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SYNTHESIS OF NITROGEN-15-LABELED 2-AMINO(GLYCOFURANO)OXAZOLINES *via* GLYCOSYLAMINE INTERMEDIATES*

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ABSTRACT

A new, efficient synthesis of doubly ^{15}N-labeled 2-amino-oxazoline derivatives of pentoses and hexoses has been delineated that involves treatment either of unprotected or *O*-isopropylidenated glycosylamines with cyanamide-$^{15}N_2$ in methanol to give 2-amino(glycofurano)oxazolines-$^{15}N_2$. A probable mechanism for these reactions is presented. These techniques provide a practical means by which a variety of stable or radioactive isotopes can be introduced into any of several known, clinically significant pyrimidine anhydronucleosides, such as 2,2′-anhydro-(1-β-D-arabinofuranosylcytosine) (cyclo-C).

INTRODUCTION

During the past ten years, oxazoline derivatives of carbohydrates have been employed by various workers[1–7] in the stereospecific synthesis of such anti-tumor nucleosides as 1-β-D-arabinofuranosylcytosine (ara-C). An objective of our work[8] was to prepare all of the 2-amino-oxazoline derivatives in the pentose and hexose series, for the purpose of spectral comparisons, and eventual conversion into anhydronucleosides. We now report a new, efficient synthesis of di-^{15}N-labeled 2-amino-oxazoline derivatives of pentoses and hexoses that involves treatment either of unprotected or *O*-isopropylidenated glycosylamines with cyanamide-$^{15}N_2$ in methanol, to yield 2-amino(glycofurano)oxazolines-$^{15}N_2$.

RESULTS AND DISCUSSION

Synthesis. — Crystalline glycosylamines **1**, **2**, and **3**, of known configuration and ring-size, were prepared from D-arabinose, D-ribose, and D-xylose, respectively, by literature methods[9,10], and were treated with cyanamide-$^{15}N_2$ in

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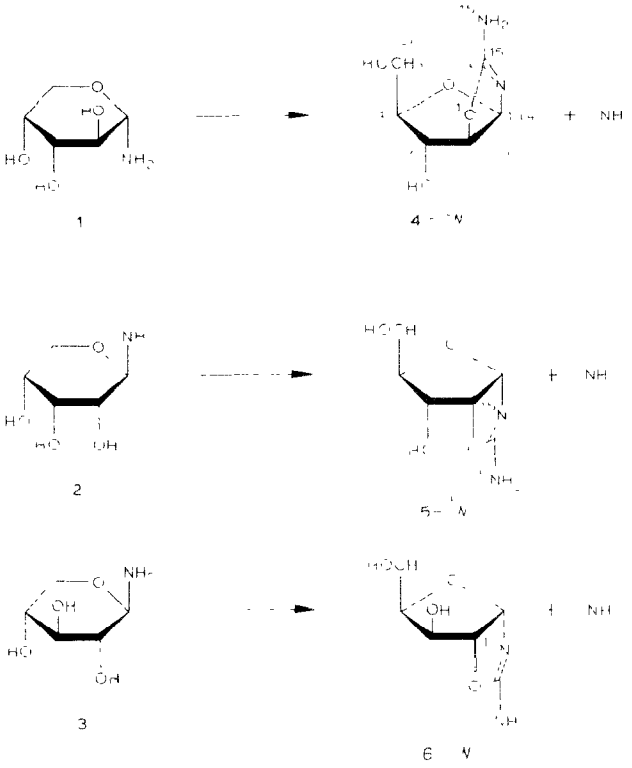
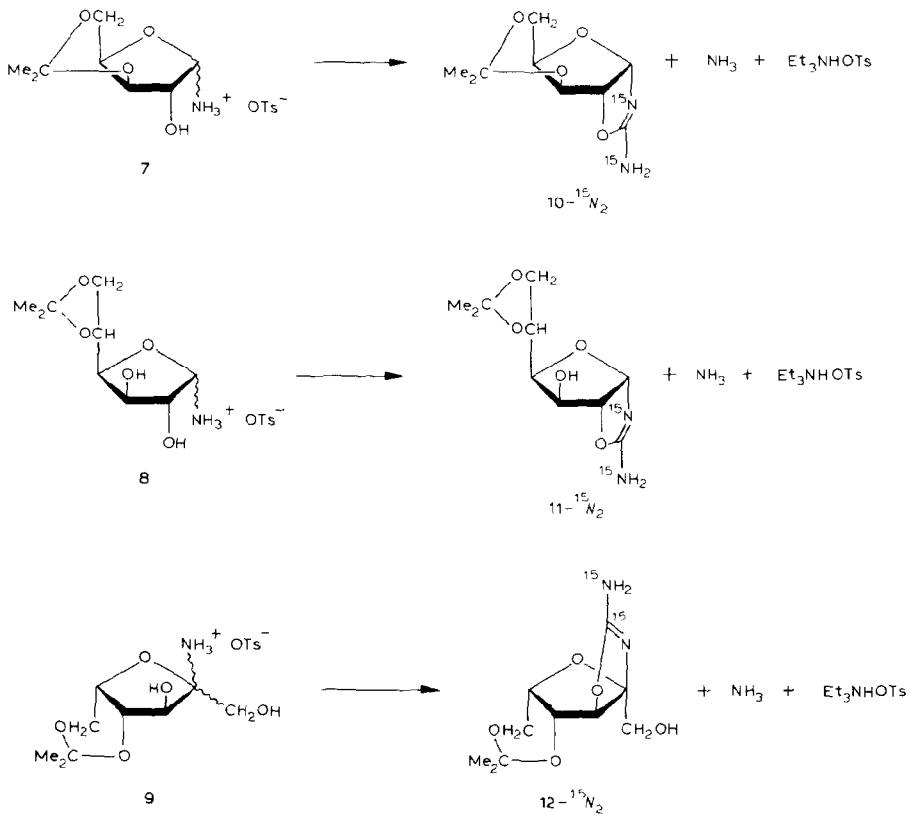
methanol to yield the corresponding 2-amino(glycofurano)oxazoline-$^{15}N_2$ derivatives (**4**-, **5**-, and **6**-$^{15}N_2$, respectively.

O-Isopropylidenated, di-^{15}N-labeled 2-amino(glycofurano)oxazoline derivatives (**10**-, **11**-, and **12**-$^{15}N_2$) of D-xylose, D-glucose, and L-sorbose, respectively, were prepared by treatment of the known, crystalline *O*-isopropylidenated glycofuranosylamine salts[11,12] (**7**, **8**, and **9**) with cyanamide-$^{15}N_2$ in methanol under basic conditions



The yields of these labeled compounds have not all been optimized, but they are, in general, good (46–92% isolated). Dicyandiamide and the free sugar were isolated, and identified, as minor by-products. Reverse-phase, liquid chromatography (l.c.) proved to be a valuable method for purification of pentose and hexose oxazoline derivatives, the structures and purities of which have been analyzed by ^{1}H-, ^{13}C-, and ^{15}N-nuclear magnetic resonance (n.m.r.) spectroscopy, and by electron-impact, mass spectrometry. The detailed, ^{15}N-n.m.r.- and mass-spectral data supported the structures assigned.

The synthesis of di-^{15}N-labeled 2-amino-oxazolines employing glycosylamine intermediates is equally well suited for the incorporation of other isotopic labels. For example, **4**-[2-^{13}C] has been prepared by treatment of **1** with cyanamide-^{13}C, and **4**-[2-^{13}C, $^{15}N_2$], by treatment of **1** with cyanamide-^{13}C, $^{15}N_2$.



Mechanism. — A probable mechanism for the formation of 2-amino-oxazoline derivatives from glycosylamines and cyanamide in methanol at room temperature is now presented.

As a change in ring size was observed for the three, unprotected glycopyranosylamines (**1, 2,** and **3**), an acyclic intermediate is suggested, instead of a cyclic, carbonium ion. Furthermore, in no instance was any methyl glycoside observed that might have arisen from reaction of a cyclic, carbonium-ion intermediate with the solvent. Therefore, it is now postulated that the key intermediate in the formation of these oxazoline derivatives is imonium ion **13**, which undergoes a transimination reaction with labeled cyanamide to give **14**-$^{15}N_2$, and this then cyclizes to give furanoid 2-amino-oxazolines-$^{15}N_2$. The retention of both labels from the cyanamide-$^{15}N_2$ indicates that the nitrogen atom of the glycosylamine is lost. Precedent for the existence, or involvement, of an imonium ion was provided by Isbell and Frush[13], who postulated an imonium-ion intermediate in the mutarotation and hydrolysis of glycosylamines. In the case of the glycosylamines derived from D-arabinose, D-ribose, and D-xylose, the formation of only furanoid 2-amino-oxazolines (**4, 5,** and **6,** respectively) was observed.

Other workers[2,4,7] observed that 2-amino(glycofurano)oxazolines are also

formed on heating free aldoses with cyanamide in the presence of a weak-base catalyst (*e.g.*, a hydrogencarbonate). We have found, however, that the use of a stronger-base catalyst results in excessive formation of dicyandiamide, and isomerization of the sugar. In the present method for the synthesis of oxazoline derivatives, it appears that glycosylamines are inherently more reactive than free aldoses in reactions with cyanamide, as neither heat nor a base catalyst is required. This minimizes the formation of dicyandiamide and other cyanamide polymers.

By monitoring the ^{1}H-n.m.r. spectra of solutions of 2-amino-(glycofurano)oxazolines in deuterium oxide at room temperature, there were detected slow changes in their spectra that could be attriuted to hydrolysis. Hence, it seems prudent to restrict the use of heat in these oxazoline syntheses whenever possible.

In a survey of various bases that might be used to catalyze the reaction of free aldoses with cyanamide, satisfactory results were obtained with either hydrogencarbonates or carbonates, in water or methanol, at room temperature. Of the various salts tried, concentrated aqueous ammonium hydrogencarbonate, or guanidinium carbonate in methanol, gave the best results. Based on the results of syntheses using glycosylamine intermediates and labeled cyanamide, it seems appropriate to postulate imonium-ion intermediates (**13** and **17**) in these oxazoline

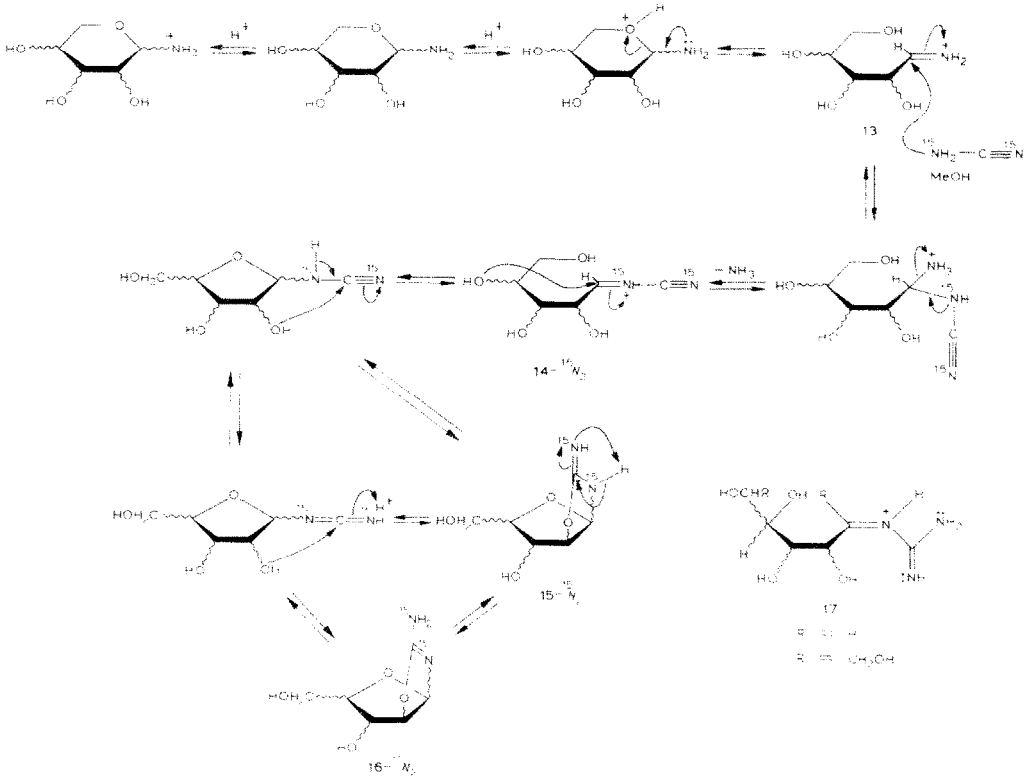


TABLE I

PROTON CHEMICAL-SHIFTS[a] (δ) AND COUPLING CONSTANTS (Hz) OF 2-AMINO(GLYCO)OXAZOLINE DERIVATIVES

Deriv-ative	Sol-vent	H-1'[b]	H-1''	H-2'	H-3'	H-4'	H-5'	H-5''[b]	H-6'	H-6''	CH_3	Exchangeable sites: NH	HO-1'	HO-3'	HO-5'
4	D_2O	5 91d[c]		4 93q	4 33q	4.01o	3 60q	3 53q	—	—	—	—	—	—	
		$J_{1',2'}$ 5 9		$J_{2',3'}$ 1 0	$J_{3',4'}$ 3 4	$J_{4',5'}$ 5 4	$J_{4',5''}$ 6 8	$J_{5',5''}$ −12 2							
	Me_2SO	5 68d		4 57q	4 01q	3 67o	3 30q	3 25q	—	—	—	6 28,2H	—	5 39d	4 69t
		$J_{1',2'}$ 5 6		$J_{2',3'}$ 1 3	$J_{3',4'}$ 3 2	$J_{4',5'}$ 5 9	$J_{1',5}$ 7 0	$J_{5',5''}$ −11 3						$^3J_{3',HO-3'}$ 4 4	$^3J_{5',HO-5'}$ 5 2
5	D_2O	5 80d		4 98t	4 12q	3 61o	3 92q	3 73q	—	—	—	—	—	—	—
		$J_{1',2'}$ 4 9		$J_{2',3'}$ 5 6	$J_{3',4'}$ 9 5	$J_{4',5'}$ 2 4	$J_{4',5''}$ 4 6	$J_{5',5''}$ −12 7							
	Me_2SO	5 59d		4 63t	3 74q	3 30o	3 66q	3 42q	—	—	—	6 27,2H	—	5 17d	4 60t
		$J_{1',2'}$ 5 1		$J_{2',3'}$ 5 5	$J_{3',4'}$ 9 3	$J_{4',5'}$ 2 4	$J_{4',5''}$ 5 8	$J_{5',5''}$ −11 7						$^3J_{3',HO-3'}$ 5 9	$^3J_{5',HO-5'}$ 5 6
6	D_2O[d]	5 94d		4 89d	4 37d	3 86m	3 89m	3 80m	—	—	—	—	—	—	—
		$J_{1',2'}$ 5 1		$J_{2',3'}$ <0 5	$J_{3',4'}$ 2 7	$J_{4',5'}$ 4 9	$J_{4',5''}$ 6 8	$J_{5',5''}$ −11 5							
	Me_2SO[e]	5 72d		4 53d	4 00d	3 50m	3 63m	3 50m	—	—	—	6 23,2H	—	5 13d	4 56t
		$J_{1',2'}$ 5 1		$J_{2',3'}$ <0 5	$J_{3',4'}$ 2 6	$J_{4',5'}$ 5 0	$J_{4',5'}$ 7 0	$J_{5',5''}$ −11 0						$^3J_{3',HO-3'}$ 4 9	$^3J_{5',HO-5'}$ 2 9
10	D_2O	5 95d	—	4 87d	4 65d	3 78m	4 34q	4 03d	—	—	1 40,3H	—	—	—	—
		$J_{1',2'}$ 5 1		$J_{2',3'}$ <0 5	$J_{3',4'}$ 2 0	$J_{4',5'}$ 2 2	$J_{4',5''}$ <0 5	$J_{5',5''}$ 13 9			1 55,3H				
	Me_2SO	5 79d	—	4 57d	4 29d	3 43m	4 08q	3 80d	—	—	1 40,3H	6 33	—	—	—
		$J_{1',2'}$ 5 4		$J_{2',3'}$ <0 5	$J_{3',4'}$ 2 0	$J_{4',5'}$ 2 9	$J_{4',5}$ <0 5	$J_{5',5}$ 13 2			1 24,3H				
	$CDCl_3$	6 08d	—	4 80d	4 37	3 69	4 14d	4 08d		—	1 47,3H	—	—	—	—
		$J_{1',2'}$ 5 0		$J_{2',3'}$ <0 5	$J_{3',4'}$ <0 5	$J_{4',5'}$ <0 5	$J_{4',5''}$ <0 5	$J_{5',5''}$ −13 1			1 40,3H				
11	D_2O	5 93d	—	4 90d	4 40d	3 76q	4 43m	—	4 21q	4 03q	1 46,3H	—	—	—	—
		$J_{1',2'}$ 5 3		$J_{2',3'}$ <0 5	$J_{3',4}$ 2 7	$J_{4',5'}$ 8 0	$J_{5',6'}$ 6 6		$J_{5',6'}$ 5 8	$J_{6',6''}$ −8 4	1 40,3H				
	Me_2SO	5 74d	—	4 53d	4 00d	3 37q	4 24m	—	3 98q	3 77q	1 30,3H	6 28	—	5 37d	—
		$J_{1',2'}$ 5 1		$J_{2',3'}$ <0 5	$J_{3',4'}$ 2 7	$J_{4',5'}$ 7 5	$J_{5',6'}$ 6 4		$J_{5',6}$ 5 9	$J_{6',6}$ −8 3	1 26,3H			$^3J_{3',HO\ 3}$ 5 1	
12'	D_2O	3 77d	3 79d	—	4 70	4 64d	3 86m	—	4 32q	4 01d	1 54,3H	—	—	—	—
		$J_{1',1''}$ −11 8			$J_{3',4'}$ <0 5	$J_{4',5'}$ 2 4	$J_{5',6'}$ 2 0		$J_{5,6'}$ <0 5	$J_{6',6'}$ −14 2	1 40,3H				
	Me_2SO	3 53d	3 39d	—	4 38	4 29d	3 50m	—	4 05q	3 77d	1 39,3H	6 24	4 58	—	—
		$J_{1',1''}$ −10 8			$J_{3',4'}$ <0 5	$J_{4',5'}$ 2 2	$J_{5',6'}$ 2 4		$J_{5',6'}$ <0 5	$J_{6',6}$ −13 3	1 24,3H				
	$CDCl_3$	3 81d	3 86d	—	4 73	4 38d	3 76m	—	4 12q	4 07d	1 46,3H		$^3J_{1',HO-1'}$ 5 6		$^3J_{1',HO-1'}$ 6 3
		$J_{1',1}$ −11 5			$J_{3',4'}$ <0 5	$J_{4',5'}$ 2 2	$J_{5,6}$ 2 5		$J_{5',6}$ <0 5	$J_{6',6}$ 13 4	1 39,3H				

[a]Measured at 400 MHz, with spectral assignments verified by selective proton-decoupling. [b]Single primes denote numbering of atoms on the sugar ring-atoms. Double primes are reserved for the labeling of the upfield proton (usually) of a methylene group. [c]Signal multiplicities: d, doublet; m, multiplet; o, octet; q, quartet; t, triplet. [d]Refined values computed by means of an iterative, best fit of the six-spin system of **6** in deuterium oxide solution. [e]Several values for **6** in dimethyl sulfoxide-d_6 are approximations, because of solvent interference and second-order coupling of H-4, H-5, and H-5'. [f]A solvent titration of a solution of **12**-$^{15}N_2$ in dimethyl sulfoxide-d_6 with $CDCl_3$ revealed a crossover of the H-1' and H-1'' signals. As H-1' and H-1'' were strongly coupled in D_2O solution, their signals were subjected to an AB analysis. The chemical-shift values reported are those calculated from this analysis.

TABLE II

HETERONUCLEAR COUPLING-CONSTANTS (Hz) OF DI-NITROGEN-15-LABELLED 2-AMINO(GLYCO)OXAZOLINES

Derivative	*Solvent*	*^{15}N-^{1}H Coupling constants*[a,b]			*^{13}C-^{1}H Coupling constants*[a,b]		*^{13}C-^{15}N Coupling constants*[b,c]		
		$^{1}J_{N-2,H}$	$^{2}J_{N-3,H-1'}$	$^{3}J_{N-3,H-1''}$	$^{3}J_{C-2,H-1'}$	$^{3}J_{C-2,H-2'}$	$^{1}J_{C-2,N-2}$	$^{1}J_{C-2,N-3}$[d]	$^{2}J_{C-1',N-3}$
4-$^{15}N_2$	D_2O	—	5.4	—	—	—	—	—	—
	Me_2SO	—[e]	5.9	—	—	—	24.4	4.9	—
4-[2-^{13}C]	D_2O	—	—	—	6.2	2.0	—	—	—
4-[2-^{13}C,$^{15}N_2$][f]	Me_2SO	—	—	—	—	—	24[f]	—[e,f]	—
5-$^{15}N_2$	D_2O	—	5.4	—	—	—	—	—	—
	Me_2SO	—[e]	5.4	—	—	—	24.4	4.9	—
6-$^{15}N_2$	D_2O	—	5.4	—	—	—	—	—	—
	Me_2SO	88.1	5.6	—	—	—	24.4	4.9	—
10-$^{15}N_2$	D_2O	—	5.4	—	—	—	—	—	—
	Me_2SO	88.9	5.3	—	—	—	24.4	4.9	—
	$CDCl_3$	—[e]	5.0	—	—	—	—	—	—
11-$^{15}N_2$	D_2O	—	5.3	—	—	—	—	—	—
	Me_2SO	86.2	5.9	—	—	—	24.4	4.9	—
12-$^{15}N_2$	D_2O	—	—	1.5,2.2	—	—	—	—	—
	Me_2SO	81.4	—	2.7	—	—	24.4	4.9[g]	6.1
	$CDCl_3$	—[e]	—	2.0	—	—	—	—	—

[a]These measurements were made by observing ^{1}H-n.m.r.spectra at 400 MHz. [b]Single primes denote numbering of atoms on the sugar ring-atom. Double primes are reserved for the labeling of the upfield proton (usually) of a methylene group. [c]These measurements were made by observing ^{13}C-n.m.r. spectra at 100.6 MHz and 316 K. [d]$^{1}J_{C-1',N-3}$ is unresolved in all cases, presumably due to broadening by exchange. [e]Unresolved, presumably due to broadening by chemical exchange. [f]This measurement was made by observing the ^{15}N-n.m.r. spectrum at 40.5 MHz and 316 K. [g]$^{1}J_{C-2',N-3}$ is unresolved, presumably due to broadening by exchange.

TABLE III

CARBON-13 CHEMICAL-SHIFTS[a] (δ_C) AND ONE-BOND, CARBON-13–PROTON COUPLING-CONSTANTS (Hz) OF 2-AMINO(GLYCO)OXAZOLINES

Derivative	*C-2*[b]	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	C(*Me*)$_2$	C(CH$_3$)$_2$
4 δ_C	162.1	99.9	88.0	75.6	84.5	61.5	—	—	—
$^1J_{13_{C,H}}$		171	160	148	147	140	—	—	—
5	163.7	98.1	80 7	71.2	77.8	60 4	—	—	—
		169	164	143	139	140, 144	—	—	—
6	162 2	98 8	86.3	73 4	78.6	58.0	—	—	—
		172	162	152	141	142	—	—	—
10	162 3	98.8	85.2	72.8	68.6	59.2	—	97.0	28.8, 18.8
		174	165	154	145	142, 150	—	—	—
11	162.6	99.2	86 2	73 2	78 5	72.1	66.4	107 9	26.6, 25.3
		166	164	155	148	152	150	—	—
12	161 7	63.7	96.9	85.3	72.9	69.5	59.3	109 4	28.9, 18.8
		142	—	163	153	146	141, 149	—	—

[a]In p.p.m. from internal tetramethylsilane in Me_2SO at 100.6 MHz and 316 K. Spectral assignments were verified by selective proton-decoupling. [b]Single primes denote numbering of atoms in the sugar chain.

syntheses as well. Hence, the imonium ion **17** is expected to show reactivity similar to that of **13** with cyanamide. Generation of the reactive, imonium ions (**13** and **17**) *in situ* serves as an adjunct to actual preforming of the glycosylamines.

N.m.r. spectroscopy. — The ^{1}H-, ^{13}C-, and ^{15}N-n.m.r. spectra (see Tables I–III) of the 2-amino(glycofurano)oxazolines (**4–6** and **10–12**) show evidence for chemical exchange of labile protons, in that certain resonances are broadened (see Figs. 1–3). In addition, these data suggest that a single tautomer (**16**-$^{15}N_2$), having an endocyclic double bond, preponderates in the equilibrium. The most compelling evidence for preponderance of the tautomer **16**-$^{15}N_2$ that contains an exocyclic NH_2 group is the observation of a triplet ($^{1}J_{15_{N,2H}}$ ~85 Hz) in the proton-coupled, ^{15}N-n.m.r. spectra[14] of **4**-, **5**-, **6**-, **10**-, **11**-, and **12**-$^{15}N_2$ (see Fig. 3). Other evidence for the endocyclic, double bond is the following: *(a)* fairly large values of $^{2}J_{15_{N,3H}}$ (~5.4 Hz), *(b)* chemical-shift equivalence of the exchange-broadened, NH signals

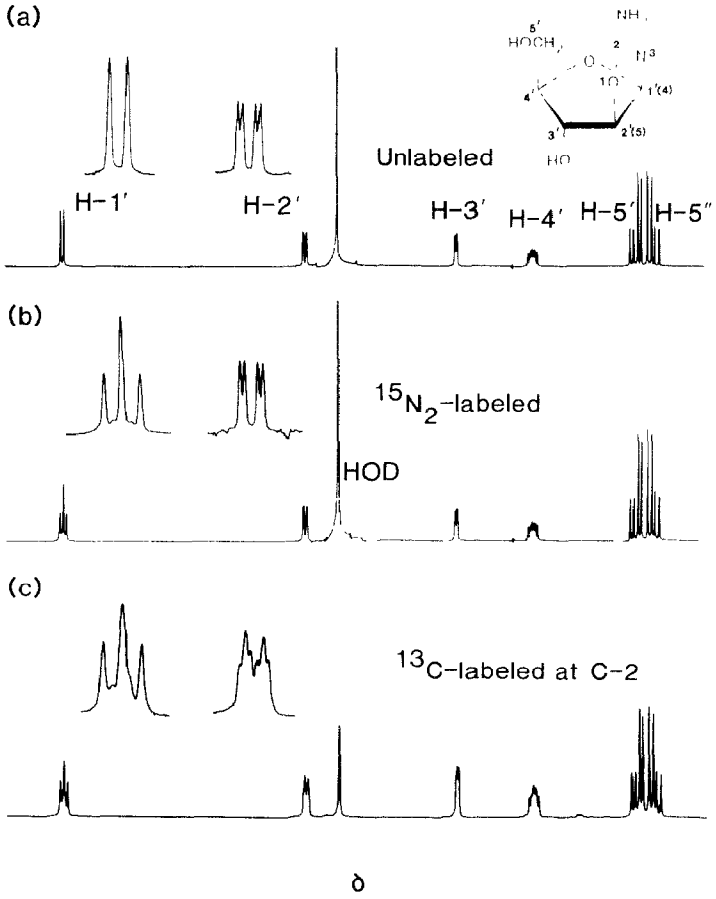


Fig. 1. ^{1}H-N.m.r. spectra at 400 MHz. (a) 2-amino-(1,2-dideoxy-β-D-arabinofurano)[1,2-*d*]-2-oxazoline (**4**), (b) its ^{15}N-labeled derivative (**4**-$^{15}N_2$), and (c) its ^{13}C-labeled derivative (**4**-[2-^{13}C]), each in solution in deuterium oxide

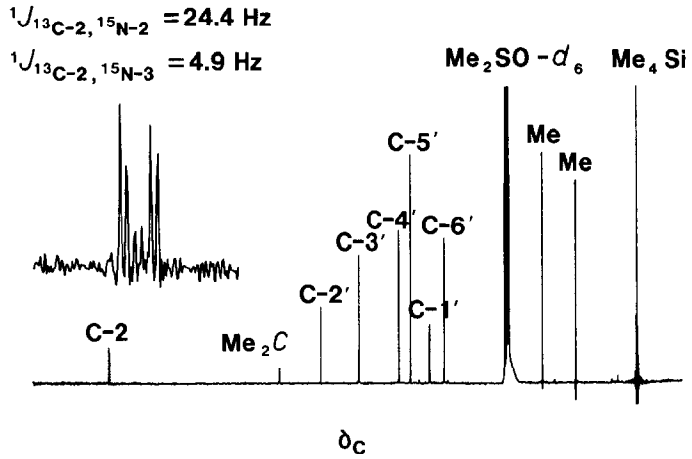


Fig. 2. Proton-decoupled, ^{13}C-n.m.r spectrum of 2-amino-(2,3-dideoxy-4,6-*O*-isopropylidene-β-L-sorbofurano)[2,3-*d*]-2-oxazoline-$^{15}N_2$ (**12**-$^{15}N_2$) in solution in dimethyl sulfoxide-d_6 (13.6mM) at 100.6 MHz and 316 K. [*Insert:* Spectrum expansion showing the one-bond ^{13}C–^{15}N coupling-constants at C-2].

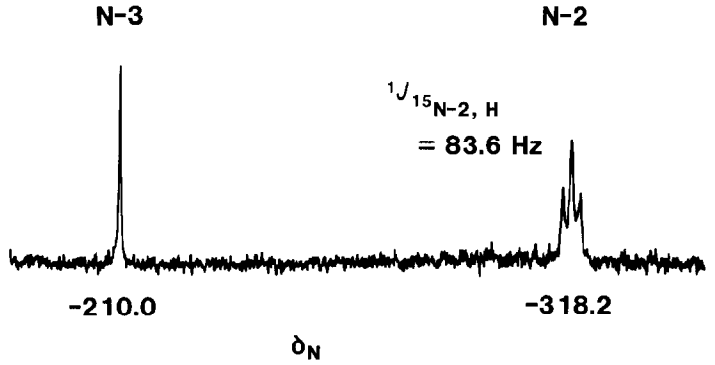


Fig. 3. Proton-coupled, ^{15}N-n.m.r. spectrum (71,833 scans) of 2-amino-(2,3-dideoxy-4,6-*O*-isopropylidene-β-L-sorbofurano)[2,3-*d*]-2-oxazoline-$^{15}N_2$ (**12**-$^{15}N_2$) in solution in dimethyl sulfoxide-d_6 (13.6mM) at 40.5 MHz and 298 K. The one-bond, ^{15}N–^{1}H coupling-constant is indicated. [The ^{15}N chemical-shifts are given in p.p.m. upfield from the nitrate ^{15}N signal of saturated, aqueous $NH_4^{15}NO_3$ in an external, reference, capillary tube]

in dimethyl sulfoxide solutions at 298 K, and *(c)* relatively large values of $^3J_{^{13}C\text{-}2,H\text{-}1'}$ and $^3J_{^{13}C\text{-}2,H\text{-}2'}$ (6.2 and 2.0 Hz, respectively).

The 2-amino(glycofurano)oxazolines (**4**, **5**, **10**, **11**, and **12**) all yielded well dispersed, first-order ^{1}H-n.m.r. spectra at 400 MHz; however, in the spectrum of compound **6**, the H-4′, H-5′, and H-5″ signals were observed as strongly coupled, overlapping multiplets. The parameters for the six-spin system of **6** in deuterium oxide solution were refined by iteration and simulation, and good agreement was obtained between experimental and theoretical spectra (see Fig. 4).

The 400-MHz, ^{1}H-n.m.r. spectra of solutions of the di-^{15}N-labeled 2-amino-(2,3-dideoxy-4,6-*O*-isopropylidene-β-L-sorbofurano)-[2,3-*d*]-2-oxazoline (**12**-$^{15}N_2$)

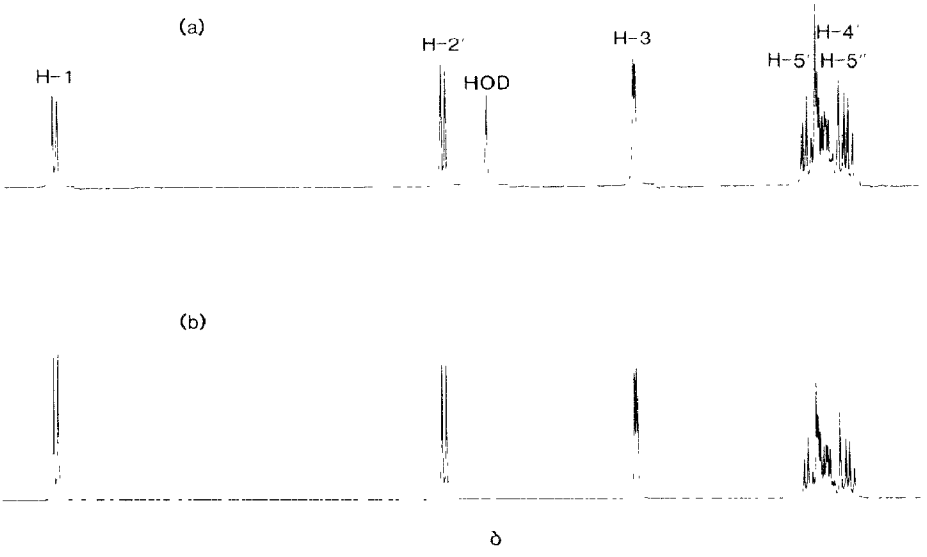


Fig 4. Experimental (a) and simulated (b) ^{1}H-n m r spectra of 2-amino-(1,2-dideoxy-α-D-xylofurano)[1,2-*d*]-2-oxazoline (**6**) in solution in deuterium oxide at 400 MHz

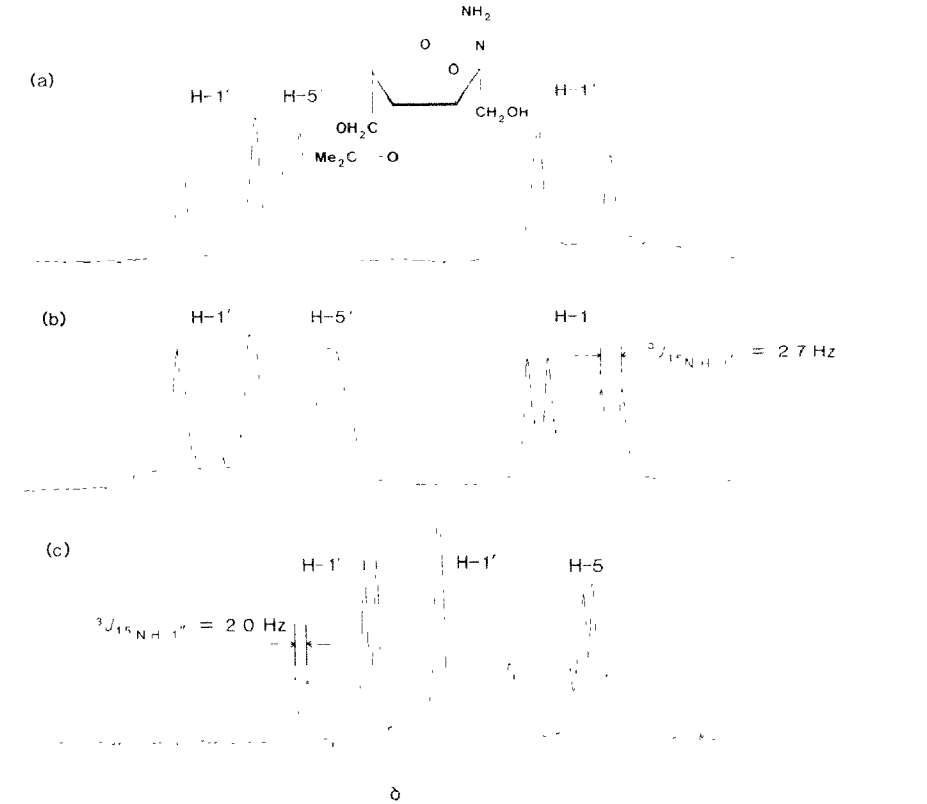


Fig 5 Partial, ^{1}H-n m r spectra at 400 MHz. (a) 2-amino-(2,3-dideoxy-4,6-*O*-isopropylidene-β-L-sorbofurano)[2,3-*d*]-2-oxazoline (**12**) and (b) its ^{15}N-labeled derivative (**12**-$^{15}N_2$), each in solution in dimethyl sulfoxide-d_6, and (c) a solution of **12**-$^{15}N_2$ in chloroform-*d* [The vicinal, ^{15}N-^{1}H coupling-constant of H-1'' is indicated in (b) and (c)]

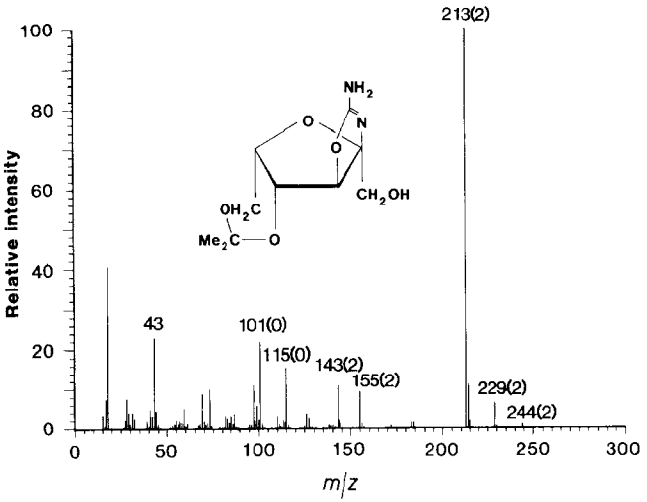


Fig 6. Electron-impact, mass spectrum of 2-amino-(2,3-dideoxy-4,6-*O*-isopropylidene-*β*-L-sorbofurano)[2,3-*d*]-2-oxazoline (**12**) [The mass shifts for the ^{15}N-labeled derivative (**12**-$^{15}N_2$) are shown in.parentheses.]

in various solvents displayed an apparent variation of vicinal coupling-constants of ^{15}N-3 with H-1′and H-1″ (see Fig. 5), suggesting the possibility of a differential solvent-shift of H-1′and H-1″. In order to confirm this possibility, a solvent titration of a solution of **12**-$^{15}N_2$ in dimethyl sulfoxide-d_6 was performed by addition of $CDCl_3$, and this revealed that the chemical shifts of H-1′ and H-1″ do, in fact, cross over as the proportion of $CDCl_3$ is increased.

Mass spectrometry. — The electron-impact, mass spectra of the ^{15}N-labeled 2-amino(glycofurano)oxazolines support the furanoid structures assigned, and confirm that *both* ^{15}N-labels of the cyanamide-$^{15}N_2$ are inserted into the 2-amino-oxazolines (see Fig. 6). The percentage incorporation of the two labels was found to be quantitative within experimental error for all compounds, except the L-sorbose derivative, for which ~9% of one of the ^{15}N-labels was not incorporated, as determined by examination of the relative abundances of the $[M - CH_2OH]^+$ fragment. This corresponds to the presence of ~16% of singly ^{15}N-labeled material. We have independently shown by ^{13}C-n.m.r. spectroscopy that the site of this isotope dilution in **12**-$^{15}N_2$ is specifically the exocyclic nitrogen atom*. The mechanism of this isotope dilution is unclear at this time.

*This is evident from the ^{13}C-n.m.r. spectrum of **12**-$^{15}N_2$ (see Fig. 2), where a low-lying doublet with a coupling constant of 4.9 Hz (~16% intensity) is observed between the doublet of doublets (C-2) at 161.7 p.p.m. The value of $^1J_{C\text{-}2,^{15}N}$ (4.9 Hz) arises from coupling of ^{15}N-3 to C-2. This small coupling was unequivocally assigned to ^{15}N-3 by observing the ^{1}H-decoupled, ^{15}N-n m.r. spectrum of 4-[2-^{13}C, $^{15}N_2$], where the coupling $J_{^{13}C,^{15}N}$ to the ^{15}N nucleus represented by the upfield signal was observed to be 24.4 Hz. The upfield, ^{15}N signal had previously been assigned to N-2 (see Fig. 3).

EXPERIMENTAL

General. — Melting points were measured with a silicone-oil, convection apparatus and are uncorrected. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tennessee. Optical rotations and infrared spectra were measured with a Perkin–Elmer model 141 polarimeter and Infracord spectrometer, respectively. Thin-layer chromatography (t.l.c.) was conducted on glass-backed layers of silica gel G (Analtech). For visibilization of the t.l.c. spots after development, 10% (v/v) H_2SO_4 in H_2O and a saturated solution of 2,6-dichloroquinone-4-chloroimide[4] in ethanol were used as spray reagents, with heating of the plates to 140°. N.m.r. spectra (^1H, ^{13}C, and ^{15}N) were recorded in the pulse, Fourier-transform (F.t.) mode at 400, 100.6, and 40.5 MHz, respectively, with a Bruker Instruments model WM-400 spectrometer. ^1H-N.m.r. spectra were recorded with a 90° pulse (13 μs), a 16,384-point data-set, and a spectral width of 4 kHz. ^{13}C-N.m.r. spectra were recorded with a 90° pulse (24 μs), broadband, 2-level proton-decoupling at 316 K, with a repetition time of 1.23 s, a 16,384-point data-set, a spectral width of 20 kHz, and field-frequency stabilization on solvent deuterium. Proton-coupled and selectively proton-decoupled, ^{13}C-n.m.r. spectra were obtained with a 45° pulse (12 μs) and a repetition time of 0.41 s. ^{15}N-N.m.r. spectra were obtained with a 20°pulse (10 μs), a 16,384-point data-set, and a spectral width of 20 kHz. ^1H-N.m.r. spectra were obtained with 5-mm sample-tubes, ^{13}C spectra with 10-mm sample-tubes, and ^{15}N spectra with 15-mm sample-tubes. Solutions for ^1H-n.m.r. spectroscopy contained 1–5 mg of solute in 0.5 mL of chloroform-*d*, deuterium oxide, or dimethyl sulfoxide-d_6, with Me_4Si or sodium 4,4-dimethyl-4-silapentanoate-2,2,3,3-d_4 as the reference, and those for ^{13}C-n.m.r. spectroscopy, with 10–40 mg of solute in 1.5–3 mL of dimethyl sulfoxide-d_6 with 25–50 μL of Me_4Si added as the reference. Solutions for ^{15}N-n.m.r. spectroscopy contained 10–40 mg of solute in 3 mL of dimethyl sulfoxide-d_6, with an external capillary of saturated, aqueous $NH_4{}^{15}NO_3$ as the reference. The assignments of the ^1H- and ^{13}C-n.m.r. spectra were confirmed by selective, homonuclear and heteronuclear proton-decoupling, respectively. The six-spin system of **6** in deuterium oxide solution was simulated by means of the PANIC program for the Bruker Instruments Aspect 2000 computer. An r.m.s. deviation of 94 mHz was obtained between experimental and calculated spectra. Electron-impact, mass spectra were obtained at 70 eV, with an ion-source temperature of 150°, using a Varian MAT 731 mass spectrometer. Liquid chromatography (l.c.) was performed at 3 MPa with either a Dupont preparative Zorbax column of octadecylsilane, or a Waters semipreparative μBondapak column. The elution solvents were: *A*, 1:9 (v/v) CH_3OH–H_2O; *B*, 1:4 (v/v) CH_3OH–H_2O; *C*, 3:7 (v/v) CH_3OH–H_2O; and *D*, 3:2 (v/v) CH_3OH–CH_2Cl_2. Ul-

*Mention of commercial products does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the products identified are necessarily the best available for the purpose.

traviolet detection was obtained simultaneously at 195 and 254 nm by means of a Schoeffel variable-wavelength detector connected in series with an LDC fixed-wavelength detector.

*2-Amino-(1,2-dideoxy-β-*D*-arabinofurano)[1,2-*d*]-2-oxazoline-*$^{15}N_2$ (**4**-$^{15}N_2$). — To a solution of dry α-D-arabinopyranosylamine[10] (**1**; 0.42 g) in dry methanol (3.5 mL) was added a solution of anhydrous cyanamide-$^{15}N_2$ (Prochem; 62.5 mg) in dry methanol (0.5 mL). The mixture was stirred for 48 h at room temperature, filtered, and the filtrate concentrated under vacuum, and refiltered. The solids were combined, successively washed on a Büchner funnel with methanol and ether, and dried under vacuum, to give a crude, white powder (321 mg). Liquid chromatography of portions (20 mg each) of the crude powder with a Waters semi-preparative μBondapak ODS column with elution by solvent *A* at 1 mL/min yielded **4**-$^{15}N_2$ as a fine, white powder (12.3 mg, 79% based on cyanamide-$^{15}N_2$); m.p. 175–177° (dec.). Unlabeled **4** was similarly prepared, and had m.p. 176–177° (dec.), undepressed on admixture with authentic[4] **4** [m.p. 175–176° (dec.); lit.[4] m.p. 175–176° (dec.)]. Identical specific rotations $[\alpha]_D^{20}$ +21.3° (*c* 1, H_2O); lit.[4] $[\alpha]_D^{25}$ +21.2° (*c* 1, H_2O), l.c. retention-times (21 min), and ^{1}H- and ^{13}C-n.m.r. spectra were found for authentic **4** and **4** prepared as described here. A molecular ion at *m/z* 176 (3.8%) was observed in the mass spectrum of **4**-$^{15}N_2$, and, in t.l.c. with solvent *D* as eluant, a single, oxazoline-positive[4] spot (R_F 0.55) was obtained that was charred by sulfuric acid. The ^{15}N atom% was calculated from the relative abundances of the $[M - CH_2OH]^+$ fragment, and was found to be consistent with complete incorporation of both ^{15}N labels from the cyanamide-$^{15}N_2$ (96.7 atom%). Infrared absorptions at ν_{max}^{Nujol} 1660 m (C=N) and 1600 w cm^{-1} (NH) were observed for **4**.

In a manner identical to that described for the preparation of **4**-$^{15}N_2$, compound **1** was also treated with cyanamide-^{13}C (91.8 atom%; Prochem) and cyanamide-^{13}C, $^{15}N_2$ (91.6 atom% ^{13}C, 99.5 atom% ^{15}N; Prochem), thus giving **4**-[2-^{13}C] and **4**-[2-^{13}C,$^{15}N_2$], respectively.

*2-Amino-(1,2-dideoxy-α-*D*-ribofurano)[1,2-*d*]-2-oxazoline-*$^{15}N_2$ (**5**-$^{15}N_2$). — Compound **5**-$^{15}N_2$ was prepared from crystalline β-D-ribopyranosylamine[9] (**2**) by the method used for preparation of **4**. Crude **5**-$^{15}N_2$ was isolated as a white powder (298 mg) that was readily purified by recrystallization from water, to give **5**-$^{15}N_2$ as prisms (226 mg, 90%). Alternatively, the crude powder could be readily purified by injection onto a Waters semipreparative, μBondapak ODS column and elution with solvent *A* at 1 mL/min, to give a retention time of 20 min. Purified **5** gave a single, oxazoline-positive spot (R_F 0.45) in t.l.c. in solvent *D*, and a mixed m.p. with authentic **5** [m.p. 195–196° (dec.); lit.[4] m.p. 197° (dec.)] which was not depressed, as well as identical ^{1}H- and ^{13}C-n.m.r. spectra, and identical optical rotation of $[\alpha]_D^{20}$ −26.5° (*c* 1, H_2O). A molecular ion at *m/z* 176 (1.7%) was observed in the mass spectrum of **5**-$^{15}N_2$. Examination of the $[M - CH_2OH]^+$ fragment gave a ^{15}N content consistent with quantitative incorporation of both ^{15}N labels from cyanamide-$^{15}N_2$. Infrared absorptions at ν_{max}^{Nujol} 1675 m (C=N) and 1625 cm^{-1} (NH) were observed for **5**.

*2-Amino-(1,2-dideoxy-α-*D*-xylofurano)[1,2-*d*]-2-oxazoline-*$^{15}N_2$ (**6**-$^{15}N_2$). — Compound **6**-$^{15}N_2$ was prepared from crystalline *β*-D-xylopyranosylamine[15] (**3**) by the method used to prepare **4**-$^{15}N_2$. However, on examination of the combined, washed, and dried crystalline product by t.l.c. (solvent *D*) and by l.c. (solvent *A*), **6**-$^{15}N_2$ was not detected. On concentration of the combined mother liquors to dryness, an oil was obtained that, by t.l.c. and l.c., consisted of significant amounts of **6**-$^{15}N_2$. After preparative, l.c. injections of crude **6**-$^{15}N_2$ onto a *μ*Bondapak ODS column, with elution by solvent *A* at 1 mL/min, **6**-$^{15}N_2$ was obtained as a white powder (116 mg, 46% based on cyanamide-$^{15}N_2$) that had a retention time of 18 min. Preparation of unlabeled **6** by this procedure gave material whose m.p. was not depressed by admixture with authentic[7b] **6** [m.p. 163–167° (dec.); lit.[7] m.p. 164° (dec.)]. Purified **6** displayed a single, oxazoline-positive spot (R_f 0.5) in t.l.c. in solvent *D*. Again, identical ^{1}H- and ^{13}C-n.m.r. spectra were obtained for **6** and authentic **6**, together with specific rotations in close agreement, $[\alpha]_D^{20}$ −26.3° (*c* 1, H_2O); lit.[7] $[\alpha]_D^{25}$ −31.5° (*c* 0.5, $HCONMe_2$). A molecular ion at *m/z* 176 (3.4%) was observed in the mass spectrum of **6**-$^{15}N_2$. Examination of the $[M - CH_2OH]^+$ fragment gave a ^{15}N content consistent with quantitative incorporation of both ^{15}N labels from cyanamide-$^{15}N_2$. Infrared absorptions at ν_{max}^{Nujol} 1660 m (C=N) and 1600 m cm^{-1} (NH) were observed for **6**.

*2-Amino-(1,2-dideoxy-3,5-*O*-isopropylidene-α-*D*-xylofurano)[1,2-*d*]-2-oxazoline-*$^{15}N_2$ (**10**-$^{15}N_2$). — To a stirred solution of crystalline 3,5-*O*-isopropylidene-D-xylofuranosylammonium *p*-toluenesulfonate[11] (**7**; 646 mg) in dry methanol (1 mL) was added freshly distilled triethylamine (250 *μ*L). After the addition of anhydrous cyanamide-$^{15}N_2$ (62.5 mg), the mixture was stirred for 24 h at room temperature, anhydrous diethyl ether was added to incipient opalescence, and the mixture was chilled, giving a white, crystalline precipitate, this was filtered off, and shown by t.l.c. analysis (solvent *D*) to be triethylammonium *p*-toluenesulfonate. [Another method for desalting crude **10**-$^{15}N_2$ consisted in isocratic elution of the mixture through a column of DEAE-cellulose (HCO_3^-) with 10mM aqueous ammonium hydrogencarbonate solution, followed by collection of the fraction having u.v. absorption at 210 nm, and freeze-drying thereof.] The mother liquor, containing crude **10**-$^{15}N_2$ desalted by the first method, was then evaporated to dryness under vacuum without heat, and redissolved in the minimal volume of ethyl acetate. Dry ether was added to incipient opalescence, and the solution was kept overnight at 5°. Prismatic crystals of **10**-$^{15}N_2$ were deposited, and were filtered off, washed with ether, and dried under vacuum, to give **10**-$^{15}N_2$ (0.264 g, 86% based on cyanamide-$^{15}N_2$); m.p. 183–184° (dec.). Alternatively, crude **10**-$^{15}N_2$ could be purified by dissolution in solvent *C* (10 mL) and then sequential injections of aliquots (0.8 mL each) onto a preparative, Zorbax ODS column, with elution by solvent *C* at 7.6 mL/min (ret. time 30 min), followed by lyophilization of the combined fractions. Crystalline **10**-$^{15}N_2$ gave a single spot (R_f 0.65) in t.l.c. in solvent *D*. A molecular ion at *m/z* 216 (1.9%) was observed in the mass spectrum of **10**-$^{15}N_2$. Examination of the ion abundances for the $[M - CH_3]^+$ fragment indicated

a ^{15}N content consistent with quantitative incorporation of both ^{15}N labels from cyanamide-$^{15}N_2$.

Unlabeled **10** was also prepared by this method, and had m.p. 183–185° (dec.), $[\alpha]_D^{20}$ +55.3° (*c* 1, H_2O) and ν_{max}^{Nujol} 1660 m (C=N) and 1600 w cm^{-1} (NH).

Anal. Calc. for $C_9H_{14}N_2O_4$: C, 50.46; H, 6.59; N, 13.08; Found: C, 50.34; H, 6.65; N, 12.86.

*2-Amino-(1,2-dideoxy-5,6-O-isopropylidene-α-*D*-glucofurano)[1,2-*d*]-2-oxazoline-*$^{15}N_2$ (**11**-$^{15}N_2$). — To a stirred solution of 5,6-*O*-isopropylidene-D-glucofuranosylammonium *p*-toluenesulfonate[11] (**8**; 489 mg) in dry methanol (1.6 mL) was added freshly distilled triethylamine (104 μL). Anhydrous cyanamide-$^{15}N_2$ (50 mg) was added, and the mixture was stirred for 2 h at room temperature, filtered, and the filtrate evaporated to dryness under vacuum without heat. The mixture was desalted by means of a column of DEAE-cellulose anion-exchange resin as described for **10**-$^{15}N_2$, and then freeze-dried. The minimal volume of dry methanol was added, together with a trace of ether, whereupon **11**-$^{15}N_2$ slowly crystallized. The crystals were successively washed with methanol and ether, and dried under vacuum without heat, to give **11**-$^{15}N_2$ (0.257 g, 92%); m.p. 191–194° (dec.). Alternatively, crude **11**-$^{15}N_2$ could be purified by dissolution in solvent *B* followed by sequential injection of aliquots of the solution onto a preparative, Zorbax ODS column, with elution by solvent *B* at 7.6 mL/min. Crystalline **11**-$^{15}N_2$ gave a single spot (R_F 0.56) in t.l.c. in solvent *D*. A molecular ion at *m/z* 246 (0.5%) was observed in the mass spectrum of **11**-$^{15}N_2$. Examination of the $[M - CH_3]^+$ fragment indicated a ^{15}N content consistent with quantitative incorporation of both ^{15}N labels from cyanamide-$^{15}N_2$.

Unlabeled **11** was also prepared by this method, and had m.p. 193–195° (dec.), $[\alpha]_D^{20}$ −26.5° (*c* 0.2, H_2O), and ν_{max}^{Nujol} 1690 (C=N) and 1610 w cm^{-1} (NH).

Anal. Calc. for $C_{10}H_{16}N_2O_5$: C, 49.18; H, 6.60; N, 11.47. Found: C, 49.30; H, 6.91; N, 11.41.

*2-Amino-(2,3-dideoxy-4,6-*O*-isopropylidene-β-*L*-sorbofurano)[2,3-*d*]-2-oxazoline-*$^{15}N_2$ (**12**-$^{15}N_2$). — To a stirred solution of 4,6-*O*-isopropylidene-L-sorbofuranosylammonium *p*-toluenesulfonate[12] (**9**; 489 mg) in dry methanol (1.6 mL) was added freshly distilled triethylamine (104 μL). Anhydrous cyanamide-$^{15}N_2$ (50 mg) was added to the mixture, which was stirred for 48 h at room temperature, filtered, and the filtrate evaporated to dryness without heat. The mixture was desalted by means of a DEAE-cellulose column as described for **10**-$^{15}N_2$, and freeze-dried. The crude **12**-$^{15}N_2$ that resulted was purified by dissolution in solvent *C* (10 mL) followed by the sequential injection of aliquots (0.6 mL each) onto a preparative, Zorbax ODS column, with elution by solvent *C* at 7.6 mL/min (ret. time 16 min). After freeze-drying of the combined fractions, the lyophilized residue was recrystallized from CH_2Cl_2–ether–hexane without heat, by chilling the opalescent solution overnight at 5°, thus giving **12**-$^{15}N_2$ as a white powder (0.238 g, 85%); m.p. 186–188° (dec.). Crystalline **12**-$^{15}N_2$ showed a single spot (R_F 0.48) in t.l.c. in solvent *D*, and a molecular ion at *m/z* 246 (1.0%) was observed in its mass spectrum.

Examination of the $[M - CH_2OH]^+$ fragment indicated a ^{15}N incorporation of only 92.0 atom% from cyanamide-$^{15}N_2$ (96.7 atom%). Thus, ~16% of **12**-$^{15}N_2$ was observed to possess only a single ^{15}N label.

Unlabeled **12**, also prepared by this method, had m.p. 187–188° (dec.), $[\alpha]_D^{20}$ −30.5° (*c* 0.08 in H_2O); ν_{max}^{Nujol} 1675 m (C=N) and 1600 w cm^{-1} (NH).

Anal. Calc. for $C_{10}H_{16}N_2O_5 \cdot 1/3\ H_2O$: C, 48.00; H, 6.71; N, 11.19. Found: C, 48.30; H, 6.49; N, 11.28

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