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THE SYNTHESIS AND N.M.R. SPECTROSCOPY OF DERIVATIVES OF 6-AMINO-6-DEOXY-D-GALACTOSE-*6*-^{15}N*

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ABSTRACT

Derivatives of 6-amino-6-deoxy-D-galactose-*6*-^{15}N have been synthesized by reaction of the 6-deoxy-6-iodo (**1**) or 6-*O*-*p*-tolylsulfonyl derivative of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose with potassium phthalimide-^{15}N. The reaction of **1** also yielded an elimination product, 6-deoxy-1,2:3,4-di-*O*-isopropylidene-β-L-*arabino*-hex-5-enopyranose. The structures of the 6-amino-6-deoxy-D-galactose derivatives and their precursors were characterized by proton- and ^{13}C-n.m.r. spectroscopy, with confirmation of the ^{13}C assignments by selective, proton decoupling. Selective broadening of the C-1, C-4, C-5, and C-6 resonances of 6-amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose by low concentrations of cupric ion was observed, and studied by computerized measurements of the ^{13}C linewidths. The application of this broadening to ^{13}C-spectral assignments of amino sugar derivatives is indicated.

INTRODUCTION

Nitrogen-15-labeled derivatives of 6-amino-6-deoxy-D-galactose were needed for measurement of the ^{15}N chemical-shifts and spin–lattice relaxation-times of ω-amino-ω-deoxy sugar derivatives. The nucleophilic substitution-reactions of 1,2:3,5-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose or its 6-deoxy-6-iodo derivative with potassium phthalimide-^{15}N in hexamethylphosphoric triamide (HMP) had previously afforded[1,2] high yields (81–84%) of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose-*6*-^{15}N having a ^{15}N enrichment of 99%. This work followed earlier studies in which unlabeled methyl 6-deoxy-6-phthalimido-α-D-glucopyranoside[3] and 1,2-*O*-cyclohexylidene-5-deoxy-5-phthalimido-3-*O*-*p*-tolylsulfonyl-α-D-xylofuranose[4] were synthesized in ~30% yield *via* reaction of methyl 6-*O*-*p*-tolylsulfonyl-α-D-glucopyranoside and 1,2-*O*-cyclohexylidene-3,5-di-*O*-*p*-tolylsulfonyl-α-D-xylofuranose, respectively, with potassium phthalimide in *N*,*N*-dimethylformamide.

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Although unlabeled 6-amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose had been synthesized by reaction of 1,2:3,4-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-galactopyranose with either ammonia[5] or sodium azide[6], these reactions were judged to be unsuitable for the preparation of the ^{15}N-labeled-amino sugar derivative, because of the requirement for either an excess of ammonia-^{15}N, or doubly labeled azide ion.

Commercially available 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) appeared to be an attractive starting-material for the desired synthesis, and so the reaction of this derivative (and of the 6-*O*-*p*-tolylsulfonyl derivative **4**) with potassium phthalimide was investigated, and the products and related derivatives were characterized by proton- and ^{13}C-n.m.r. spectroscopy.

RESULTS AND DISCUSSION

Synthesis. — The 6-deoxy-6-iodo derivative **1** was found to react with 1.2 molecular equivalents of potassium phthalimide in HMP at 145–150°, to give a mixture of a major component (**2**) and a minor component (**3**). Quantitative analysis of the extracted mixture by proton-n.m.r. spectroscopy of the H-1 signals of **2** and **3** indicated the ratio of products **2**:**3** to be 7:3. Fractional recrystallization of the mixture afforded 6-deoxy-1,2:3,4-di-*O*-isopropylidene-6-phthalimido-α-D-galactopyranose (**2**) in 59–64% yield and 6-deoxy-1,2:3,4-di-*O*-isopropylidene-β-L-*arabino*-hex-5-enopyranose (**3**) in 8% yield. The structures of these products were indicated

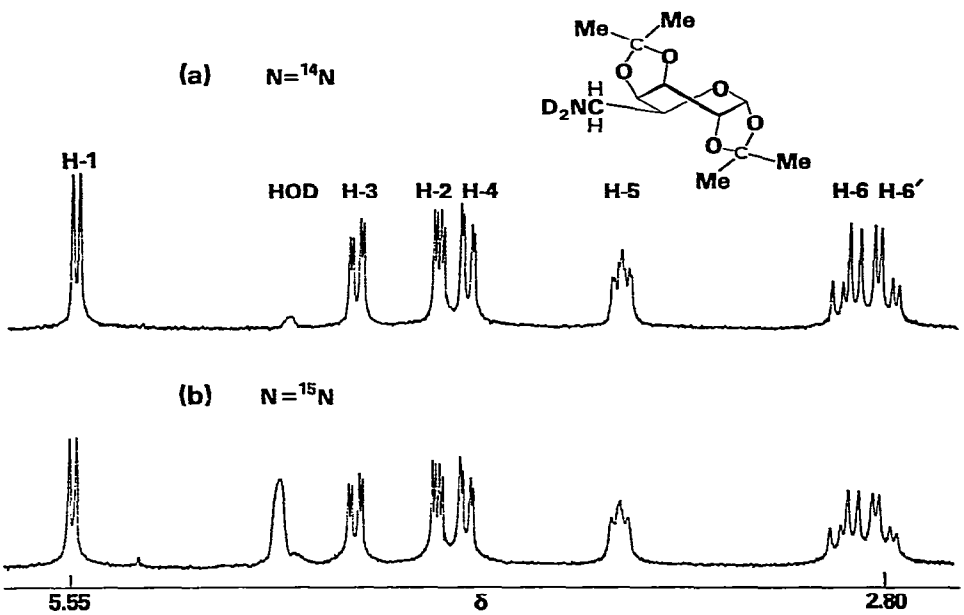


Fig. 1. Partial, proton-n.m.r. spectra of solutions in chloroform-*d* at 220 MHz: (a) 6-amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose-d_2 (**5**-d_2), and (b) its ^{15}N-labeled derivative (**5**-^{15}N-d_2).

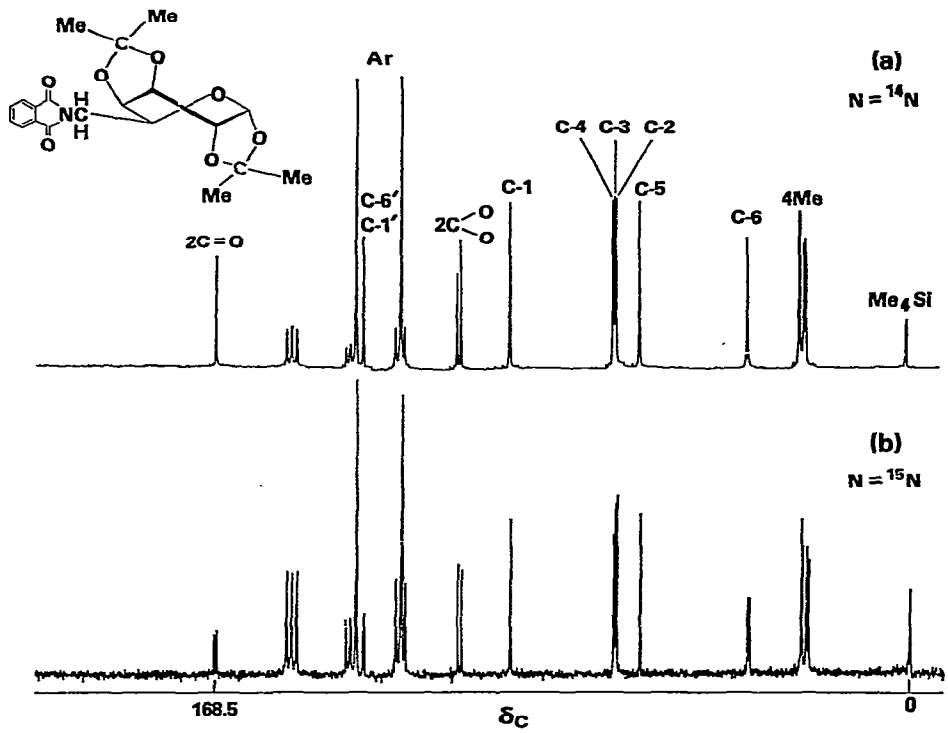
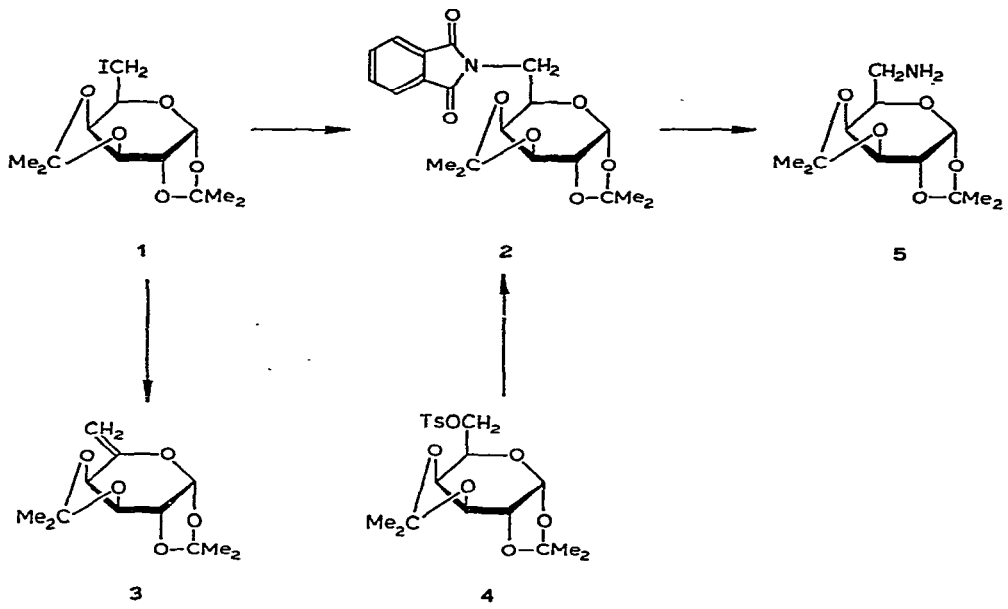


Fig. 2. Fourier-transform, ^{13}C-n.m.r. spectra of solutions in pyridine-d_5 at 22.6 MHz: (a) 6-deoxy-1,2:3,4-di-*O*-isopropylidene-6-phthalimido-α-D-galactopyranose (**2**), and (b) its ^{15}N-labeled derivative (**2**-^{15}N).

by their proton- and ^{13}C-n.m.r. spectra (for example, see Figs. 1 and 2, respectively). After the synthetic phase of this work had been completed, a report appeared of the preparation of **2** by reaction of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose with a mixture of phthalimide, triphenylphosphine, and diethyl azodicarboxylate[7].

The 5-enopyranose derivative **3** is evidently formed from **1** by elimination of the elements of hydrogen iodide, catalyzed by the phthalimide anion. Derivative **3** was identified by comparison with an authentic specimen of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-β-L-*arabino*-hex-5-enopyranose which was synthesized in 57% yield by reaction of the 6-deoxy-6-iodo derivative **1** with potassium *tert*-butoxide in bis(2-methoxyethyl) ether. Methoxide ion had been employed in the original synthesis[8] of **3** in variable yield (20–70%). In the present work, *tert*-butoxide ion was used as the base in an effort to minimize the possibility of nucleophilic substitution of the iodine atom by the alkoxide ion. However, isolation of **3** is complicated by its volatility under vacuum. Compound **3** had also been encountered[9] during the sodium hydroxide-catalyzed photolysis of **1** in *tert*-butyl alcohol at 25°.

Reaction of 1,2:3,4-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-galactopyranose (**4**) with potassium phthalimide in HMP proceeded more sluggishly than the reaction of the 6-iodo derivative **1**, and the use of a higher temperature (172°) and a



higher proportion (1.3 molecular equivalents) of potassium phthalimide was found to be necessary, so as to insure reasonably complete conversion of **4**. Such extensive conversion was found to be desirable, because of inhibition of the crystallization of **2** by unchanged **4**. The 6-*O*-*p*-tolylsulfonyl derivative **4** is significantly less reactive towards phthalimide ion than the 1,2:3,5-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose derivative studied earlier[1,2]. Diminished reactivity of 6-*O*-sulfonyl-galactopyranose derivatives towards *other* nucleophilic species had been reported previously[10–12], and rationalized in terms of polar, repulsive forces in the SN2 transition-state[11–13]. Nevertheless, reaction of **4** with potassium phthalimide gave the crystalline 6-deoxy-6-phthalimido derivative **2** in 68–70% yield, without significant formation of the elimination product **3**. Although derivative **2** was found to crystallize readily in the presence of **3** (for example, when prepared from **1** as a mixture), the method preferred for the synthesis of **2**-^{15}N involved treatment of the 6-*O*-*p*-tolylsulfonyl derivative **4** with potassium phthalimide-^{15}N, which afforded a 70% yield of **2**-^{15}N.

The 6-deoxy-6-phthalimido derivatives **2** and **2**-^{15}N were readily converted into 6-amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**5**) and its labeled analog (**5**-^{15}N), respectively, by treatment with hydrazine hydrate in ethanol.

The optical rotation of **5** has been the subject of some confusion, as the original report of this compound [5] did not specify the solvent used for the rotatory measurement, and a later report[6] of the synthesis of **5** by the azide route incorrectly compared the optical rotation of **5** with that of 6-(*n*-decylamino)-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose.

Proton-n.m.r. spectroscopy. — The chemical shifts of the olefinic protons of derivative **3** are not markedly characteristic of their chemical environment, as these

protons resonate in the same spectral region as H-3 and H-4, at ~4.6 p.p.m. However, the proton integral (measured at 90 MHz) confirmed the absence of H-5. The position of the H-6 and H-6′ signals of **3** at high field may be attributed to increased electron charge-density at C-6 due to electromeric release from the oxygen atom of the pyranose ring.

Dispersion of the proton-n.m.r. spectrum of the 6-deoxy-6-phthalimido derivative **2** was inadequate at 100 MHz, and so this compound was re-examined at 270 MHz. At the latter frequency, the spectrum of **2** and **2**-^{15}N were sufficiently well dispersed that a first-order, spectral analysis could be performed, even though the H-2 and H-6 signals were superposed. For derivative **2**-^{15}N, the coupling constants between the ^{15}N nucleus and H-5, H-6, and H-6′ were found to be small, and not fully resolved by pulse-Fourier transform techniques at 270 MHz. The coupling constants $J_{6,^{15}N}$ and $J_{6',^{15}N}$ were detected only as shoulders on the H-6 and H-6′ resonances, respectively, and were estimated to be of the order of 1 Hz each, in agreement with previous work[14] on 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose-*6*-^{15}N. For compound **2**-^{15}N, evidence for the presence of small couplings of the ^{15}N nucleus with neighboring protons, in the form of broadening of its proton-coupled, ^{15}N-n.m.r. spectrum, has been reported[15].

The proton-n.m.r. spectra of the 6-amino-6-deoxy derivatives **5** and **5**-^{15}N were found to be completely dispersed at 220 MHz (see Fig. 1), and first-order analysis gave the chemical shifts and coupling constants recorded in Table I. Even in the single-scan, continuous-wave mode, the coupling constants $J_{5,^{15}N}$, $J_{6,^{15}N}$, and $J_{6',^{15}N}$ were not resolved, and were detected only as slight broadenings of the H-5, H-6, and H-6′ multiplets, respectively. The proton–proton coupling-constants of **2**, **2**-^{15}N, **5**, and **5**-^{15}N are similar to those reported previously[16] for non-nitrogenous 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose derivatives, including **1** and **4**. The magnitudes of $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ indicate that these 6-amino-6-deoxy-D-galactopyranose derivatives also adopt a skew conformation (**2a** or **5a**) in which H-2 and H-3 have a *gauche* orientation. The values $J_{5,6}$ 10.3 and $J_{5,6'}$ 3.5 Hz for the 6-deoxy-6-phthalimido derivative **2** are more extreme than those ($J_{5,6}$ 7.9 and $J_{5,6'}$ 5.1 Hz) of the 6-amino derivative **5**, and indicate greater rotameric homogeneity about the C-5–C-6 bond of **2**. Steric considerations[17] suggest that the favored rotamer of **2** would be that (**2b**) in which the bulky phthalimido substituent is antiperiplanar to C-4. In this rotamer, H-6 has a *quasi-syn*-axial relationship with O-4, and should therefore be deshielded more than H-6′. Thus, the chemical-shift difference between H-6 and H-6′ is large for **2** (0.58 p.p.m.), but is much smaller for the amino derivative **5** (0.13 p.p.m.) in which significant populations of rotamers other than **5b** cause more extensive averaging of the chemical shifts of H-6 and H-6′.

Carbon-13 and nitrogen-15 n.m.r. spectroscopy. — The ^{13}C-n.m.r. spectra of the 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose derivatives **1**–**5** display a pair of well resolved resonances at low field (~109 p.p.m.) which were assigned to the quaternary, 2-carbon nuclei of the isopropylidene groups by means of off-resonance, proton decoupling and proton-coupled spectra. The signals of the four methyl carbon

TABLE I

PROTON CHEMICAL-SHIFTS[a] AND COUPLING-CONSTANTS (Hz) OF 1,2:3,4-DI-*O*-ISOPROPYLIDENE-α-D-GALACTOPYRANOSE DERIVATIVES

Derivative	*R-6*	*Solvent*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6′*	*Me*	*Ar*	*NH₂*
2[b]	$C_8H_4NO_2$[c]	C_5D_5N	5.67d[d]	4.50q	4.78q	4.37q	4.70o	4.51q	3.93q	1.60, 1.57, 1.38, 1.25	7.79m, 7.57m	
			$J_{1,2}$ 4.8	$J_{2,3}$ 2.4	$J_{3,4}$ 8.0	$J_{4,5}$ 1.8	$J_{5,6}$ 10.3	$J_{5,6'}$ 3.5	$J_{6,6'}$ 13.8			
2-^{15}N[b]	$C_8H_4{}^{15}NO_2$[c]	C_5D_5N	5.70d	4.51q	4.79q	4.37q	4.73m[e]	4.54m	3.97m	1.61, 1.57, 1.37, 1.24	7.80m, 7.55m	
			$J_{1,2}$ 4.8	$J_{2,3}$ 2.4	$J_{3,4}$ 7.9	$J_{4,5}$ 1.7	$J_{5,6}$ 10.3	$J_{5,6'}$ 3.3	$J_{6,6'}$ 13.6	$J_{6,{}^{15}N} \sim 1$	$J_{6',{}^{15}N} \sim 1$	
3[f]	5-ene	$CDCl_3$–C_6F_6 (4:1 v/v)	5.57d	4.25d	~4.58	~4.58	—	~4.74	~4.65	1.48, 1.48, 1.38, 1.37		
			$J_{1,2}$ 3.7	$J_{2,3} < 2$								
5[g]	NH_2	$CDCl_3$	5.56d	4.31q	4.60q	4.22q	3.70sp	2.95q	2.82q	1.53, 1.45, 1.33, 1.33		1.46
			$J_{1,2}$ 5.0	$J_{2,3}$ 2.2	$J_{3,4}$ 8.0	$J_{4,5}$ 1.8	$J_{5,6}$ 7.9	$J_{5,6'}$ 5.1	$J_{6,6'}$ 13.5			
5-^{15}N[g]	$^{15}NH_2$	$CDCl_3$	5.56d	4.31q	4.60q	4.22q	3.70m[h]	2.96q[h]	2.82q[h]	1.53, 1.44, 1.33, 1.33		1.62
			$J_{1,2}$ 5.1	$J_{2,3}$ 2.3	$J_{3,4}$ 8.0	$J_{4,5}$ 1.8	$J_{5,6}$ 7.8	$J_{5,6'}$ 5.1	$J_{6,6'}$ 13.2			

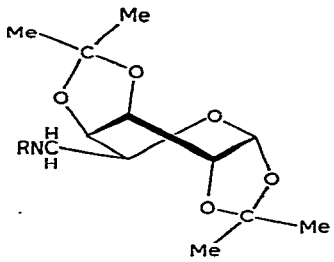
[a]In p.p.m. from internal tetramethylsilane. [b]At 270 MHz. [c]Phthalimido. [d]Signal multiplicities: d, doublet; m, multiplet; o, octet; q, quartet; and sp, septet. [e]$J_{5,{}^{15}N}$ unresolved. [f]At 90 MHz. [g]At 220 MHz. [h]$J_{5,{}^{15}N}$, $J_{6,{}^{15}N}$, and $J_{6',{}^{15}N}$ were not resolved, but were evident as a slight broadening of the H-5, H-6, and H-6′ multiplets, respectively.

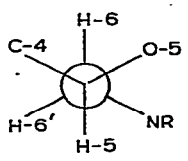
TABLE II

CARBON-13 CHEMICAL-SHIFTS[a] AND COUPLING-CONSTANTS (Hz) OF 1,2:3,4-DI-*O*-ISOPROPYLIDENE-α-D-GALACTOPYRANOSE DERIVATIVES

Derivative	*R-6*	*Solvent*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCO*	*Isopropylidene Me*	*Others*
1	I	$CDCl_3$	96.7	70.6	71.1	71.6	69.0	2.3	109.4, 108.7	26.0, 26.0, 24.9, 24.5	
2	$C_8H_4NO_2$[b]	C_5D_5N	96.8	71.0	71.3	71.8	65.3	39.0	109.7, 108.9	26.2, 26.0, 25.0, 24.6	168.4 (C=O), 132.4 (C-1′, C-6′)[c], 134.2, 123.3 (C-2′–C-5′)
			$^1J_{^{13}CH}$[d] 179.7	151.4	155.0	151.4	~145	139.2		126.7, 126.7, 124.8, 125.8	164.0, 166.2
2-^{15}N	$C_8H_4{}^{15}NO_2$[b]	C_5D_5N	96.9	71.1	71.4	71.8	65.4	39.0d[e]	109.7, 108.9	26.2, 26.1, 25.0, 24.5	168.5d (C=O), 132.5d (C-1′, C-6′), 134.2, 123.2 (C-2′–C-5′)
								$J_{C-6,^{15}N}$ 10.0		$J_{C=O,^{15}N}$ 13.4, $J_{C-1'(C-6'),^{15}N}$ 7.4
3	5-ene	$CDCl_3$	97.3	73.2	70.9[f]	72.0[f]	152.4	100.4	110.3, 109.7	26.8, 26.5, 25.6, 24.5	
4	OTs	C_5D_5N	96.6	70.8[f]	71.0	70.9[f]	66.6	69.5	109.6, 108.9	26.0[g], 26.0[g], 24.9, 24.3	145.2 (C-4′), 130.2, 128.3 (C-2′, C-3′, C-5′, C-6′), 133.5 (C-1′), 21.3 (Ar*Me*)
5	NH_2	$CDCl_3$	96.5	70.7	70.9	71.8	69.5	42.4	109.2, 108.4	26.1, 26.1, 25.0, 24.4	
			$^1J_{^{13}CH}$[d] 181.6	153.8	153.8	150.2	138.7	133.9		127.0, 127.0, 126.0, 126.1	
5-^{15}N	$^{15}NH_2$	$CDCl_3$	96.5	70.7	70.9	71.8	69.5	42.4d	109.1, 108.4	26.0, 26.0, 24.9, 24.4	
								$J_{C-6,^{15}N}$ 3.7		

[a]In p.p.m. from internal tetramethylsilane, at 22.6 MHz. [b]Phthalimido. [c]A prime indicates carbon nuclei of an aromatic ring. [d]Values to the right of this symbol indicate coupling constants of the respective carbon-13 nuclei with their directly bonded protons. [e]d, doublet. [f]Assignments interchangeable. [g]Resolved in $CDCl_3$ solution.





2a R = phthaloyl
5a R = H_2

2b R = phthaloyl
5b R = H_2

nuclei of the isopropylidene groups were fully resolved at high field (~25 p.p.m.) for derivatives **2**, **2**-^{15}N, **3**, and **4** (for ^{13}C chemical-shifts and coupling-constants, see Table II). Assignment of the ^{13}C-n.m.r. spectra of carbohydrates by the chemical-shift correlation-technique has recently been shown to be unreliable[18], and, for **1**–**5**, this technique was particularly inappropriate for the C-2, C-3, and C-4 resonances, which were invariably observed as a closely spaced group of singlets at ~71 p.p.m. Unequivocal evidence for assignment of the ^{13}C resonances of C-1–C-6 was obtained in most instances by selective decoupling of each of the protons, H-1 to H-6′, in turn. With the exception of the 5-enopyranose derivative **3**, which probably adopts a conformation that differs somewhat from that of the saturated derivatives, the chemical shifts of C-2, C-3, and C-4 increase in the same order (see Table II).

In general, the chemical shift of C-6 was found to vary markedly, depending on the nature of the substituent attached to C-6 (range, 2.3–100.4 p.p.m.). The C-6 resonance of the iodo derivative **1** appeared at very high field (2.3 p.p.m.), as had been observed previously for the α-^{13}C nucleus of simple alkyl iodides[19]. The chemical shifts of C-5 and C-6 of the enopyranose derivative **3** are more characteristic of its olefinic structure than are the proton chemical-shifts of H-6 and H-6′. However, these carbon-13 shifts are at the extremes of the range of chemical shifts for typical olefinic carbons (152.4 and 100.4 p.p.m., for C-5 and C-6, respectively), apparently because electromeric release of electrons from the oxygen atom of the pyranose ring causes the charge densities on C-5 and C-6 to be markedly different. The C-6 resonances of the nitrogen-substituted derivatives **2** and **5** appear at relatively high field (~40 p.p.m., see Figs. 2 and 3), in agreement with results reported for 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose[14].

Variation of the substituent at C-6 of derivatives **1**, **2**, **4**, and **5** produced smaller changes in the ^{13}C shifts of C-5 (β-shifts) than in those of C-6 (α-shifts). The dependence of these β-shifts on the nature of the substituent at C-6 was less obvious than that of the α-shifts.

One particular preparation of the 6-amino-6-deoxy derivative **5** was unexpectedly contaminated by an impurity that caused this sample to be blue or green, depending on the conditions under which it was isolated. For this sample, the C-5 and C-6 resonances were found to be absent from the ^{13}C-n.m.r. spectrum obtained from

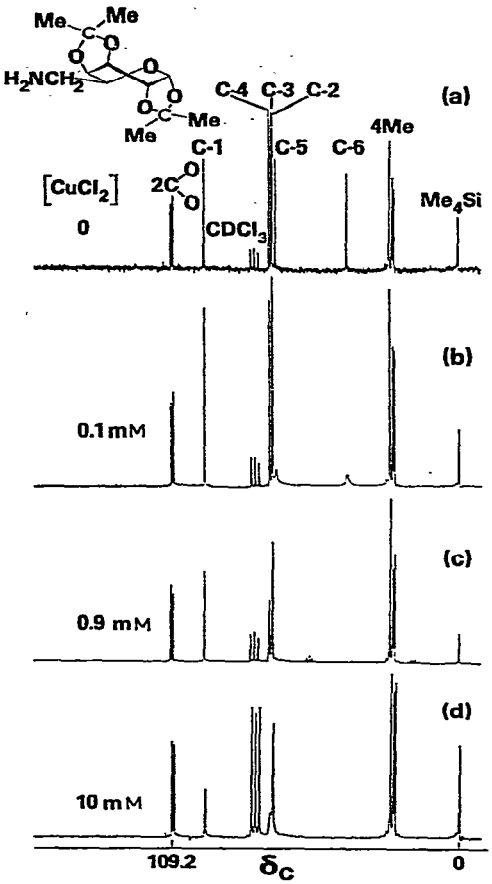


Fig. 3. Effects of increasing concentrations of cupric chloride on the Fourier-transform, ^{13}C-n.m.r. spectra of 6-amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**5**) in chloroform-*d* solution at 22.6 MHz.

4096 scans of its solution in 9:1 chloroform-*d*–hexafluorobenzene. However, longer-term scanning revealed the C-6 resonance as an extremely broad band that was almost hidden in the base-line noise. Under these conditions, the C-5 resonance was obscured by those of C-2–C-4. In the ^{13}C-n.m.r. spectrum of a colorless sample of 5-^{15}N that was prepared at the same time as the unlabeled sample, the C-5 and C-6 resonances were broadened, but were readily visible. On cooling the solution of 5-^{15}N to −50°, the C-5 resonance sharpened up, although the C-6 resonance remained somewhat broad, probably because of coupling of the C-6 and ^{15}N nuclei. Similar effects were observed in the ^{15}N-n.m.r. spectrum of 5-^{15}N, which was a sharp singlet at −50°, but which gradually broadened with increasing temperature until, at ambient probe-temperature (30°), a very broad singlet was observed, and then, at 60°, the ^{15}N resonance was so broad that it was indistinguishable from base-line noise.

On equilibration of the solution of **5** with aqueous, tetrasodium (ethylene-

TABLE III

LINEWIDTHS[a] OF CARBON-13 RESONANCES OF 6-AMINO-6-DEOXY-1,2:3,4-DI-*O*-ISOPROPYLIDENE-α-D-GALACTOPYRANOSE[b] (5) AS A FUNCTION OF THE CONCENTRATION OF CUPRIC CHLORIDE

$[CuCl_2]$ (mM)	*C-1*	*C-4*	*C-5*	*C-6*	*Broadening of ^{13}C resonance sets in*
0	1.3[c]	1.5	1.9[d]	2.1	
0.01	1.6	1.2	3.6	3.6	C-5 and C-6
0.03	1.3	1.5	7.2	6.6	
0.1	1.8	1.5	34.2	24.3	
0.5	2.3	3.4	—[e]	86.7	
0.9	2.1	4.5	∞[f]	∞[f]	C-4
1.7	2.8	9.6	∞	∞	C-1
2.6	3.9	16.1	∞	∞	
10	6.1	—[e]	∞	∞	

[a]Width of resonance at half-height in Hz ± 0.6 Hz, computed from Lorentzian fit. [b]Initial concentration, 1M, in chloroform-*d*. [c]Other linewidths: C-2, 1.5; C-3, 1.2; *C*Me, 1.2 and 1.0; Me, 4.1 (× 2), 2.8, and 3.0 Hz. [d]The corresponding linewidth of 5-^{15}N is 3.4 Hz, which suggests the presence of an unresolved coupling of ^{13}C-5 with the ^{15}N nucleus. [e]Obscured. [f]Resonance not visually detectable.

dinitrilo)tetraacetate solution, the blue color was extracted into the aqueous layer, and the ^{13}C-n.m.r. spectrum of the organic layer now displayed sharp resonances for C-5 and C-6.

These phenomena indicated contamination of **5** and 5-^{15}N by varying proportions of a paramagnetic metal ion, probably by means of the scavenging effect of complexation with the amino function. This hypothesis was confirmed by the results of a systematic study in which the ^{13}C linewidths ($\Delta\nu_{\frac{1}{2}}$) of **5** were measured by Lorentzian line-fitting, in the presence of increasing concentrations of cupric chloride. The gradual addition of this paramagnetic salt to a 1M solution of pure **5** in chloroform-*d* produced the same effects on the ^{13}C linewidths (see Fig. 3 and Table III) as had been observed for the contaminated amine samples. Broadening of the C-5 and C-6 resonances of **5** (to 3.6 Hz each) was initially detectable at a concentration of cupric chloride of only 10μM, at which concentration, the solution was colorless. At higher concentrations of cupric chloride (0.03–0.5mM), the linewidths of the C-5 and C-6 resonances continued to increase (for example, see Fig. 3b), until, at 0.5mM, the C-6 resonance was 86.7-Hz wide. In this range of concentrations, the linewidth of the C-5 resonance increased at a slightly higher rate than that of the C-6 signal (see Table III). At $[CuCl_2] = 0.9$mM, the C-5 and C-6 resonances disappeared, and broadening of the C-4 resonance set in (see Fig. 3c). Broadening of the C-1 resonance was detected at $[CuCl_2] = 1.7$mM, and, at higher concentrations, up to 10mM, the linewidths of C-1 and C-4 continued to increase (see Fig. 3d). At the latter concentration, the solution was bright blue. The linewidths of the C-2 and C-3 signals of **5** could not readily be measured for solutions that contained cupric chloride, because of peak overlaps.

The foregoing data indicate the existence of specific complexing of the amino

group of **5** with the cupric ion. The insignificant broadening of the isopropylidene carbon signals suggests that this coordination does not involve the oxygen atoms of the isopropylidene groups, and the sluggish broadening of the C-1 signal suggests that the ring-oxygen atom is probably not involved either. Thus, **5** appears to form a simple ammine complex with cupric ion. However, the fact that the ^{13}C signals of a molar solution of **5** are broadened by traces of cupric ion indicates that this ion undergoes rapid exchange between different amino sites.

Solutions containing a high ratio of the amine **5** to cupric ion would, presumably, contain a large proportion of the tetrammine complex, which is known to be favored in the equilibria of simple aliphatic amines with cupric ions[20].

The linewidth of a paramagnetically broadened, Lorentzian, ^{13}C resonance is described[21] by $\Delta\nu_{\frac{1}{2}} = 1/\pi T_2$, where, in the limit of rapid isotropic motion of the molecule, the transverse relaxation-time, T_2, is given by[21,22]

$$\frac{1}{T_2} = \frac{2S(S+1)Nn}{3N_c}\left[\frac{2g^2\beta^2\gamma_C^2\tau_e}{r^6} + \frac{A^2\tau_e}{\hbar^2}\right], \qquad (1)$$

where N and N_c are the molar concentrations of paramagnetic ions and ^{13}C nuclei, respectively, n is the number of ^{13}C nuclei in the solvation sphere of the paramagnetic ion, r is the distance between the paramagnetic ion and nucleus, A is the isotropic, hyperfine coupling-constant between the unpaired electron and nucleus, and the remaining symbols have their usual meanings[21,22]. The first term in the brackets of equation *1* represents the interaction between the magnetic dipoles of the unpaired electron and nucleus, whereas the second term describes the Fermi-contact interaction, which is manifested by the existence of the scalar coupling A.

Equation *1* requires a linear dependence of linewidth on the concentration of paramagnetic ion added, and graphical plotting of the linewidths of the C-1, C-4, C-5, and C-6 resonances (from Table III) confirmed this dependence (within the limits of experimental error). Although dilution of a molar solution of **5** in chloroform-*d* with an equal volume of ethanol-d_6 caused small shifts ($\leqslant$0.5 p.p.m.) of the ^{13}C resonances of C-1–C-6, no significant chemical-shifts were produced by the addition of cupric chloride up to a concentration of 10mM.

No significant, ^{15}N isotope-effect was observed, either for the ^{13}C chemical-shifts of derivatives **2** and **2**-^{15}N, or for those of **5** and **5**-^{15}N.

In many previous investigations of paramagnetic, metal complexes[23], the contact term in equation *1* was assumed to be negligible, and the distance r between the paramagnetic ion and the nucleus in question was calculated from the relationship

$$\Delta\nu_{\frac{1}{2}} \propto r^{-6}, \qquad (2)$$

which implies that the dipolar term of equation *1* predominates. Recently, however, for a number of cupric complexes, including those of simple aliphatic amines[24,25], it has been demonstrated that the scalar interaction predominates, and, therefore, that equation *2* is invalid[23]. Expression of the hyperfine coupling-constant A as an explicit function of r is difficult[24], because of the dependence of A on a wave function

that describes the spin density of the unpaired electron at the nucleus[22,26]. Thus, the existence of significant, hyperfine coupling[27] between the unpaired spin-density of the cupric ion and the ^{13}C nuclei at C-5 or C-6 of **5** could provide an alternative explanation of the selective broadening of its C-5 and C-6 signals, in addition to that based on variation of the dipolar term with distance. The magnitude of the contact term may be estimated[24] by comparative measurements of the spin–lattice relaxation-time (T_1) and the spin–spin relaxation-time (T_2), but this is beyond the scope of the present paper. The effect, on the linewidth, of the chemical exchange of cupric ions between different amine molecules[25] is also undefined.

For these reasons, no attempt has been made to calculate relative, internuclear distances from the ^{13}C linewidths of **5**. Regardless of the actual mechanism of line-broadening, there is clearly a proximity effect of complexation of the cupric ion with the amino group of **5**, and, therefore, the selective broadening of ^{13}C resonances by added paramagnetic ions may be a useful technique for assignment of the ^{13}C-n.m.r. spectra of amino sugar derivatives, including the aminoglycoside antibiotics[28].

Carbon-13 and nitrogen-15 coupling constants. — The ^{13}C–proton coupling-constants over one bond were measured from the proton-coupled, ^{13}C-n.m.r. spectra of **2** and **5** (see Table II), and were found to correlate approximately with the electro-negativities of the carbon substituents[29,30]. Thus, the largest value of $^1J_{CH}$ observed (~180 Hz) was that of C-1, and the values for C-5 and C-6 were found to be significantly smaller than those of C-2, C-3, and C-4 (see Table II).

The coupling constants $^1J_{C\text{-}6,^{15}N}$ 10.0 Hz and $^1J_{C=O,^{15}N}$ 13.4 Hz for **2**-^{15}N are similar to the values (9.8 and 13.4–14.6 Hz, respectively) reported for other ω-deoxy-ω-phthalimido-aldose derivatives[14,31]. These values are well described by the Binsch relationship $S_N S_C = 80\ ^1J_{^{13}C^{15}N}$, where S_N and S_C are the percentage *s*-characters of the bonding orbitals of the nitrogen and carbon atoms, respectively[32]. However, the value $^1J_{C\text{-}6,^{15}N}$ 3.7 Hz for the aminodeoxy derivative **5**-^{15}N is substantially smaller than that of **2**-^{15}N, and does not agree with the value, $^1J_{^{13}C^{15}N}$ 7.8 Hz, calculated from the Binsch equation by insertion of the reasonable values $S_N = S_C = 25\%$. In this respect, **2**-^{15}N resembles methylamine, for which the value $^1J_{^{13}C^{15}N}$ −4.5 Hz has been reported[33]. By means of semi-empirical, molecular-orbital methods, a theoretical coupling-constant of −2.6 Hz has recently been calculated[34,35] for methylamine, as the sum of Fermi-contact, orbital, and spin-dipolar contributions (−2.7, +0.2, and −0.1 Hz, respectively). For methylamine and other nitrogenous derivatives, the deviation of their experimental and theoretical coupling-constants from the Binsch equation was interpreted as being due to positive contributions of the nitrogen lone-pairs to the Fermi-contact term, which partially offset the usually dominant, negative contributions of the CN localized-bonding orbitals. It was concluded that the Binsch equation cannot be used for molecules that have *s*-containing, lone pairs on nitrogen or carbon atoms[35]. The results for **5**-^{15}N support this view.

The ^{15}N chemical-shifts of a series of ω-amino-ω-deoxy and 2-amino-2-deoxy sugar derivatives have been reported elsewhere[15,31], together with a limited number of ^{15}N spin–lattice relaxation-times[31].

EXPERIMENTAL*

General. — Reactions were monitored by thin-layer chromatography on plates coated with silica gel G (Analtech) which were developed in either dichloromethane containing 1% of methanol (derivatives **1–4**) or 4:1 (v/v) dichloromethane–methanol (compound **5**), with detection by charring with aqueous, 10% sulfuric acid. All compounds were isolated in chromatographically pure form. Optical rotations were measured for solutions in chloroform, unless otherwise stated, by use of a Perkin–Elmer polarimeter, model 141. Cupric chloride was added to molar solutions of **5** in chloroform-*d*, either as weighed quantities of the dried solid, or as aliquots of dilute solutions in ethanol-d_6.

Proton-n.m.r. spectra were recorded in the continuous-wave mode at 60, 100, and 220 MHz by use of Varian Associates spectrometers, models A-60, HA-100, and HR-220, respectively, and in the pulse-Fourier transform (F.t.) mode at 90 and 270 MHz by means of Bruker spectrometers, models HFX-11 and WH-270, respectively. ^{13}C-N.m.r. spectra were obtained in the F.t. mode at 22.6 MHz with the Bruker HFX-11 spectrometer, by using a 45° pulse (5 μs), a repetition time of 2 s, an 8,192-point data-set, field-frequency stabilization on solvent deuterium, and quadrature detection. ^{15}N-N.m.r. spectra were obtained at 9.12 MHz as described previously[21]. Solutions for proton-n.m.r. contained 4–400 mg of solute in 0.1–1.0 mL of solvent (5-mm sample-tubes), the lower limits representing use of a microcell for compound **3**. Solutions for ^{13}C-n.m.r. spectroscopy generally contained 263–416 mg of solute in 0.8–1.0 mL of solvent (10-mm sample-tubes).

Spectral assignments and analysis. — Assignment of the high-field, proton-n.m.r. spectra of **2** and **5** was straightforward, and the spectra were analyzed by first-order methods. The assignment of H-5 of compound **2** was confirmed by spin decoupling of H-6′ at 100 MHz.

The ^{13}C assignments for derivatives **1–5** were confirmed by selective decoupling of H-1 to H-6′, in turn, at 90 MHz. Supplemental evidence for assignments was obtained by off-resonance, proton decoupling, and from proton-coupled, ^{13}C-spectra acquired with retention of the nuclear Overhauser effect by gated, low-power irradiation of the protons at 90 MHz during the period (4.2 s) between data acquisitions.

Linewidths of the ^{13}C resonances of **5** and **5**-^{15}N were computed automatically, using the least-squares, Lorentzian fitting-routine of the NTCFT program for Nicolet series 1080 minicomputers (Nicolet Technology Corporation).

*6-Deoxy-1,2:3,4-di-*O*-isopropylidene-6-phthalimido-α-*D*-galactopyranose* (**2**). — (*A*) *From* **1**. A mixture of the 6-deoxy-6-iodo derivative **1** (1.0 g), potassium phthalimide (600 mg, 1.2 mol. equiv.), and dry HMP (10 mL) was heated for 3 h at 145–150°. The suspension was cooled, diluted with cold dichloromethane (400 mL), and washed successively with cold, 5% sodium hydroxide solution (75 mL), dilute sodium sulfate

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solution, water (5 × 150 mL), and dilute sodium thiosulfate solution (150 mL). The organic layer was dried (Na_2SO_4), and evaporated to a syrup, an aliquot of which was dissolved in 4:1 (v/v) chloroform-*d*–hexafluorobenzene, and the resulting solution analyzed by proton-n.m.r. spectroscopy at 90 MHz. Integration of the H-1 signals of **2** and **3** indicated a ratio of products **2**:**3** of 7:3.

Crystallization of the syrup from aqueous ethanol afforded crude **2**, 677 mg (64%), which, on recrystallization from ethanol and then from hexane–dichloromethane, yielded **2** as fine needles, m.p. 122–123°, $[\alpha]_D^{21}$ +14° (*c* 0.61); lit.[7] m.p. 145–146° (see later).

Anal. Calc. for $C_{20}H_{23}NO_7$: C, 61.7; H, 6.0; N, 3.6. Found: C, 61.8; H, 6.0; N, 3.6.

A different preparation afforded two crops of a dimorph of **2**, totalling 625 mg (59%), m.p. 145–146° and m.p. 143–144.5°. Crystallization of the material in the mother liquor from aqueous ethanol yielded the 5-ene **3** as lustrous plates, 54 mg (8%), m.p. 86–87°, undepressed on admixture with authentic **3**; $[\alpha]_D^{20}$ −160.8° (*c* 0.77), and proton-n.m.r. spectrum (at 90 MHz) identical with that of authentic **3** {lit.[8] m.p. 85°, $[\alpha]_D^{20}$ −128° $(CHCl_2)_2$, lit.[9] m.p. 85–86°}.

(*B*) *From* **4**. A mixture of **4** (1.0 g), potassium phthalimide (583 mg, 1.3 mol. equiv.), and dry HMP (10 mL) was stirred and heated for 4 h at 172°. The suspension that resulted was cooled in a refrigerator, diluted with cold dichloromethane (150 mL), and the mixture washed once with cold, 5% sodium hydroxide solution, four times with sodium sulfate solution, and twice with water. Evaporation of the organic layer afforded a pale-yellow syrup that, on crystallization from aqueous ethanol, yielded **2** as brilliant laths, 637 mg (68%), m.p. 121–122°. Recrystallization from 2-propanol or hexane–dichloromethane gave fine needles having m.p. 122–123°, $[\alpha]_D^{20}$ +14.2° (*c* 5.50).

Similar treatment of **4** with potassium phthalimide-^{15}N (enrichment, 99.2 atom-%) afforded a 70% yield of **2**-^{15}N, m.p. 121–122°, $[\alpha]_D^{20}$ +14.3° (*c* 5.2). Dimorphic forms of **2** and **2**-^{15}N were observed that melted either partially at 122–123° and then completely at 146–147°, or entirely at the higher temperature.

(*C*) *From* **4** *by a more rapid procedure.* A mixture of **4** (10 g), potassium phthalimide (5.8 g, 1.3 mol. equiv.), and dry HMP (100 mL) was stirred and heated for 5 h at 172°. The pale-brown suspension that resulted was cooled in ice, poured into ice–water (2 L), and the mixture nucleated, stirred, and kept overnight in a refrigerator. The flocculent precipitate resulting was filtered off (S & S 589, black-ribbon, filter paper), washed with water, and dissolved in ethanol; the solution was decolorized (alkaline charcoal), and the solute crystallized, to give **2** as lustrous needles, 4.8 g (51%), initial m.p. 122–123°, final m.p. 146–147°. The mother liquor (aqueous HMP) deposited a further quantity of precipitate which, when combined with the ethanolic mother liquor, afforded a second crop of chromatographically pure **2**, 0.84 g (9%), initial m.p. 122–123°, final m.p. 145–146°.

By the same method, reaction of **4** with potassium phthalimide-^{15}N afforded **2**-^{15}N in 45–47% yield (first crop), m.p. 146–147°.

*6-Deoxy-1,2:3,4-di-*O*-isopropylidene-β-*L-arabino*-hex-5-enopyranose* (**3**). — A mixture of the 6-deoxy-6-iodo derivative **1** (1.0 g), potassium *tert*-butoxide (1.0 g), and redistilled bis(2-methoxyethyl) ether (20 mL) was boiled under reflux (nitrogen atmosphere) for 1 h, cooled, and diluted with dichloromethane (100 mL). The dark-brown suspension was washed successively with water (2 × 300 mL), sodium thiosulfate solution (2 × 300 mL), and water (2 × 300 mL), and the resultant, yellow solution dried (Na_2SO_4) and evaporated, to give a syrupy residue (0.44 g) and a sublimate (0.14 g) which, when combined and crystallized from aqueous ethanol, afforded **3** as colorless leaflets, 372 mg (57%), m.p. 81–85°. Two recrystallizations from aqueous ethanol gave **3** as iridescent plates, m.p. 86.5–87°, $[\alpha]_D^{20}$ −159.2° (*c* 0.82).

*6-Amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-*D*-galactopyranose* (**5**). — A mixture of the phthalimido derivative **2** (4.8 g), ethanol (50 mL), and aqueous, 85% hydrazine hydrate (1.8 mL, 2.5 mol. equiv.) was stirred and boiled under reflux for 1 h; the white magma that had formed was then broken up, and diluted with more ethanol (20 mL). The mixture was boiled and stirred for a further 0.5 h, cooled, and evaporated to a solid residue that was dissolved in a mixture of dichloromethane (100 mL) and aqueous, 5% sodium hydroxide solution (200 mL). The mixture was shaken, the aqueous layer extracted with dichloromethane (50 mL), and the organic layers were combined, washed with water (50 mL), dried (Na_2SO_4), and evaporated to a colorless, mobile syrup that was dried at 50°/25 kPa, to give **5**, 3.04 g (96%). The syrup was strongly basic, it reacted with ninhydrin, and had $[\alpha]_\lambda^{20}$ −50.6, −55.0, −63.0, −105.1, and −160.7° at λ 589, 578, 546, 436, and 365 nm, respectively (*c* 2.5, methanol); lit.[6] $[\alpha]_D^{28}$ −44.0° (in methanol), and lit.[5] $[\alpha]_\lambda^{19}$ −50.4, −63.1, −70.85, and −127.2° at λ 633, 578, 546, and 434 nm, respectively (solvent not reported).

Treatment of **2**-^{15}N with hydrazine hydrate in a similar way yielded **5**-^{15}N as a colorless, mobile syrup (99%), $[\alpha]_\lambda^{20}$ −49.9, −54.4, −62.5, −103.8, and −159.0° at λ 589, 578, 546, 436, and 365 nm, respectively.

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