monosaccharides were more effective than polyols as acrosin inhibitors. These data suggest that the aldehyde and ketone moieties are not absolute requirements for inhibitory activity, although the presence of these groups renders the carbohydrates more effective acrosin inhibitors. The most important determinant of inhibitory activity was the spacial configuration of the hydroxy groups attached to the last four asymmetric carbons of the monosaccharide. Preliminary studies have indicated that the interaction between monosaccharides and acrosin is not common to other serine proteinases such as trypsin. Structure–activity studies of acrosin inhibition by monosaccharides and related compounds could be useful in the design of a specific acrosin inhibitor. A specific acrosin inhibitor would be particularly useful in elucidation of the role of acrosin in essential enzymatic processes related to fertilization.

Experimental Section

D-Seduheptulose was a product of P.L. Biochemicals (Milwaukee, WI). Other carbohydrates, as well as N^{α}-benzoyl-L-arginine ethyl ester (BAEE) and N^{α}-benzoyl-DL-arginine-*p*-nitroanilide (BAPA), were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the finest quality commercially available.

Measurement of Enzyme Activity. Esterolytic activity of acrosin was determined spectrophotometrically with either BAEE or BAPA as substrates. Assay mixtures consisted of 50 mM sodium phosphate, pH 7.5, the K_m concentration of substrate (empirically determined as 3×10^{-5} and 3×10^{-4} M for BAEE and BAPA, respectively), and enzyme protein (0.04–0.2 μg), in a total volume of 1.0 mL. A change in absorbance at 253 nm of 1.15 and 9.9 corresponded to the hydrolysis of 1.0 μmol of BAEE and BAPA, respectively.[14]

Isolation and Purification of Human Acrosin. α-Acrosin was isolated from frozen human spermatozoa by acetic acid extraction and purified by sequential steps of Sephadex G-150 and CM-cellulose chromatography, as previously described.[9,15] The purification procedure resulted in α-acrosin preparations with specific activities which ranged from 80 to 140 μmol of BAEE hydrolyzed min^{-1} (mg of protein)$^{-1}$, when assayed in the presence of 0.5 mM BAEE. The protein content of the purified acrosin preparations was estimated by reaction with fluorescamine.[16] Bovine serum albumin was used as a protein standard. Polyacrylamide gel electrophoresis of the preparation at pH 4.3[17] yielded a single band of protein after staining with Coomassie brilliant blue.[18]

Inhibition Studies. Monosaccharides and related compounds were evaluated as acrosin inhibitors by preincubation with acrosin at pH 7.5 for 5 min at ambient temperature prior to initiating the reaction by the addition of either BAEE or BAPA. All inhibitors were present in reaction mixtures at a concentration of 200 mM, unless otherwise indicated.

Kinetic Studies. Michaelis constants were determined by use of plots of reciprocal velocity vs. reciprocal substrate concentration.[19] Kinetic analysis of acrosin inhibition by fructose was performed by varying the substrate concentration (BAEE) in the presence of fixed concentrations of fructose. Enzyme was preincubated in the presence of inhibitor for 5 min prior to the addition of substrate to initiate the reaction.

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2-L-Rhamnopyranosyl[1,2,4]triazolo[1,5-*a*]pyridine. 4′ and 3′ Oxidation Products. Synthesis and Structure–Activity Relationships[1a,b]

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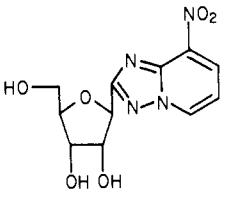
A series of 2-α-L-rhamnopyranosylnitro[1,2,4]triazolo[1,5-*a*]pyridine C-nucleosides was synthesized from the condensation of a thioiminoether with nitro-2-pyridylhydrazines. Catalytic reduction afforded the corresponding amino derivative. A 1′,2′ unsaturated C-nucleoside was also obtained by two different routes. Selective oxidation gave the 3′- and 4′-ketonucleosides. The cytotoxic properties of the nucleosides, as well as their effect on viral transformation and replication, were described. The nitro derivatives inhibit viral replication, but at toxic doses; the introduction of a keto function leads to a product which inhibits the replication of murine leukemia virus (MuLV) at noncytotoxic concentrations. The amino derivatives have no significant antiviral effect.

In the last decade, after the discovery of the pharmacological properties of C-nucleosides,[2] a number of studies have been devoted to the synthesis of such products.[3] We recently described the obtention of some structural analogues of the formycines, including 2-β-D-ribosyl[1,2,4]-triazolo[1,5-*a*]pyridines and 3-β-D-ribosyl[1,2,4]triazolo-[4,3-*a*]pyrazines.[4] One of these products, the 2-β-D-ribofuranosyl-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (1), presented interesting cytotoxic and antiviral properties.



1 (CT 5394)

In order to establish some structure-activity relationships, we decided to prepare a new series of compounds containing the same base, [1,2,4]triazolo[1,5-*a*]pyridine, and the L-rhamnopyranose as the glycon. Our purpose in choosing this sugar was to determine if modifications of

(1) (a) Synthesis of C-Nucleosides. 19. For part 18, see ref 5. (b) The chemical part of this work was abstracted from the third cycle thesis of P.A.

(2) R. J. Suhadolnik, "Nucleoside Antibiotics", Wiley Interscience, New York, 1970.

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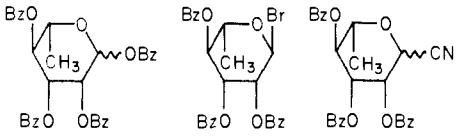
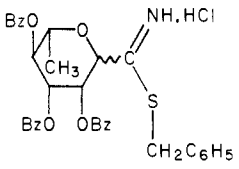
the carbohydrate structure of the nucleoside could change its biological activity as was reported in the case of N-nucleosides of L-rhamnose after selective oxidation of the hydroxyl group in the 4′ position.[6]

A theoretically suitable product for our synthesis was already described, the 1-cyano-2,3,4-tri-*O*-acetyl-6-deoxy-L-*manno*-hexopyranose,[7] but it was proved, in our laboratory, that the synthesis of this product in good yield from the corresponding 1-bromo compound was impossible.

We decided to start from the 1,2,3,4-tetra-*O*-benzoyl-6-deoxy-L-*manno*-hexopyranose (**2**).[8] **2**, by reaction with



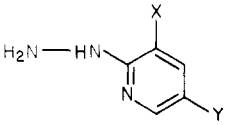
2 **3** **4**



5

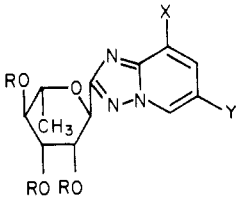
gaseous hydrobromic acid in glacial acetic acid, gave the 1-bromo derivative **3**, which reacted with mercuric cyanide in nitromethane during 70 h and yielded a mixture of α and β anomers of **4**, isolated after column chromatography (72%, α/β = 4.5). Each anomer of **4** could be converted to the thioiminoether hydrochloride **5** by the action of benzyl mercaptan in anhydrous ether with hydrochloric acid at 0 °C in high yield.

Condensation of **5**, obtained from the α anomer of **4**, with 3-nitro-2-pyridylhydrazine (**6**) in refluxing pyridine



6, X = NO_2; Y = H
7, X = H; Y = NO_2

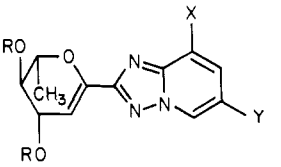
resulted in the formation of two compounds. The major derivative (40%) was the 2-(2′,3′,4′-tri-*O*-benzoyl-6′-deoxy-α-L-*manno*-hexopyranosyl)-8-nitro[1,2,4]triazolo-[1,5-*a*]pyridine (**8**). **8** could be deprotected (CH_3OH/NH_3) and gave the free nucleoside **10** (76%).



8, R = Bz; X = NO_2; Y = H
10, R = H; X = NO_2; Y = H
11, R = Bz; X = NH_2; Y = H
12, R = H; X = NH_2; Y = H
16, R = Bz; X = H; Y = NO_2
22, R = H; X = H; Y = NO_2
23, R = Bz; X = H; Y = NH_2
24, R = H; X = H; Y = NH_2

As previously reported in the case of the D-ribose series, we observed the formation of a [1,2,4]triazolo[1,5-*a*]-pyridine instead of the expected isomer, [1,2,4]triazolo-[4,3-*a*]pyridine, as the result of a Dimroth-like rearrangement.[9]

The second product (14%) was identified as the 2-(3′,4′-di-*O*-benzoyl-2′,6′-dideoxy-L-*arabino*-hex-1′-enopyranosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (**9**). The



9, R = Bz; X = NO_2; Y = H
13, R = H; X = NO_2; Y = H
14, R = Bz; X = NH_2; Y = H
15, R = H; X = NH_2; Y = H
17, R = Bz; X = H; Y = NO_2

NMR spectra obtained with **9** (or with the deprotected product **13**) showed no signal for $H_{1'}$, while $H_{2'}$ appeared as a deshielded doublet (6.53 ppm). In mass spectra, the deprotected product **10** presented the major peak at B + 30, as commonly observed with C-nucleosides having a free 2′-hydroxyl group; with the unsaturated product **13**, the most important fragment was B + 72, and the B + 30 peak (generally weak with 2-deoxynucleosides[10]) was totally absent. **9** is the first example of a 1′,2′ unsaturated C-nucleoside. Such N-nucleosides have been obtained by M. J. Robins and co-workers by indirect methods.[11]

The same synthesis was made from the β anomer of **4** by an identical synthetic pathway. We finally obtained **10** with the same yield. This proved that a prototropy occurred at the formation stage of **5** as previously described in the case of derivatives of 2-deoxy-D-ribose.[12]

The nitro group of **8** was catalytically reduced to the corresponding amino group. After chromatography, we obtained **11** (56%), which was treated with methanolic ammonia to give the 2-(6′-deoxy-α-L-*manno*-hexopyranosyl)-8-amino[1,2,4]triazolo[1,5-*a*]pyridine **12** (80%). Deprotection of **9** was achieved by using sodium methylate in methanol. The free nucleoside **13** was obtained in 63% yield. Catalytic hydrogenation of **9** (1 kg/cm^2, 2 h) gave us compound **14** after purification on silica (45%). Attempts to prepare the completely reduced product failed with increasing reaction time or pressure.

We also directly deprotected the crude product obtained after reduction of **9**, without purification, in order to increase the yield of **15**; thus, we obtained, after treatment with methanolic ammonia and purification, the 8-amino product **15** in 60% yield from **9**.

In order to obtain structure–activity relationships, we decided to modify the position of the nitro (amino) group on our products and to observe their influence on the biological properties. We condensed the rhamnosyl thioformimidate **5** with 5-nitro-2-pyridylhydrazine. We observed on TLC two products, but only **16** was isolated after chromatography (35%). Since the unsaturated product

(6) K. Antonakis and I. Chouroulinkov, *Biochem. Pharmacol.*, **23**, 2095 (1974).
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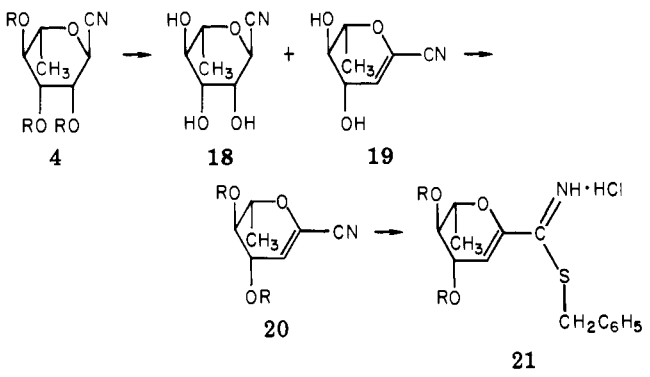
(8) R. K. Ness, H. G. Fletcher, and C. S. Hudson, *J. Am. Chem. Soc.*, **73**, 296 (1951).
(9) K. T. Potts and C. R. Surapaneni, *J. Heterocycl. Chem.*, **7**, 1019 (1970).
(10) J. A. McCloskey, "Basic Principles in Nucleic Acid Chemistry", Vol. I, P. O. P. Ts'o, Ed., Academic Press, New York, 1974, p 209.
(11) (a) M. J. Robins and E. M. Trip, *Tetrahedron Lett.*, 3369 (1974); (b) M. J. Robins, R. A. Jones, and R. Mengel, *J. Am. Chem. Soc.*, **98**, 8213 (1976).
(12) A. Kolb, C. Gouyette, T. Huynh Dinh, and J. Igolen, *Tetrahedron*, **31**, 2914 (1975).

Scheme I[a]



[a] R = $-COC_6H_5$.

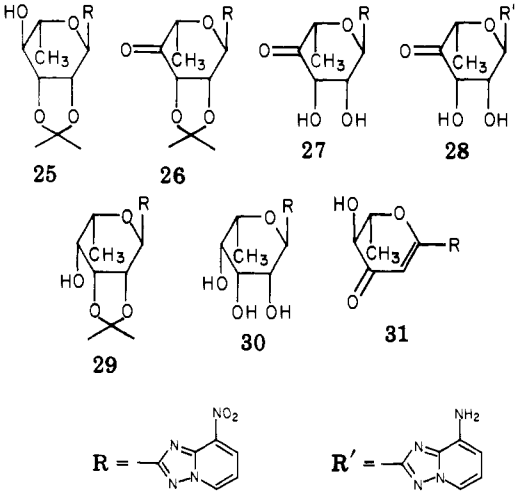
17 could not be obtained in such a way, we developed an indirect synthesis.

It has been demonstrated in our laboratory[13] that treatment of the α anomer of 4 with methanolic ammonia will give two compounds: the 1-cyano-6-deoxy-α-L-*manno*-hexopyranose (18) and the 1-cyano-2,6-dideoxy-L-*arabino*-hex-1-enopyranose (19) (see Scheme I). These two compounds were separated on a silica column. The second one (44%) was benzoylated into 20 and then transformed into the thioiminoether hydrochloride 21, which was condensed with 7. Though the crude product was relatively pure, we could not obtain more than 7% of 17 after column chromatography. This could be explained by a retention or a degradation on the silica columns used for purification. This poor yield precluded any attempts of reduction or deprotection of 17.

In the case of 16, we deprotected the hydroxyl groups and obtained the saturated 6-nitro product 22 in 72% yield. Catalytic hydrogenation of 16 gave the corresponding amino compound 23 (66%), which was deprotected into 24, isolated after chromatography (60%).

We then decided to modify specifically the sugar part of our nucleosides in order to observe the relationship of the hydroxyl group to biological activity. In recent years, many laboratories have worked on oxidation of nucleosides. It has been recently proved, in the case of deoxyhexosyl N-nucleosides, that selective oxidation of the sugar moiety will give an active product while the parent nucleoside will remain inactive.[6] Most oxidation methods are based on dimethyl sulfoxide as the oxidizing agent in the presence of an activator. Moffat and coworkers first described this type of reaction[14] (Me_2SO/*N,N'*-dicyclohexylcarbodiimide). Similar oxidations with acetic anhydride or phosphorous pentaoxide as activators were then used.[15] Recently, Swern and co-workers have described a new improvement to this method. They chose oxalyl chloride as the activator and obtained carbonyl derivatives from a large number of alcohols in high yield.[16] Furthermore, this reaction did not give byproducts difficult to eliminate, like dicyclohexylurea in the Pfitzner–Moffat reaction.

10 was first protected in the 2′ and 3′ positions by an isopropylidene group to give 25, which was allowed to react with the couple $Me_2SO/(COCl)_2$ (a fivefold excess of reagents was used): 25 was completely oxidized to the



4′-keto derivative 26, which could be obtained analytically pure after column chromatography (62%). On scale-up it was more efficient to recover 26 by simple filtration after treatment of the crude with a minimum of dichloromethane/ethyl ether (80%).

The free ketonucleoside 27 was obtained by reacting 26 at room temperature with 99% formic acid (93%). The nitro function of 26 was catalytically reduced to the corresponding amino group. After purification on a silica column, we obtained the 2-(6′-deoxy-α-L-*lyxo*-hexopyranos-4′-ulosyl)-8-amino[1,2,4]triazolo[1,5-*a*]pyridine (28; 65%).

The reduction of the keto function with sodium borohydride alone, as previously described in the literature for keto-N-nucleosides,[17] failed. In the presence of cerium chloride,[18] a reagent known to allow selective reduction of a keto group in the presence of an aldehyde, the reduction of 26 was complete in 2 h. Two products were formed: the minor product (TLC) was 25, and the major one was purified on a silica column and identified as 2-(6′-deoxy-2′,3′-*O*-isopropylidene-α-L-*tallo*-hexopyranosyl)-8-nitro-[1,2,4]triazolo[1,5-*a*]pyridine (29), which was deprotected with 99% formic acid for 2 h and gave a 72% yield of 30 after purification of Bio-Gel.

We also oxidized our 1′,2′-unsaturated product 13. With activated manganese dioxide, which allowed selective oxidation of allylic alcohols,[19] we obtained 2-(2′,6′-dideoxy-L-*erythro*-hex-1′-enopyranos-3′-ulosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (31) in 40% yield after column chromatography.

The structures of the described compounds were assigned by classical physical methods. The structures of the heterocycles were assumed on the basis of their UV spectra: as expected, we observed the same absorbtion maximum as in the case of the D-ribose or D-arabinose series previously prepared,[4,5] which proved unambiguously that the base moiety was [1,2,4]triazolo[1,5-*a*]pyridine.

The structure of the nucleosides was confirmed by mass spectrometry. Their spectra presented the well-known

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(19) A. J. Fatiadi, *Synthesis*, 65 (1976).

Table I. NMR Spectra of the Sugar Moiety of the Deprotected Derivatives (Me_2SO, D_2O)[a]

no.	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$(CH_3)_{6'}$	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6'CH_3}$	field freq
10	4.77 (d)	4.04 (dd)		3.05-3.54 (br)		1.16 (d)	1.0	2.9	ND	ND	6.0	80
12	4.80 (s)	4.18 (d)	3.60 (dd)	3.27-3.48 (br)		1.32 (d)	0	3.0	9.0	ND	6.0	250
22	4.95 (s)	4.22 (br)	3.60 (br)	3.25-3.50 (br)		1.33 (d)	~0	ND	ND	ND	6.5	400
24	4.68 (s)	4.08 (br)		3.20-3.58 (br)		1.30 (d)	~0	ND	ND	ND	6.0	400
30	4.79 (d)	4.05 (t)		3.25-3.80 (br)		1.48 (d)	1.4	1.4	ND	ND	6.25	80
13		6.03 (d)	4.23 (br)	ND (HDO)	4.03 (m)	1.47 (d)		2.5	ND	8.5	6.5	400
15		5.85 (d)	4.20 (br)	ND (HDO)	4.00 (m)	1.47 (d)		2.5	ND	8.5	6.5	400
27	5.46 (s)	4.50-4.73 (br)			4.32 (qd)	1.18 (d)	0	ND			6.2	80
28	5.28 (d)	4.45-4.66 (br)			4.35 (qd)	1.16 (d)	0.3	ND			6.30	80

[a] Abbreviations used: s, singlet; d, doublet; t, triplet; qd, quadruplet; dd, doublet of doublet; m, multiplet; br, broad; ND, no determination. Chemical shifts are in δ units (parts per million) and field frequency and J values are in hertz.

Table II. Biological Properties of the Products[a]

				% inhibition			
				MSV assay on C_3H cells			
		cytotoxic effect: % colony formation on		without addition of helper virus		with optimal amount of helper virus,	MLV assay
product	concn, μg/mL	C_3H cells	S^+L^- cells	day +5	day +10	day +5	on S^+L^- cells
13	3			15	0	39	30
	5	71.5	49	44	30	26	50
	10	27	18	64	58	50	82.5
	20	2	0	91	70	76	
12	100	115	83				
	200			0	0	11	50
	250	108	94				
27	30			35	0	31	35
	50	109	81	54	14	46	55
	100	76	25	67	42	78	80
	250	21	0				
1	2.5					47	
	5		79.6			62	
	10		46				

[a] Percent colony formation was calculated comparatively to control cells which are considered to have a 100% plating efficiency. In these experiments, MSV titer on C_3H cells was 7×10^6 and MLV titer on S^+L^- cells was 2×10^5.

pattern of degradation of C-nucleosides, i.e., weakness of ions like B^+ or BH^+ and predominance of fragments such B + 30, B + 44, or B + 72. The major peak was observed at different values as a function of the structure of the glycon.

The anomeric identities were determined from the NMR spectra. The complete analysis of the spectra, where the protons of the sugar part are forming an ABC system, was impossible, but the signal of $H_{1'}$ always appeared, as a deshielded singlet or like a very small coupled doublet with $J_{1',2'} \leq 1$ Hz (except for **30**, which was 1.4 Hz). This was in agreement of a 1',2' equatorial–equatorial arrangement, i.e., an α configuration (Table I). NMR spectra of protected substances, easier to analyze, confirmed the attributed configuration.

Biological Results

In Vitro Cytotoxic Determination. Cytotoxicities toward normal mouse fibroblasts (C_3H cells) and MSV transformed cells (S^+L^- cells) were studied as described under Experimental Section. Most of the products with an amino function (**12**, **15**, and **24**) were without any effect on the cellular growth at 500 μg/mL. At this concentration, the multiplication of the normal fibroblasts was strongly inhibited by the compounds with a nitro function (**10**, **13**, and **30**); 50% cytotoxic doses were about 500, 62.5, and 400 μg/mL, respectively. The presence of a keto function (**27**) in addition to the nitro group seems to decrease their toxicity; only a delay of the cellular growth was observed with 500 μg/mL of the product **27**. One product in each group was chosen to study in detail their biological effect in vitro: **12**, **13**, and **27**.

The action of these compounds on the plating efficiency is summarized in Table II. No toxic effect was observed with **12** on C_3H cells as well as on S^+L^- cells. Fifty percent toxic doses (TD_{50}) were obtained, respectively, with 7 μg/mL of **13** and with 150 μg/mL of **27** on C_3H colonies formation. On S^+L^- cells, TD_{50}'s were 5 μg/mL or **13** and 73 μg/mL of **27**.

Action of the Compounds on Focus Formation Induced by MSV and on MuLV Replication. The effect of the products on focus formation induced by MSV was studied using C_3H cells. C_3H cells were infected with MSV either without addition of MuLV or in the presence of an optimal amount of MuLV. In the first case, focus formation on day +5 was dependent on the replication of MuLV always present in MSV stock, but on day +10, the MSV genome was completely expressed with the formation of nonproducer foci independent of MuLV replication.[20]

In the second case, MSV focus formation was dependent on the replication of helper virus added in excess and was entirely expressed on day +5.

In order to study the effect of these compounds on MuLV replication, MuLV titers were determined on S^+L^- cells treated after viral adsorption with varying amounts of each product.

The data presented on Table II show an inhibition of MSV titer on day +5 in the presence of **13** or **27**. The fifty percent inhibitory doses (ID_{50}) were respectively about 10 and 50 μg/mL either in the absence of helper virus or in

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the presence of an excess of MuLV. In the absence of MuLV, when foci were counted on day +10, the ID_{50}'s were 9 μg/mL of **13** and 145 μg/mL of **27**. No effect on the MSV assay was observed in the presence of **12**.

MuLV titer on S^+L^- cells was decreased in the presence of **13**, **12**, or **27**; ID_{50} values were, respectively, 50, 200, and 43 μg/mL, but MuLV replication was inhibited with cytotoxic doses of **13**.

Table II shows that **27** has antiviral properties similar to those of **1**[5] but is more cytotoxic.

Conclusion

Taking in account all our results, some general conclusions can be pointed out: (1) The derivatives with a nitro function are cytotoxic compounds in contrast to the nucleosides with an amino function. The addition of a keto function to the nitro derivatives decreases their toxicity. (2) The amino compounds have no effect on cell transformation induced by MSV and are very low inhibitors of MuLV replication. On the other hand, the nitro derivatives are able to inhibit the MSV focus formation and MuLV replication. This inhibition was obtained with cytotoxic doses of nitro products (**13**), but the presence of a keto function in these nucleosides led to a compound (**27**) able to inhibit MuLV replication at noncytotoxic doses, as suggested by the data on MSV focus formation on day +5 and on S^+L^- assay.

Experimental Section

Biological Assays. Cell Cultures and Virus. C_3H cell line[21] and S^+L^- 3T3 FL cell lines[22] were gifts of Dr. R. H. Bassin (National Cancer Institute). The cells were grown in Mac Coy's 5a media supplemented with 10% heat-inactivated fetal calf serum (Gibco) and antibiotics (Gibco). The NB tropic murine leukemia virus (MuLV) was the IC isolate of Moloney[23] propagated in NIH/3T3 cell line. The murine sarcoma virus (MSV) was obtained by superinfection of S^+L^- cells (C243 clone) with MuLV.

Cytotoxicity Assays. Cellular growth: 10^4 C_3H cells were seeded into microtest wells (Costar, 24 wells, 16-mm diameter), and 24-h later, different doses of product were added. Cellular growth and morphology were examined on day +5 as described previously.[5]

Plating efficiency:[24] 200 C_3H or S^+L^- cells were seeded in 35-mm wells of Costar plate. Various concentrations of product were added 24 h after cell seeding. Cell colonies were counted on day +9 after staining with May Grunwald Giemsa.

Virus Infectivity Assays. For S^+L^- focus assay[25] or for MSV assay,[20] 8×10^4 cells were seeded into 60-mm Falcon petri dishes. Twenty-four hours later, cells were pretreated with 20 μg/mL of DEAE-dextran (Pharmacia) and infected with 0.5 mL of diluted virus [MSV or MuLV or MSV (0.3 mL) + MuLV helper (0.2 mL)]. Virus adsorption took place for 30 min at 37 °C. Plates were then refed with 3.5 mL of fresh medium. Aqueous solutions of nucleosides were freshly prepared, filtered through a 0.22-μm Millipore filter, diluted in medium, and added to cells immediately after virus infection. The foci were counted 5 days later and also 10 days later for MSV assay.

Chemistry. Melting points were taken in capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 221 or 237 spectrometer, and ultraviolet spectra were recorded on a Perkin-Elmer 137, Beckman DB-T, or Cary 14 spectrometer. Optical activities were measured with a Perkin-Elmer 241 MC polarimeter. NMR spectra were obtained at 80 MHz on a Varian FT 80 and at 250 and 400 MHz at the Institute d'Electronique (Orsay, France) with Me_4Si as internal standard. Mass spectra were recorded on MS 50 or VG micromass 70–70 F. TLC plates (0.25-mm thick) were prepared with Merck Kieselgel $HF_{254+366}$ and visualized with a UV light at 254 nm. For column chromatography we used Kieselgel 60 (Merck Art 7729) or Bio-Gel P_2 (200–400 mesh).

2,3,4-Tri-*O*-benzoyl-1-bromo-6-deoxy-α-L-*manno*-hexopyranose (3). **2** (69.5 g, 0.12 mol) was dissolved in 250 mL of glacial acetic acid. Anhydrous hydrobromic acid was allowed to bubble in the solution kept at 0 °C. When 5 equiv was absorbed, the solution was stored at 4 °C overnight. The white solid formed was dissolved in CH_2Cl_2 and washed with cold H_2O, saturated sodium bicarbonate, and then with cold water. The organic phase, dried over sodium sulfate, was evaporated. The pure compound was obtained by precipitation (CH_2Cl_2–petroleum ether): yield 50.1 g (77%); mp 174 °C (lit. 163–164 °C); TLC (petroleum ether–EtOAc, 3:1) R_f 0.86. Anal. ($C_{27}H_{23}O_7Br$) C, H.

2,3,4-Tri-*O*-benzoyl-1-cyano-6-deoxy-α- and -β-L-*manno*-hexopyranose (4). **3** (25 g, 46 mmol) and $Hg(CN)_2$ (25 g, 110 mmol) were suspended in 140 mL of dry CH_3NO_2 and stirred for 3 days. The excess of $Hg(CN)_2$ was filtered off, and the solution was brought to dryness, dissolved in CH_2Cl_2, and washed with an aqueous solution of KI (1 M) containing 10% of methanol. The organic phase dried over Na_2SO_4 was adsorbed on silica and column chromatographed (petroleum ether–AcOEt, 9:1).

The α anomer was collected first: yield 14.1 g (62%); mp 80 °C; TLC (petroleum ether–AcOEt 3:1) R_f 0.86; IR no nitrile band; $[\alpha]_D$ +176.8° (*c* 0.16, $CHCl_3$). Anal. ($C_{28}H_{23}O_7N$) C, H, N.

The column was then eluted with petroleum ether-AcOEt (8.5:1.5), and the β anomer was collected: yield 3.20 g (14%); mp 85 °C; TLC (petroleum ether–AcOEt, 3:1) R_f 0.38; IR no nitrile band; $[\alpha]_D$ +218.9° (*c* 0.19, $CHCl_3$). Anal. ($C_{28}H_{23}O_7N$) C, H, N.

Benzyl (2,3,4-Tri-*O*-benzoyl-6-deoxy-L-*manno*-hexopyranosyl)thioimidate Hydrochloride (5). A solution of 22 g of the α anomer of **4** (45 mmol) and 5.7 mL of benzyl mercaptan (46 mol) in 220 mL of anhydrous ether was saturated with hydrochloric acid at 0 °C and kept overnight at 4 °C. Ether was then evaporated and the foam formed was treated with heptane. The white powder obtained was filtered and washed with dry ether: yield 22.4 g (83%); mp 74–76 °C. Anal. ($C_{35}H_{31}O_7NS$·HCl) C, H, N.

Using the same method, 3.00 g of the β anomer of **4** gave 3.29 g (82%) of **5**, mp 74–76 °C.

2-(2′,3′,4′-Tri-*O*-benzoyl-6′-deoxy-α-L-*manno*-hexopyranosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (8) and 2-(3′,4′-Di-*O*-benzoyl-2′,6′-dideoxy-L-*arabino*-hex-1′-enopyranosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (9). **5** (7.4 g, 11 mmol) and **6**[9] (1.76 g, 11 mmol) in 70 mL of dry pyridine were heated to reflux for 4 h. After evaporation, the residue was suspended in methanol and neutralized with 8 N NaOH. The residue was filtered off and purified on silica ($CHCl_3$) to yield 2.47 g of **8** (35%): mp 250 °C (AcOEt); TLC ($CHCl_3$–EtOH, 99:1) R_f 0.46; IR 1725, 1640, 1530, 710 cm^{-1}; $[\alpha]_D$ +79.2° (*c* 0.16, $CHCl_3$). Anal. ($C_{33}H_{26}O_9N_4$) C, H, N.

The methanolic solution, brought to dryness, was chromatographed on silica ($CHCl_3$). The first fractions were discarded and then 1.09 g of crude **9** was isolated and purified on a short column of silica; 0.76 g of **9** (13%) was obtained: mp 133 °C (Et_2O); TLC ($CHCl_3$) R_f 0.57; IR 1720, 1640, 1530, 710 cm^{-1}; $[\alpha]_D$ +58.7° (*c* 0.18, $CHCl_3$). Anal. ($C_{26}H_{20}O_7N_4$) C, H, N.

A second crop of **8** was obtained from the column: yield 0.35 g (5%) (same characteristics as those described above).

5, obtained from the β anomer of **4**, gave identically the products **8** and **9** in the same proportions.

2-(6′-Deoxy-α-L-*manno*-hexopyranosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (10). The debenzoylation of 2 g (3.2 mmol) of **8** in methanolic ammonia gave, after treatment with charcoal and recrystallization, 776 mg of **10** (76%): mp 237 °C (EtOH); TLC ($CHCl_3$–EtOH, 9:1) R_f 0.17; MS, *m/e* 310 (1.5), 293 (29), 221 (8), 207 (20), 205 (13) [$BC(=O)CH_2^+$], 193 (100) (HBCHO), 178 (15), 177 (27) ($B{=}CH_2$), 164 (5), 163 (32.5)[$(B)^+$· or (B + 30) – NO]; IR 1650, 1540 cm^{-1}; UV ϵ_{236} 13 600, ϵ_{330} 6200; $[\alpha]_D$ –87.6° (*c* 0.19, H_2O). Anal. ($C_{12}H_{14}O_6N_4$) C, H, N.

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(25) R. H. Bassin, N. Tuttle, and P. J. Fishinger, *Nature (London)*, **229**, 564 (1971).

Debenzoylation of 8 obtained by condensation of 6 and 5 (from β anomer of 4) gave 10, presenting the same characteristics of that above.

2-(2′,3′,4′-Tri-*O*-benzoyl-6′-deoxy-α-L-*manno*-hexopyranosyl)-8-amino[1,2,4]triazolo[1,5-*a*]pyridine (11). 8 (500 mg, 0.8 mmol) in 15 mL of CH_3COCH_3 was hydrogenated over 10% Pd/C under 5 kg/cm² pressure at room temperature during 8 h. After filtration, the solvent was evaporated and the residue was chromatographed on silica ($CHCl_3$). Pure fractions, treated with pentane, gave 257 mg (56%) of 11: mp 222 °C (EtOH); TLC ($CHCl_3$–EtOH, 98:2) R_f 0.37; IR 1720, 1640 cm⁻¹; $[\alpha]_D$ +127.6° (*c* 0.17, $CHCl_3$). Anal. ($C_{33}H_{28}O_7N_4$) C, H, N.

2-(6′-Deoxy-α-L-*manno*-hexopyranosyl)-8-amino[1,2,4]triazolo[1,5-*a*]pyridine (12). The debenzoylation of 975 mg (1.61 mmol) of 11 in methanolic ammonia during 3 days at room temperature gave, after evaporation, a white powder purified on Bio-Gel. The pure fractions were lyophilized and crystallized in methanol: 362 mg (80 %) of 12 was obtained; mp 241 °C (MeOH); TLC ($CHCl_3$–EtOH, 8:2) R_f 0.23; MS *m/e* 280 (37) ($M^{+\cdot}$), 262 (<1), 205 (2), 191 (15), 177 (34) (BCH=CHOH), 175 (19), 163 (100) (HBCOH), 148 (14), 147 (12) ($B{=}CH_2$), 134 (7) (B^+H), 133 (<1) ($B^{+\cdot}$); IR 1625 cm⁻¹; UV ϵ_{272} 9300, ϵ_{295} 6800; $[\alpha]_D$ −44.6° (*c* 0.18, H_2O). Anal. ($C_{12}H_{16}O_4N_4$) C, H, N.

2-(2′,6′-Dideoxy-L-*arabino*-hex-1′-enopyranosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (13). A suspension of 412 mg of 9 (0.82 mmol) and 100 mg of CH_3ONa (1.85 mmol) in 75 mL of anhydrous methanol was heated to reflux during 1.5 h under nitrogen. The solution was neutralized by the addition of Amberlite CG 50 (H^+ form), then filtered, and evaporated. The residue was recrystallized in AcOEt: yield 150 mg (63%); mp 210 °C (AcOEt); TLC ($CHCl_3$–EtOH, 9:1) R_f 0.25; MS, *m/e* 292 (5) ($M^{+\cdot}$), 275 (3), 235 (100) [HB–C(OH)=CHCHO], 207 (14), 205 (2), 191 (7), ($HBCHCH_2$), 178 (3), 177 (<1), 164 (<1), 163 (<1) ($B^{+\cdot}$); IR 1640, 1530 cm⁻¹; UV ϵ_{239} 17 400, ϵ_{332} 7400; $[\alpha]_D$ −46.6° (*c* 0.16, H_2O). Anal. ($C_{12}H_{12}O_5N_4$) C, H, N.

2-(3′,4′-Di-*O*-benzoyl-2′,6′-dideoxy-L-*arabino*-hex-1′-enopyranosyl)-8-amino[1,2,4]triazolo[1,5-*a*]pyridine (14). 9 (762 mg, 1.56 mmol) in 75 mL of CH_3COCH_3 was hydrogenated over 10% Pd/C during 2 h under atmostpheric pressure. After filtration, the solvent was evaporated and the residue was chromatographed on a silica column (CH_2Cl_2–EtOH, 99.5:0.5). The pure fractions were collected, and the product crystallized with Et_2O: yield 324 mg (45%); mp 100 °C (Et_2O–petroleum ether); TLC (CH_2Cl_2–EtOH 99:1) R_f 0.30; IR 1720, 1620 cm⁻¹; $[\alpha]_D$ +98.9° (*c* 0.10, $CHCl_3$). Anal. ($C_{26}H_{22}O_5N_4$) C, H, N.

2-(2′,6′-Dideoxy-L-*arabino*-hex-1′-enopyranosyl)-8-amino[1,2,4]triazolo[1,5-*a*]pyridine (15). 9 (762 mg, 1.56 mmol) was hydrogenated as described above. The green residue (697 mg) obtained after filtration was treated with methanolic ammonia at room temperature during 80 h. The solvent was evaporated and the solid was chromatographed on Bio-Gel. Pure fractions were collected, lyophilized, and crystallized in methanol: yield 239 mg (60% from 9); mp 221 °C (EtOH); TLC (CH_2Cl_2–EtOH, 9:1) R_f 0.26; MS, *m/e* 262 (38) ($M^{+\cdot}$), 244 (31) ($M^{+\cdot} - H_2O$), 205 (100) [HBC(OH)=CHCHO], 177 (28), 161 (29) ($BCH{=}CH_2$), 148 (18) ($HBCH_2^+$), 134 (27) (BH), 133 (3) ($B^{+\cdot}$); IR 1625 cm⁻¹; UV ϵ_{241} 7500, ϵ_{297} 5100; $[\alpha]_D$ −38.2° (*c* 0.10, H_2O). Anal. ($C_{12}H_{14}O_3N_4$) C, H, N.

2-(2′,3′,4′-Tri-*O*-benzoyl-6′-deoxy-α-L-*manno*-hexopyranosyl)-6-nitro[1,2,4]triazolo[1,5-*a*]pyridine (16). 5 (7.76 g, 12 mmol) and 7[9] (1.85 g, 12 mmol) were refluxed in 50 mL of pyridine and 50 mL of CH_2Cl_2 for 5 days. A solid was filtered off, and the solution, concentrated to dryness, was dispersed in methanol and neutralized with 8 M NaOH. The solution was evaporated and the residue was chromatographed on a column of silica (CH_2Cl_2). The first fractions were discarded, then 2.95 g of crude 16 were recovered, triturated in a mixture (1:1) of Et_2O and petroleum ether, and gave 2.61 g (35%) of pure 16: mp 238 °C (EtOH); TLC (CH_2Cl_2–EtOH, 99:1) R_f 0.46; IR 1720, 1640, 1525, 710 cm⁻¹; $[\alpha]_D$ +118.2° (*c* 0.17, $CHCl_3$). Anal. ($C_{33}H_{26}O_9N_4$) C, H, N.

3,4-Di-*O*-benzoyl-1-cyano-2,6-dideoxy-L-*arabino*-hex-1-enopyranose (20). To a mixture of dry pyridine (15.6 mL) and 6.6 mL (57 mmol) of benzoyl chloride cooled at −10 °C was added, with stirring, by small portions in 2 h 1.31 g (8.5 mmol) of 19.[13] The mixture was then heated for 2 h at 60 °C and then treated at room temperature with 1.1 mL of H_2O, and stirring continued during 15 min. CH_2Cl_2 (20 mL) was added, and the solution was washed with cold water, 3 N H_2SO_4, a saturated solution of $NaHCO_3$, and finally with water. The organic phase, dried over Na_2SO_4, was brought to dryness. The residue was purified on silica (petroleum ether–AcOEt, 25:1). 20 (2.24 g, 74%) was recovered as a white oil pure on TLC. No satisfactory analysis could be obtained. Anal. Calcd for $C_{21}H_{17}O_5N$: C, 69.41; H, 4.72; N, 3.86. Found: C, 69.92; H, 5.91; N, 4.23.

Benzyl (3,4-Di-*O*-benzoyl-2,6-dideoxy-L-*arabino*-hex-1-enopyranosyl)thioimidate Hydrochloride (21). A solution of 2.07 g (5.7 mmol) of 20 and 0.7 mL of benzyl mercaptan in 21 mL of anhydrous Et_2O was saturated with dry HCl. The solution was kept in a refrigerator for 3 days. The crystalline product was isolated and washed with *n*-pentane and dry Et_2O: yield 2.74 g (92%); mp 137 °C. Anal. ($C_{28}H_{25}O_5N_5$·HCl) C, H, N.

2-(3′,4′-Di-*O*-benzoyl-2′,6′-dideoxy-L-*arabino*-hex-1′-enopyranosyl)-6-nitro[1,2,4]triazolo[1,5-*a*]pyridine (17). A mixture of CH_2Cl_2 and pyridine (1:1) (32 mL) containing 2.70 g of 21 (5.1 mmol) and 0.79 g (5.1 mmol) of 7 was refluxed for 3 days. The insoluble portion was filtered off, and the solution was brought to dryness, then dissolved in methanol, and neutralized with 8 N NaOH. The solution was evaporated, and the residue was chromatographed on a silica column (CH_2Cl_2). The purest fraction, 239 mg, was purified on a medium-pressure silica column (Lobar, Merck) (CH_2Cl_2–petroleum ether, 3:1): yield 168 mg (7%) after crystallization in Et_2O; mp 110 °C; TLC (CH_2Cl_2–EtOH, 99:1) R_f 0.65; IR 1720, 1640, 1525, 710 cm⁻¹; $[\alpha]_D$ +71.2° (*c* 0.10, $CHCl_3$). Anal. ($C_{26}H_{20}O_7N_4$) C, H, N.

2-(6′-Deoxy-α-L-*manno*-hexopyranosyl)-6-nitro[1,2,4]triazolo[1,5-*a*]pyridine (22). 16 (500 mg, 0.8 mmol) and CH_3ONa (250 mg, 4.6 mmol) were dispersed in 50 mL of methanol and stirred for 20 h. CG 50, 1 g (H^+ form), was added and after neutralization, the solution was filtered and evaporated; the residue was chromatographed on Bio-Gel. The pure fractions were lyophilized and crystallized in methanol: yield 180 mg (72%); mp 212 °C (EtOH); TLC (CH_2Cl_2–EtOH, 25:15) R_f 0.67; MS, *m/e* 310 (4) ($M^{+\cdot}$), 292 (<1) ($M^{+\cdot} - H_2O$), 221 (3), 207 (10), 205 (5), 193 (100) (HBCHO), 178 (6), 177 (6), 163 (8) ($B^{+\cdot}$); IR 1640, 1525 cm⁻¹; UV ϵ_{250} 17 300, ϵ_{315} 3900; $[\alpha]_D$ −25.8° (*c* 0.17, H_2O). Anal. ($C_{12}H_{14}O_6N_4$) C, H, N.

2-(2′,3′,4′-Tri-*O*-benzoyl-6′-deoxy-α-L-*manno*-hexopyranosyl)-6-amino[1,2,4]triazolo[1,5-*a*]pyridine (23). 16 (1.02 g, 1.6 mmol) was hydrogenated in 90 mL of CH_3COCH_3 over 10% Pd/C at room temperature and atmospheric pressure during 4 h. After filtration, the solution was evaporated and the residue chromatographed on a silica column (CH_2Cl_2–EtOH, 97:3) to yield 640 mg of 23 (66%): mp 237 °C; TLC (CH_2Cl_2–EtOH, 95:5) R_f 0.37; IR 1720, 1600 cm⁻¹; $[\alpha]_D$ +133.5° (*c* 0.17, $CHCl_3$). Anal. ($C_{33}H_{28}O_7N_4$) C, H, N.

2-(6′-Deoxy-α-L-*manno*-hexopyranosyl)-6-amino[1,2,4]triazolo[1,5-*a*]pyridine (24). 23 (640 mg, 1.1 mmol) treated with methanolic ammonia for 3 days gave after treatment a clear yellow powder, which was chromatographed on Bio-Gel and yielded 185 mg (62%) of 24: mp 205 °C (EtOH); TLC (CH_2Cl_2–EtOH, 25:15) R_f 0.27; MS, *m/e* 280 (8) ($M^{+\cdot}$), 205 (1), 191 (6), 177 (10) (BCH=CHOH), 175 (6), 163 (100) (HBCHO), 148 (5), 147 (3), 134 (4) ($BH^{+\cdot}$); IR 1620 cm⁻¹; UV ϵ_{255} 4700, ϵ_{309} 2600; $[\alpha]_D$ −28.7° (*c* 0.12, H_2O). Anal. ($C_{12}H_{16}O_4N_4$) C, H, N.

2-(6′-Deoxy-2′,3′-*O*-isopropylidene-α-L-*manno*-hexopyranosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (25). 10 (350 mg, 1.1 mmol) and *p*-toluenesulfonic acid (1 mg, 5.2×10^{-3} mmol) were stirred in 50 mL of CH_3COCH_3 and 3 mL of 2,2-dimethoxypropane overnight at room temperature. After treatment with sodium bicarbonate and filtration, the solvent was evaporated; the white foam obtained crystallized with ethyl acetate: yield 335 mg (85%); mp 120 °C; TLC (CH_2Cl_2–EtOH, 95:5) R_f 0.48; IR 1640, 1520 cm⁻¹; $[\alpha]_D$ −133.9° (*c* 0.17, $CHCl_3$). Anal. ($C_{15}H_{18}O_6N_4$) C, H, N.

2-(6′-Deoxy-2′,3′-*O*-isopropylidene-α-L-*lyxo*-hexopyranos-4′-ulosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (26). To a stirred mixture of 10.5 mL of CH_2Cl_2 and 0.39 mL of $(COCl)_2$ (5.0 mmol), cooled to −60 °C, was added 0.71 mL of dry Me_2SO (10 mmol) in 5 min; the stirring was continued for 5 min at −60 °C and then for 5 min at −40 °C. 25 (367 mg, 1.08 mmol) in 4.2 mL of CH_2Cl_2 was added (5 min) and the reaction, kept at −40

°C during 15 min, was stopped by the addition of 3.6 mL of *N,N*-diisopropylethylamine (21 mmol). After warming up to room temperature, the solution was diluted with 13.5 mL of H_2O, stirred for 10 min, and decanted.

The organic phase was dried over Na_2SO_4 filtered, and evaporated. The residue was chromatographed on a silica column (CH_2Cl_2–EtOH, 99:1): 225 mg of pure **26** was obtained (62%); mp 241 °C; TLC (CH_2Cl_2–EtOH, 97:3) R_f 0.54; IR 1725, 1630, 1520 cm^{-1}; $[\alpha]_D$ −212.5° (*c* 0.16, $CHCl_3$). Anal. ($C_{15}H_{16}O_6N_4$) C, H, N.

2-(6′-Deoxy-α-L-*lyxo*-hexopyranos-4′-ulosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (27). **26** (200 mg, 0.5 mmol) was stirred in 10 mL of 99% formic acid for 1.5 h. The solution was evaporated and the residue crystallized in methanol: yield 165 mg (93%); mp 180 °C (EtOH); TLC (CH_2Cl_2–EtOH, 8:2) R_f 0.69; MS, *m/e* 308 (1) ($M^{+\cdot}$), 290 (7) ($M^{+\cdot} - H_2O$), 235 (15) [HBC(O-H)=CHCHO], 207 (100) (BCH=CHCHO), 205 (16), 193 (96) (HBCHO), 191 (30) (BCH=CH_2), 178 (11), 177 (16), 164 (9), 163 (6) ($B^{+\cdot}$); IR 1730, 1630, 1520 cm^{-1}; UV ϵ_{225} 8700, ϵ_{327} 4800; $[\alpha]_D$ −102.7° (*c* 0.13, $CHCl_3$). Anal. ($C_{12}H_{12}O_6N_4$) C, H, N.

2-(6′-Deoxy-α-L-*lyxo*-hexopyranos-4′-ulosyl)-8-amino[1,2,4]triazolo[1,5-*a*]pyridine (28). **27** (375 mg, 1.2 mmol) dissolved in 100 mL of MeOH was hydrogenated over 10% Pd/C at room temperature and 1.5 kg/cm^2 of pressure during 4 h. After filtration, the solution was evaporated and the residue was chromatographed on Bio-Gel. Pure fractions crystallized in methanol: yield 220 mg (65%); mp 170 °C (EtOH–*n*-C_5H_{12}); TLC (CH_2Cl_2–EtOH, 25:15) R_f 0.58; MS, *m/e* 278 (11) ($M^{+\cdot}$), 260 (33) ($M^{+\cdot} - H_2O$), 205 (12), 177 (100) (BCH=CHOH), 175 (20), 163 (87) (HBCHO), 161 (52) (BCH=CH_2), 148 (14), 147 (10), 134 (24), 133 (5), ($B^{+\cdot}$); IR 1730, 1625 cm^{-1}; UV ϵ_{270} 7300, ϵ_{297} 5700; $[\alpha]_D$ −59.9° (*c* 0.10, H_2O). Anal. ($C_{12}H_{14}O_4N_4$) C, H, N.

2-(6′-Deoxy-2′,3′-*O*-isopropylidene-α-L-*tallo*-hexopyranosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (29). To a stirred suspension of 1.02 g of **26** (2.9 mmol) and 1.08 g (2.9 mmol) of $CeCl_3$ (7 H_2O) in 24 mL of MeOH, kept at 0 °C, was added in portions, in 2 h, 200 mg of $NaBH_4$ (5.3 mmol). The solution was diluted with 50 mL of CH_2Cl_2 and washed with water saturated with NaCl. The organic phase, dried over Na_2SO_4, was evaporated and the residue chromatographed on silica (CH_2Cl_2–EtOH, 98.5:1.5): 424 mg of pure **29** (41%) was obtained; mp 115 °C (Et_2O–*n*-C_5H_{12}); TLC (CH_2Cl_2–EtOH, 96:4) R_f 0.50; IR 1635, 1530 cm^{-1}; $[\alpha]_D$ −150.9° (*c* 0.11, $CHCl_3$). Anal. ($C_{15}H_{18}O_6N_4$) C, H, N.

2-(6′-Deoxy-α-L-*tallo*-hexopyranosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (30). **29** (220 mg, 0.63 mmol) was treated with HCOOH as described above. The residue obtained by evaporation was chromatographed on Bio-Gel. The pure product, after lyophilization, crystallized in methanol: yield 141 mg (72%); mp 159 °C (AcOEt–*n*-C_5H_{12}); TLC (CH_2Cl_2-EtOH, 8:2) R_f 0.44; MS, *m/e* 310 (<1) ($M^{+\cdot}$), 293 (6) ($M^{+\cdot} - 17$), 235 (2), 221 (6), 207 (51) (BCH=CHOH), 205 (9), 193 (100) (HBCHO), 178 (7), 177 (14) (B=CH_2), 163 (5) ($B^{+\cdot}$); IR 1650, 1540 cm^{-1}; UV ϵ_{227} 9800, ϵ_{328} 5100; $[\alpha]_D$ −109.1° (*c* 0.13, H_2O). Anal. ($C_{12}H_{14}O_6N_4$) C, H, N.

2-(2′,6′-Dideoxy-L-*erythro*-hex-1′-enopyranos-3′-ulosyl)-8-nitro[1,2,4]triazolo[1,5-*a*]pyridine (31). To a stirred solution of 500 mg (1.7 mmol) of **13** in 15 mL of DMF was added 5 g (58 mmol) of activated MnO_2.[26] After 16 h the solvent was evaporated. The powder was treated with CH_2Cl_2 and then with a mixture CH_2Cl_2–EtOH (1:1) and decanted. The organic solutions were brought to dryness and chromatographed on a silica column (CH_2Cl_2-EtOH, 99.5:0.5); pure fractions crystallized: yield 201 mg (40%); mp 211 °C (EtOH); TLC (CH_2Cl_2–EtOH, 95:5); R_f 0.53; CIMS (isobutane, 160 °C, 5.5 eV), *m/e* 291 (MH^+), 261 ($MH^+ - NO$); IR 1675, 1640, 1540 cm^{-1}. Anal. ($C_{12}H_{10}O_5N_4 \cdot 0.25C_2H_5OH$) C, H, N.

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Preparation and Analgesic Properties of Amino Acid Derivatives of (−)-5,9α-Diethyl-2′-hydroxybenzomorphan

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The *N*-arginyl derivative of methionine-enkephalin (fragment 60–65 of β-lipotropin) has been shown to be equiactive with the parent pentapeptide, despite the fact that the tyrosine amino group in this compound has been neutralized by the formation of an amide linkage. A series of *N*-(amino acid) derivatives of (−)-5,9α-diethyl-2′-hydroxybenzomorphan was prepared and evaluated for analgesic activity. In vitro activities were found to vary greatly, depending on the nature of the amino acid used. The *N*-arginyl derivative was found to be equipotent to (−)-5,9α-diethyl-2′-hydroxybenzomorphan and also to methionine-enkephaline in the naloxone binding assay.

The discovery of the "endogenous opioid substances", the enkephalins[1] and the endorphins,[2] as proposed neurotransmitters or neuroregulators involved in the perception of pain was rekindled interest in the field of opioid analgesics. It has strengthened the belief that agents can be found which will be both potent analgesics and free of the deleterious side effects (tolerance, physical dependance, etc.) normally associated with opioid analgesics. In the past several years, a massive body of evidence has been accumulated on structure–activity relationships of the enkephalins[3] such that a fairly clear picture has emerged

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