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NUCLEOSIDES, 45. — ASSIGNMENT OF GLYCOSYLATION SITES IN O-HEXOPYRANOSYL-RIBONUCLEOSIDES BY ^{13}C-NMR [1]

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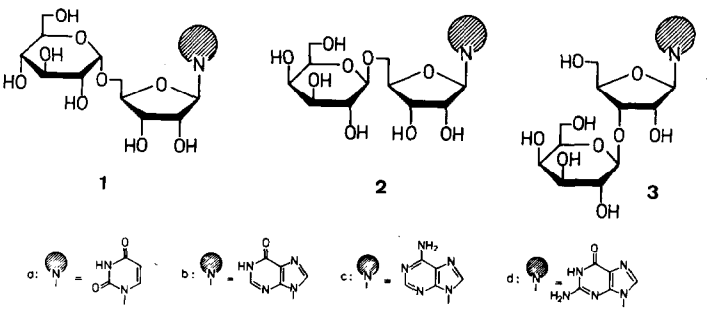
Summary: Evidence is presented that glycosylation of a ribose-OH group in nucleosides results in a significant downfield shift for the appended ^{13}C nucleus and smaller upfield displacements for the adjacent carbons, providing an efficient tool for differentiating between 2'-O-, 3'-O- and 5'-O-glycosyl-ribonucleosides. Therewith, the products formed on enzymatic galactosylation of uridine, inosine, and adenosine[4-6] are unequivocally assigned β(1→3)-glycosidic linkages (3a - 3c).

The glycosidase-induced transfer of a hexose unit onto a ribose is marked by low regioselectivity yielding (1→3)- and (1→5)-linked hexosyl-riboses as major, the (1→2)- and (1→4)-coupled isomers as minor products[2]. A higher selectivity appears to apply to ribonucleosides as hexosyl acceptors, since α-glucosidases of either fungal, bacterial or plant origin transfer glucosyl moieties from maltose (or soluble starch) to the ribose-5'-oxygen exclusively, resulting in glucosyl-nucleosides of type 1[3]. E.coli-derived β-galactosidase, however, combined with o-nitrophenyl β-D-galactopyranoside as the hexosyl donor, glycosylates adenosine[4], inosine[5], or uridine[6] with a high (e.g. 10:1 [5,6]) preference for the secondary ribose hydroxyls. The resulting minor components, i.e. 5'-O-galactosyl-nucleosides 2b, 2c and 2d have been structurally clarified by identification of hydrolysis products, by periodation studies as well as by unequivocal syntheses of 2d[7] and the underlying galactosyl-β(1→5)-ribose[8]; in contrast, the nature of the major products, i.e. whether galactosylation occured at the O-2' or O-3' of the ribose portion, remains to be unambiguously established, although some synthetic studies[9] and some subtile differences in ^{1}H-NMR chemical shifts[9] strongly indicated them to be the galactopyranosyl-β(1→3)-ribonucleosides 3a - 3c. Unequivocal proof thereof is now provided by comparative evaluation of ^{13}C-chemical shifts of the ribose carbons in 3a and 3b with those of their parent nucleosides.



In 3a and 3b[10], ^{13}C-NMR resonances for galactosyl and ribose carbons fall within the same range, yet the former are readily identified by comparison with the signals obtained for methyl β-D-galactopyranoside (cf. Fig. 1), chemical shift differences for C-2 through C-5 of galactose being between 0.07 and 0.32 ppm only. Direct additional proof for the assignments in Fig. 1 is provided by selective decoupling of the galactose protons[11], which, at 380 Hz (cf. Fig. 2), leaves the galactosyl carbons as discrete singlets. On increasing the decoupling frequency, the ribose carbons successively become decoupled (Fig. 2), whereby the signal for C-3′ turns into a distinct singlet at lower frequency (410 Hz)[12] than that for C-2′, thus clearly establishing their sequence.

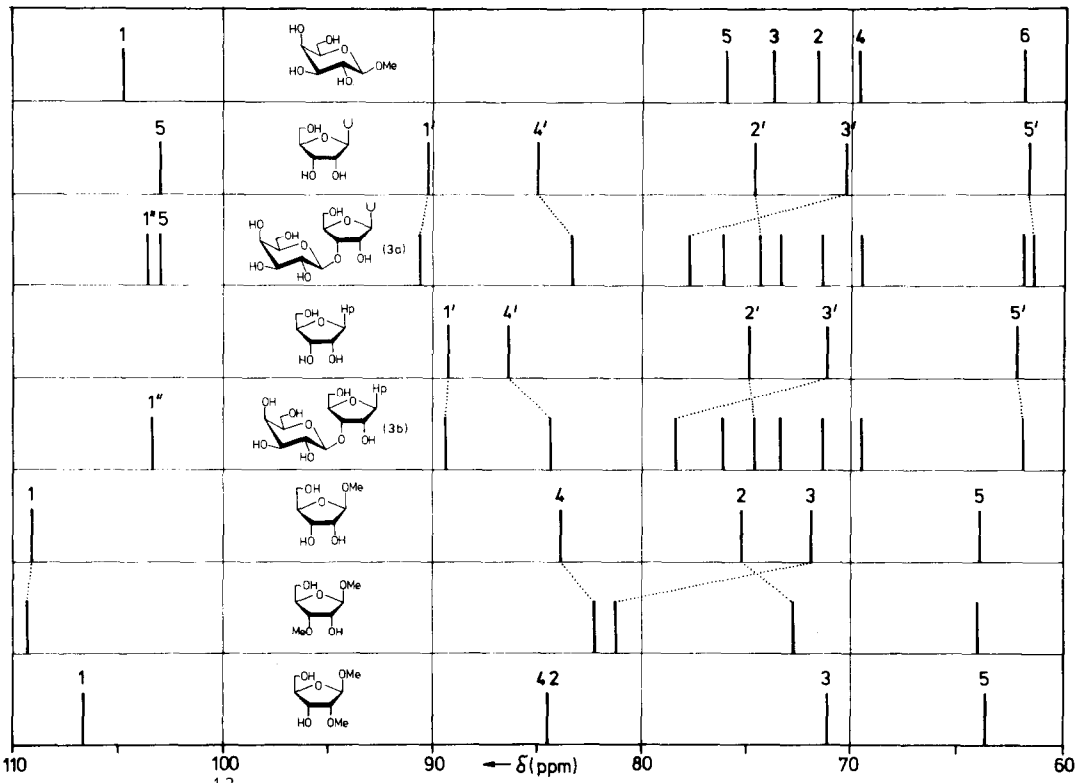


Fig. 1. Relevant ^{13}C-NMR resonances of galactosyl-β(1→3)-ribonucleosides 3a and 3b, and of model compounds, in D_2O with dioxane (δ = 67.4 ppm) as internal standard (25.16 MHz, Varian XL 100). Data of methyl ribosides are from Gorin and Mazurek[13], and were measured against external tetramethylsilane. (Abbreviations: U = uracil; Hp = hypoxanthine)

Comparing now the ribose carbons of 3a and 3b with those of their parent nucleosides, i.e. uridine and inosine, resp., (cf. Fig. 1), it is clearly apparent that the galactosyl residues are linked to O-3′, since the appended C-3′ signals are displaced downfield by a sizable 7.5 and 7.2 ppm, respectively, as contrasted to an adverse, considerably smaller upfield shift for the C-4′-resonances and only negligible effects on the other ribose carbons. This is in sweeping accord with the shifts observed for methyl β-D-ribofuranoside and its 3-O-methyl (cf. Fig. 1) or 3-O-isopropyl derivatives[13], establishing a close parallel of effects found on O-alkylation and O-glycosylation. In contrast, 2′-O-substitution results in an entirely different shift pattern, as exemplified by the last example in Fig. 1.

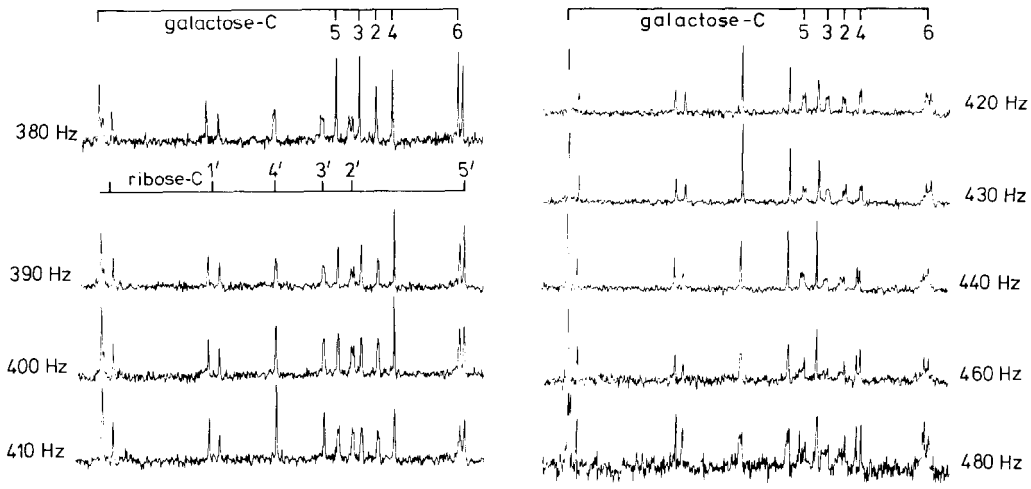


Fig. 2. Selectively ^{1}H-decoupled ^{13}C-NMR spectrum of 3a (D$_2$O, Varian XL 100). The proton decoupling frequencies given are relative to sodium D$_4$-trimethylsilyl-propionate in D$_2$O.

Closely analogous shift displacements are found for β(1→5)- or α(1→5)-linked glycosyl-ribonucleosides as evidenced by the data given in Fig. 3 for 2a, 1b and the corresponding model compounds. Here, the C-5′ signal shows a distinct downfield shift as compared to the smaller, yet clearly noticeable adverse effect on C-4′ and essentially negligible consequences for the other ribose carbons. Again, displacements in the same direction and the same order of magnitude are observed for methyl β-D-riboside and its 5′-O-methyl derivative (Fig. 3).

For characterization purposes, glycosyl-ribonucleosides have often been converted into their well-crystallizing per-O-acetates[3-7], thus a comparative evaluation of shift displacements between tri-O-acetyl-uridine and the peracetate of 2a was made, with methyl tetra-O-acetyl-β-D-galactopyranoside for signal identification. Not unexpectedly, in view of comparing carbons carrying acetoxy and tetraacetyl-glycosyloxy groups the effects are different, yet nevertheless significant. When going from peracetyl-uridine to 2a-hexacetate downfield shifts are observed for C-5′ (63.2→68.0 ppm) and C-4′ (80.0→81.9), whilst the anomeric C-1′, is reversely displaced (87.6→85.8 ppm).

In summary, the shift displacement patterns observed between ribonucleosides and their O′-glycosylation products are seen to provide an efficient tool with which to assign glycosylation sites. It also seems likely that the approach outlined herein will prove applicable to other ribonucleoside-derived products such as ethers, acetals and esters, most opportune being the differentiability between 2′-O- and 3′-O-aminoacyl derivatives.

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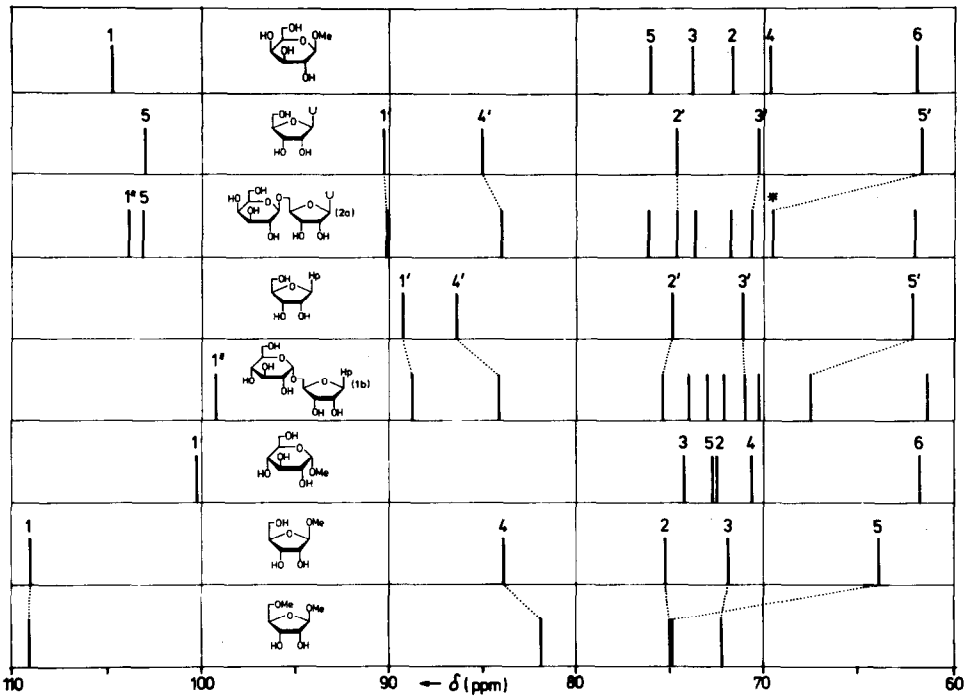

Fig. 3. ^{13}C-NMR data of galactosyl-β(1→5)-uridine (2a) and glucosyl-α(1→5)-inosine (1b) in comparison with model compounds. The signal marked * contains the resonances for C-4" (galactose) and C-5' (ribose).

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10. Samples of enzymatically prepared 3a[6] and 3b[5] were kindly provided by Prof. Y. Suzuki, Okayama University, Kurashiki.
11. In the ^{1}H-NMR spectra of 3a (cf. Fig. 1 in ref. 9) and 3b, ribose and galactose protons are well separated (except those at the anomeric centre), thus considerably facilitating the selective decoupling.
12. Presupposition for the C-3'/C-2'-differentiation was, of course, the unequivocal, double resonance-based assignment of H-3' (dd of J = 5.1 and 6.1 Hz at δ = 4.39 ppm) and H-2' (dd of J = 4.1 and 5.1 Hz at 4.50).
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