-*O*-Acetyl-6-azido-4-*O*-benzoyl-2,6-dideoxy-α-D-*arabino*-hexopyranoside (13). A solution of the 6-bromo derivative **12**[34] (8 g, 28 mmol) in anhydrous *N*,*N*-dimethylformamide (50 mL) containing sodium azide (8 g, 120 mmol) was stirred for 8 h at 80 °C. The cooled mixture was poured into water (100 mL) and extracted with ether (750 mL). Evaporation of the solvent gave a crude product (6.8 g) which was chromatographed on silica gel with hexane–ethyl acetate (4:1) as eluent. This gave 6.5 g (95%) of pure **13** as a crystalline compound. A sample was recrystallized from hexane–acetone: mp 68 °C; $[\alpha]^{20}_D$ +85° (*c* 1 chloroform); IR 2100 (azide), 1725, 1280, 1050 (ester), and 1610, 1590 cm^{-1} (Ar); ^1H NMR ($CDCl_3$) δ 8.10–7.40 (m, Ar H), 5.50 (m, 3-H), 5.15 (dd, $J = J' = 10$, 4-H), 4.90 (d, $J = 4$, $J' < 1$, 1-H), 4.08 (m, 5-H), 3.43 (s, OCH_3), 2.16–1.76 (m, 2e-H and 2a-H). Anal. ($C_{16}H_{19}N_3O_6$) C, H, N.

Methyl 6-Azido-2,6-dideoxy-α-D-*arabino*-hexopyranoside (14). To a solution of compound **13** (4.96 g, 14 mmol) in absolute methanol (50 mL) was added 1 M sodium methoxide (25 mL), and the mixture was stirred for 18 h at room temperature. The solution was deionized with Amberlite IR-50 (H^+) cation-exchange resin, and evaporation of the filtrate gave a pale yellow syrup. Flash chromatography with dichloromethane–methanol as eluent afforded 2.8 g (98%) of pure **14** as a colorless syrup: $[\alpha]^{20}_D$ +104° (*c* 1, chloroform); IR (film) 3400 (OH), 2100 cm^{-1} (azide); ^1H NMR ($CDCl_3$) δ 4.77 (d, $J = 3$, $J' < 1$, 1-H), 3.85 (m, 5-H), 3.63 (m, 3-H), 3.52 (dd, $J = 14$, $J' = 2.5$, 6-Ha), 3.44 (dd, $J = 14$, $J' = 5$, 6-Hb), 3.33 (s, OCH_3), 3.30 (dd, $J = J' = 10$, 4-H), 2.13 (dd, $J = 13$, $J' = 5$, 2e-H), 1.67 (dd, $J = 13$, $J' = 10$, $J'' = 3$, 2a-H). Anal. ($C_7H_{13}N_3O_4$) C, H, N.

Methyl 6-Amino-2,6-dideoxy-α-D-*arabino*-hexopyranoside (15). A solution of **14** (2.4 g, 6.85 mmol) was treated under the same conditions as **10** to give **11**. This afforded 2.3 g (98%) of **15** as a syrup. Crystallization in methanol as a picrate gave the following: mp 156 °C; $[\alpha]^{20}_D$ +75° (*c* 1.2, chloroform); ^1H NMR (as a base; in DMSO-d^6) δ 4.66 (d, $J = 4$, $J' < 1$, 1-H), 3.67–3.50 (m, 6-CH_2), 3.28 (m, 5-H), 3.21 (s, OCH_3), 3.01–2.82 (m, 3-H and 4-H), 1.79 (m, $J = 13$, $J' = 4$, 2e-H), 1.44 (m, $J = 13$, $J' = 12$, $J'' = 4$, 2a-H). Anal. picrate salt ($C_{13}H_{18}N_4O_{11}$) C, H, N.

Methyl 3-azido-2,3,6-trideoxy-β-D-*arabino*-hexopyranoside (16) was prepared according to Bartner et al.[35]

Methyl 3-Azido-2,3-dideoxy-β-D-*arabino*-hexopyranoside (17). To a solution of **7** (19.2 g, 95 mmol) in methanol (200 mL) was added *p*-toluenesulfonic acid (1 g), and the mixture was stirred for 48 h at room temperature. After evaporation under reduced pressure, the syrup was dissolved in anhydrous pyridine (60 mL), and while the temperature of the resulting solution was maintained at 15 °C, acetic anhydride (30 mL) was added dropwise. After the mixture was stirred for 6 h, standard workup afforded a crude syrup which was chromatographed on silica gel with hexane–acetone (4:1) as eluent. This gave 19.5 g of starting material **7** as its 4,6-di-*O*-acetyl derivative and 2.5 g of **17** as its 4,6-di-*O*-acetyl derivative. This later was dissolved in a mixture of anhydrous methanol (45 mL) and 1 M sodium methoxide (5 mL). After the mixture was stirred for 4 h, neutralization was realized by addition of Amberlite IR-50 (H^+) ion-exchange resin. Filtration and evaporation of the filtrate gave 2.2 g of **17** as a crystalline compound: mp 90–92 °C; $[\alpha]^{20}_D$ −11° (*c* 0.9, chloroform); ^1H NMR ($CDCl_3$) δ 4.43 (dd, $J = 10$, $J' = 2$, 1-H), 3.85–3.74 (m, 6-CH_2, 4-H), 3.52–3.36 (m, 3-H), 3.44 (s, OCH_3), 3.32–3.21 (m, 5-H), 2.17 (m, 2e-H), 1.58, (m, 2a-H). Anal. ($C_7H_{13}N_3O_4$) C, H, N.

Methyl 3-Amino-2,3,6-trideoxy-β-D-*arabino*-hexopyranoside (18). Catalytic hydrogenation of **16** (0.5 g) under the conditions previously used to prepare **11** gave 0.47 g (95%) of **18** as a syrup: $[\alpha]^{20}_D$ −45° (*c* 1, chloroform) (lit.[36] (L series) $[\alpha]^{20}_D$ +47° (*c* 0.75, chloroform)).

Methyl 3-Amino-2,3-dideoxy-β-D-*arabino*-hexopyranoside (19). Catalytic hydrogenation of **17** (1 g) under the conditions previously used to prepare **11** or **18** gave 0.9 g (90%) of **19** as a crystalline product: mp 140–142° C; $[\alpha]^{20}_D$ −62° (*c* 0.55, methanol); ^1H NMR (methanol) δ 4.54 (dd, $J = 9$, $J' = 2$, 1-H), 3.81–3.59 (m, 3-H, 6-CH_3), 3.49 (s, OCH_3), 3.36 (dd, $J = J' = 9$, 4-H), 3.02 (m, 5-H), 2.18 (m, 2e-H), 1.51 (m, 2a-H). Anal. ($C_7H_{15}NO_4$) C, H, N.

Methyl 3-Amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside (20). See ref 36.

Methyl 3-Amino-2,3,6-trideoxy-β-L-*arabino*-hexopyranoside (21). See ref 36.

Typical Procedure for the Synthesis of 3-[(2-Chloroethyl)ureido]hexopyranosides. To a cold (0 °C) stirred solution of amino sugar **5** (800 mg, 5 mmol) in anhydrous dimethylformamide (2 mL) was added dropwise, 2-chloroethyl isocyanate (0.4 mL, 5 mmol). After the addition, the reaction solution was stirred at 0 °C for an additional 5 h and then concentrated under reduced pressure. The residue was column chromatographed on silica gel with a mixture of CH_2Cl_2–MeOH (95:5) as eluent. This afforded 800 mg (60%) of a crystalline compound characterized as the ureido derivative (mp 125–127 °C).

To the cold solution of this urea (1.2 g, 4.5 mmol) in formic acid (8 mL) was added, portionwise, sodium nitrite (2.5 g, 36 mmol). After the addition and stirring for 30 min, water was added, and after additional stirring for 30 min, the mixture was poured into ethyl acetate (100 mL) and the organic layer was washed, dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography with CH_2Cl_2–MeOH (9:1) as eluent gave 600 mg (45%) of **22**.

In Vivo Tests. The experimental procedures, the type of mice used (B6D2F1 or C57B1/6), 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).

Test against L1210 Leukemia in Oily Suspension (Table I). On day 0, mice (B6D2F1) were inoculated intraperitoneally with 10^5 L1210 leukemia cells. On days 1, 5, 9, the mice (15 for each group treated) received intraperitoneally the dose of 20 mg/kg of compounds **22–26** in 0.2 mL of neutralized and sterilized olive oil suspension.

The control group (30 mice) received only the same volume of solvent. The mortality of the mice was monitored daily, and autopsies were performed to find out whether deaths were due to leukemia or to a toxic action of the drug. The observation period lasted at least 60 days.

Test against Lewis Lung Carcinoma (3LL) (Table II) and B16 Melanocarcinoma (Table III) in Oily Suspension. On day 0, mice (C57BL/6) were inoculated intraperitoneally either with 2×10^6 Lewis lung carcinoma (3LL) cells or with 2×10^6 B16 melanocarcinoma cells. The treatment protocol, the dose used and the observation period are similar to the above procedure. Twenty mice for each treated group and 40 mice for control groups were used.

Tests against L1210 Leukemia in Aqueous Solution (Tables IV–VI). The protocol of mice leukemia inoculation is the same as in Table I. Ten mice for each treated group and 20 mice for control group were used (Tables IV and VI). Eight mice for each treated group and 16 mice for control group were used (Table V). In Table IV, the mice received intraperitoneally on days 1, 5, 9 the dose of 5 mg/kg of each drug in 0.2 mL of water. In Table V, the mice received on days 1, 5, 9 various doses (0.62 to 10 mg/kg) of each drug in 0.2 mL of water. In Table VI, various doses of compound **24** (2.5 to 30 mg/kg) were administered intraperitoneally in a single injection on day 1 in 0.2 mL of water. The observation period was for all antitumor testings as above at least 60 days.

Tests against B16 Melanocarcinoma in Aqueous Solution (Table VII). On day 0, mice (C57B1/6) were inoculated intraperitoneally with 2×10^6 B16 melanocarcinoma cells. On day 1, various doses of compound **24** (5 to 30 mg/kg) were administered intraperitoneally in a single injection in 0.2 mL of water.

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Registry No. 1, 6386-19-2; **2**, 18933-62-5; **3**, 98383-20-1; **4**, 98383-21-2; **5**, 67693-33-8; **5** [*N*-(*N'*-(2-chloroethyl)ureido) derivative], 98383-15-4; **6**, 98383-22-3; **7**, 20379-53-7; **7** (4,6-di-*O*-acetyl derivative), 20379-54-8; **8**, 16697-56-6; **9**, 98383-24-5; **10**, 98383-25-6; **11**, 98383-26-7; **12**, 98383-28-9; **13**, 98383-29-0; **14**, 98383-30-3; **15**, 98383-31-4; **15**·picrate, 116724-65-3; **16**, 72075-77-5; **17**, 116724-59-5; **17** (4,6-di-*O*-acetyl derivative), 116724-64-2; **18**, 116836-60-3; **19**, 116724-60-8; **20**, 54623-23-3; **21**, 85439-77-6; **22**, 98383-16-5; **23**, 98383-23-4; **24**, 98383-18-7; **25**, 98383-27-8; **26**, 98383-32-5; **27**, 116724-61-9; **28**, 116724-62-0; **29**, 98383-36-9; **30**, 116724-63-1; $ClCH_2CH_2NCO$, 1943-83-5.

Hypoxia-Selective Antitumor Agents. 1. Relationships between Structure, Redox Properties and Hypoxia-Selective Cytotoxicity for 4-Substituted Derivatives of Nitracrine

William R. Wilson,† Robert F. Anderson,‡ and William A. Denny*,§

Section of Oncology, Department of Pathology, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand, Cancer Research Campaign, Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN U.K., and Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand. *Received March 7, 1988*

The nitroacridine derivative 9-[[3-(dimethylamino)propyl]amino]-1-nitroacridine (nitracrine) is selectively cytotoxic to hypoxic tumor cells in culture. However, the compound undergoes reductive metabolism too rapidly, with the reduction not being sufficiently inhibited by molecular oxygen in aerobic tissues, for it to demonstrate the same activity in vivo. In a search for derivatives with lower reduction potentials, we have synthesized and evaluated a series of derivatives bearing 4-substituents with a wide range of electronic properties. The one-electron reduction potentials ($E(1)$) of these compounds, when compared under conditions of equivalent ionization, were highly correlated with σ_p values. However, at pH 7 the influence of substituent electronic properties was modified by prototropic equilibria, with the basic nature of the acridine limiting the extent to which ring substituent electronic effects can be used to modulate reduction potential of the 1-nitro group. Nevertheless, comparison of the kinetics of the killing of AA8 cells under hypoxia suggests that some metabolic stabilization of the compounds can be achieved by the use of electron-donating substituents, with such compounds retaining the hypoxia-selective toxicity of nitracrine in cell culture. However, the 4-substituted nitracrines show no clear relationship between $E(1)$ and cytotoxic potency, in distinct contrast to simpler nitroheterocycles such as nitroimidazoles.

The nitroacridine nitracrine (**4**) shows antitumor activity in some experimental systems and has been used clinically in Poland for the treatment of mammary, lung, ovarian, and colon tumors.[1,2] Nitracrine was briefly investigated by the National Cancer Institute in 1975 (as NSC 247561), but was not advanced to clinical trial because of its restricted antitumor spectrum and high toxicity.[3] Since then we have shown[4] nitracrine to be an extremely potent hy-

† Department of Pathology.
‡ Gray Laboratory.
§ Cancer Research Laboratory.

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