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Two-bond $^{13}C-^{13}C$ spin-coupling constants in carbohydrates: effect of structure on coupling magnitude and sign

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Abstract

An empirical projection method is described to predict the magnitudes and signs of two-bond $^{13}C-^{13}C$ spin-coupling constants ($^2J_{CC}$) in aldopyranosyl rings. The method has been applied primarily to the interpretation of $^2J_{CCC}$ values, although the behavior of $^2J_{COC}$ has also been examined in light of the new approach, producing results which may prove useful in the conformational analysis of *O*-glycosidic linkages in oligosaccharides. High-level ab initio calculations of $^2J_{CC}$ values in model compounds were found to be in agreement with the predictions.

Keywords: Coupling constants, two-bond $^{13}C-^{13}C$ spin–spin; Conformational analysis

1. Introduction

Continued progress in defining the structure–function relationships of biologically important carbohydrates will depend, in part, on improved methods to detect and quantify the conformational and motional characteristics of these molecules in solution and bound to biological receptors. Apart from computational approaches [1], NMR spectroscopy is well suited to the task, since it provides several parameters that are affected by molecular structure and dynamics. The effect of motional averaging on NMR parameters, however, can be complex, as discussed by Jardetzky [2], thereby making the translation of experimentally observed parameters into a motional model potentially difficult. Thus, in cases where molecular motion (i.e., conformational

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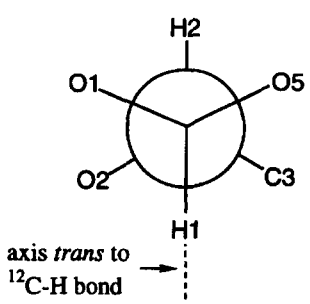
averaging) is suspected, use of an array of different NMR parameters may prove helpful if not essential. For example, structural analysis of the conformationally flexible β-D-ribofuranose ring of RNA should benefit from an integrated treatment of the abundant $^{13}C-^{1}H$ and $^{13}C-^{13}C$ spin-coupling constants within this ring [3,4]. The same argument can be applied to flexible aldopyranosyl rings, such as those having the α-D- or L-*ido* configuration [5].

With the above considerations in mind, we have been examining J_{CH} and J_{CC} in carbohydrates [6–12] and nucleosides [3,4,13,14] in order to better define their dependencies on molecular structure. Recent work has suggested that one-bond $^{13}C-^{1}H$ spin-coupling ($^{1}J_{CH}$) in aldofuranosyl rings correlates with C–H bond length, with the latter in turn depending on C–H bond orientation (quasi-axial vs. quasi-equatorial) [4,8]. One-bond $^{13}C-^{13}C$ spin-coupling ($^{1}J_{CC}$) involving carbons bearing hydroxyl groups appears to depend, in part, on the O–C–C–O torsion angle [12]. Vicinal spin-couplings, $^{3}J_{CH}$ and $^{3}J_{CC}$, depend on dihedral angles as expected, although factors such as the orientation of terminal electronegative substituents relative to the coupling pathway also affect the observed coupling [7,9,10,15,16].

Two-bond $^{13}C-^{1}H$ spin-coupling ($^{2}J_{CCH}$) in carbohydrates can be positive or negative in sign and has been shown to depend on the orientation of electronegative substituents relative to the C–H bond, with substituents anti to this bond making a positive contribution to the coupling [17,18]. Bock and Pedersen [18] have proposed a projection rule to predict these couplings for specific C–C–H coupling pathways. Thus, for example, this rule yields a projection sum of −0.5 Hz and predicts a large negative value of $^{2}J_{C1,H2}$ in methyl β-D-allopyranoside (**1**) (Scheme 1); experimental measurements of this coupling (−6.5 Hz) confirm this prediction [6,7].

In contrast to $^{2}J_{CCH}$, few studies have addressed the dependence of two-bond $^{13}C-^{13}C$ spin-coupling ($^{2}J_{CC}$) on carbohydrate structure [9,10,19,20]. Previous work has shown that $^{2}J_{C1,C3} \approx 0$ Hz in α-D-glucopyranose (**2**), while $^{2}J_{C1,C3} = 4.5$ Hz in β-D-glucopyranose (**3**) [9,19] (Table 1). These and related observations in other monosaccharides have led to the suggestion that the orientation of the terminal hydroxyl substituents along a HO–C–C–C–OH coupling fragment is a critical determinant of coupling magnitude [9,10]. Furthermore, $^{2}J_{C1,C5} \approx 0$ Hz in β-D-glucopyranose (**3**) while $^{2}J_{C1,C5} = 1.8$ Hz in α-D-glucopyranose (**2**) [9,19] (Table 1), but the origin of this different behavior is not well understood [9]. This report describes an empirically derived



Projection sum calculation [18] for methyl β-D-allopyranoside 1

O1: cos 120° = -0.5
O2: cos 60° = 0.5
O5: cos 120° = -0.5
projection sum = -0.5

Scheme 1.

Table 1
Two-bond $^{13}C-^{13}C$ spin-coupling constants [a] in several monosaccharides

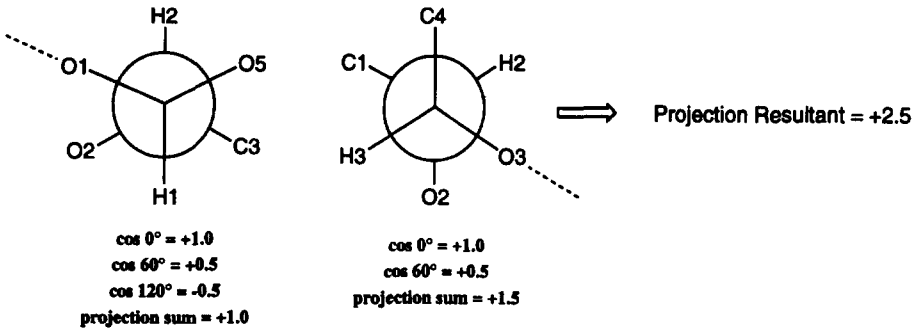
Compound	$^2J_{C1,C3}$ [b]	$^2J_{C1,C5}$ [b]	$^2J_{C2,C4}$ [c]	$^2J_{C3,C5}$ [c]
α-D-Glucopyranose (**2**)	nc	(−) 1.8	(+) 3.0	
β-D-Glucopyranose (**3**)	(+) 4.5	nc	(+) 2.8	
β-D-Mannopyranose (**4**)	(+) 4.0	nc	nc	
α-D-Allopyranose (**5**)	(−) 2.4			
β-D-Allopyranose (**6**)	nc			
α-D-Mannopyranose (**7**)	nc	(−) 2.0	nc	
β-D-Altropyranose (**8**)	nc			
α-D-Altropyranose (**9**)	nc			
2-Deoxy-α-D-*arabino*-hexopyranose (**10**)	(−) 2.3			
2-Deoxy-β-D-*arabino*-hexopyranose (**11**)	(+) 1.8			
α-D-Galactopyranose (**15**)	nc	(−) 1.9	nc	
β-D-Galactopyranose (**16**)	(+) 4.6	nc	nc	
α-D-Xylopyranose (**17**)	nc	(−) 2.0		(+) 0.9
β-D-Xylopyranose (**18**)	(+) 4.0	nc		(+) 2.0
α-D-Arabinopyranose (**19**)	(+) 4.2	nc [d]		nc

[a] Couplings are accurate to within ±0.1 Hz. Indicated coupling signs in parentheses are predicted from projection resultants (see text and Fig. 1). An "nc" entry denotes no coupling was observed ($J < 0.8$ Hz).
[b] Several values taken from ref. [9].
[c] Several values taken from ref. [10].
[d] Compound **19** exists primarily in the $^1C_4$ conformation, and thus geometry about the C-1–O-5–C-5 coupling pathway is similar to that observed in β anomers in the $^4C_1$ form (e.g., **3**, **4**, **16**, **19**).

projection model to predict the magnitudes and signs of $^2J_{CCC}$ and $^2J_{COC}$ in carbohydrates, and to provide a framework on which to base future investigations of these coupling constants in these molecules.

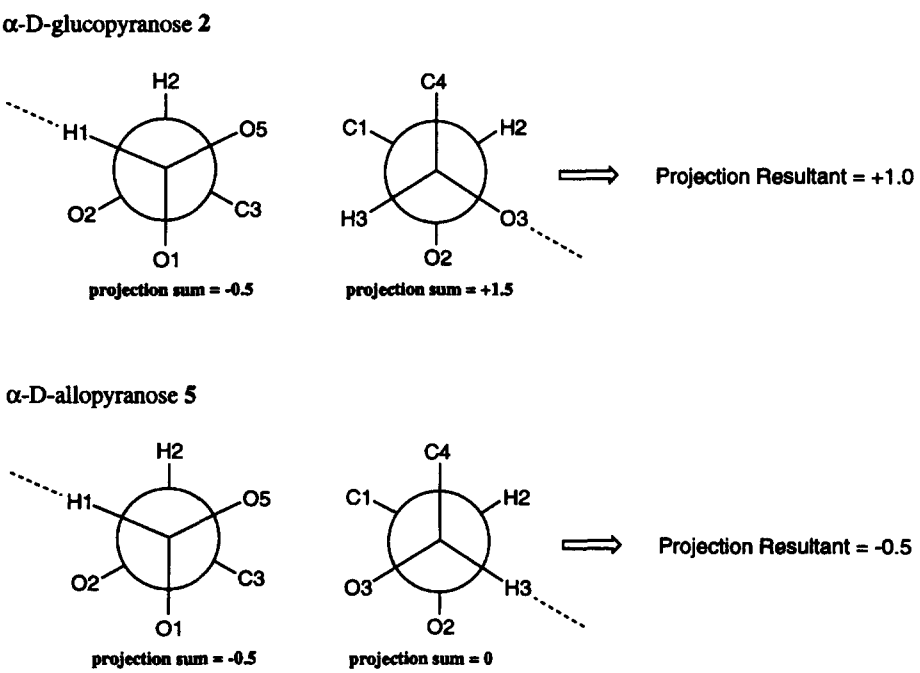
2. Results and discussion

The projection rule for $^2J_{CH}$ [18] involves an inspection of the angles each electronegative substituent on the C–C–H coupling pathway makes with an axis trans to the C–H bond (Scheme 1). The cosines of each of these angles are then combined to yield a projection sum, which is used to estimate coupling magnitude and sign. Since $^2J_{CC}$ is expected to be influenced by the same factors as $^2J_{CH}$ [19,21], a modification of the projection method might prove applicable in predicting these couplings. However, unlike $^2J_{CH}$ where only one of the coupled nuclei is terminal (i.e., H), $^2J_{CC}$ involves two non-terminal nuclei, and thus two projections are likely to be required to treat this situation. Thus, for $^2J_{C1,C3}$ in β-D-glucopyranose (**3**), the two projections are obtained by viewing along the C-1–C-2 bond from C-1 to C-2, and along the C-2–C-3 bond from C-3 to C-2 (Scheme 2). The projection sums for each projection (+1.0 and +1.5) are combined to give a projection resultant of +2.5, which is related to the observed absolute $^2J_{CCC}$ in **3** of 4.5 Hz (Table 1). A similar treatment of $^2J_{C1,C3}$ in β-D-mannopyranose (**4**) also gives a projection resultant of +2.5, which corresponds to an absolute $^2J_{C1,C3}$ of 4.0 Hz (Table 1). On the other hand, α-D-glucopyranose (**2**) gives a projection



Scheme 2.

resultant of +1.0, corresponding to $^2J_{C1,C3} \approx 0$ Hz, and α-D-allopyranose (**5**) gives a value of −0.5, corresponding to an absolute $^2J_{C1,C3}$ of 2.4 Hz (Scheme 3, Table 1). By analogy to $^2J_{CH}$ [17,18], the projections for **3** (Scheme 2) show electronegative substituents trans to the coupled carbons, and thus the observed $^2J_{C1,C3}$ is likely to be positive in sign. Applying this assumption and the $^2J_{C1,C3}$ data in Table 1, a projection plot can be constructed as shown in Fig. 1. This plot predicts that $^2J_{C1,C3}$ in **3** and **4** are positive in sign, whereas $^2J_{C1,C3}$ in **5** is negative (Table 1). The small or zero $^2J_{C1,C3}$ values in β-D-allopyranose (**6**), α-D-mannopyranose (**7**) and β-D-altropyranose (**8**) are



Scheme 3.

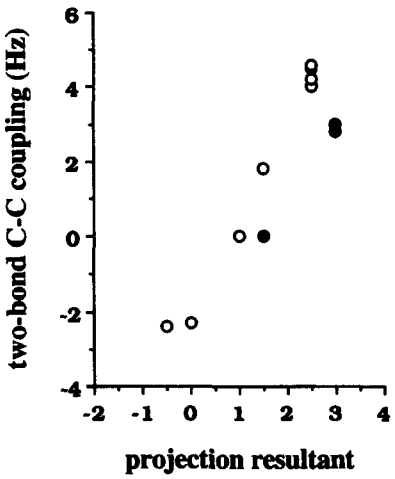


Fig. 1. Plot of projection resultant vs. $^2J_{CC}$ using $^2J_{C1,C3}$ (open circles) and $^2J_{C2,C4}$ (closed circles) coupling data given in Table 1.

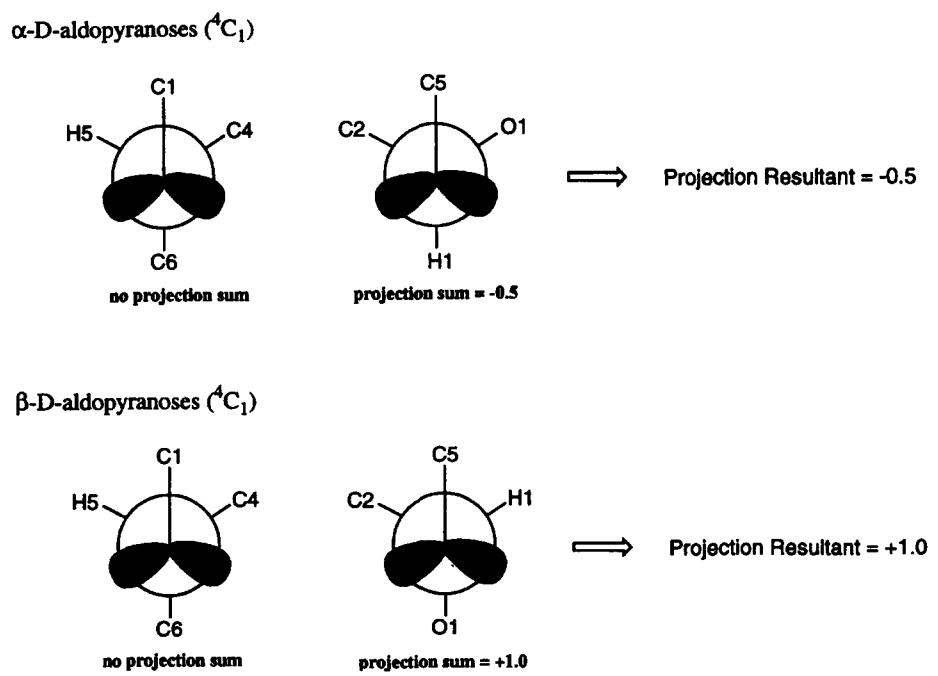
consistent with the projection resultants of +1.0 obtained for these configurations. Interestingly, the projection resultant of −0.5 for the $^4C_1$ form of α-D-altropyranose (**9**) predicts a negative $^2J_{C1,C3}$, but no coupling is observed (Table 1). This result can be explained, however, by noting that **9** exists in both $^4C_1$ and $^1C_4$ forms in solution [22,23], and in the $^1C_4$ conformer the projection resultant (+2.5) predicts a positive $^2J_{C1,C3}$. Thus, conformational averaging of both ring forms in solution would be expected to lead to the observed very small or zero value of $^2J_{C1,C3}$.

The effect of C-2 substitution on $^2J_{C1,C3}$ was examined by measuring $^2J_{C1,C3}$ in 2-deoxy-D-[1-^{13}C]*arabino*-hexopyranose. A projection resultant of 0 is obtained for the α-pyranose **10**, which is consistent with the observed absolute coupling of 2.3 Hz (Table 1, Fig. 1). In the β-pyranose **11**, a projection resultant of +1.5 is obtained, which is consistent with the observed absolute coupling of 1.8 Hz (Table 1, Fig. 1). Furthermore, $^2J_{C1,C3}$ is predicted to be negative in **10** and positive in **11** (Table 1). This predicted change in sign is consistent with computed trends in $^2J_{CC}$ observed in the model compound, $HOCH_2–CH_2–CH_2OH$ (1,3-dihydroxypropane, **12**). Two geometries of this diol were optimized at the HF/3-21G level of theory using the Gaussian 92 suite of programs [24]; the *tGt* conformer **13** corresponded to the geometry observed about the C-1–C-2–C-3 fragment of **10**, whereas the *tTt* conformer **14** corresponded to the geometry about the same fragment in **11**. Using a $[5s2p1d|2s]$ basis set and methods described previously [12,25], $^2J_{CC}$ values (QCISD(T) treatment) of −0.7 Hz and 6.6 Hz were computed for **13** and **14**, respectively. These results provide theoretical evidence supporting the contention that $^2J_{CC}$ becomes more negative when **11** is converted to **10**. We found that, unlike the behavior of $^1J_{CC}$ in carbohydrates [12], rotation about the C–O bonds of **13** and **14** had a relatively small effect on $^2J_{CC}$ values. Furthermore, the addition of a second hydroxyl substituent to one of the terminal carbons of **12**, which yields an improved structural model for evaluating coupling behavior in **10** and **11**, gave

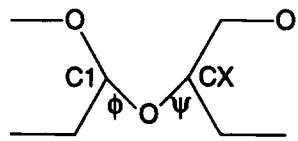
the same coupling trends as found for **12**. This latter result is consistent with experimental data indicating that $^2J_{C1,C3}$ and $^2J_{C2,C4}$ in aldopyranosyl rings show similar dependencies on ring structure [10].

Coupling between C-1 and C-3 in aldopyranosyl rings involves terminal carbons bearing one (C-3) and two (C-1) electronegative substituents. The applicability of Fig. 1 was also examined for $^2J_{CCC}$ values involving terminal carbons each bearing one electronegative substituent. The projection resultants for $^2J_{C2,C4}$ in **2** and **3** are +3.0, and couplings of 3.0 and 2.8 Hz are observed, respectively (Table 1) [10]. On the other hand, a projection resultant of +1.5 is observed for **4** and **7**, and α- (**15**) and β- (**16**) D-galactopyranoses, corresponding to couplings of essentially 0 Hz (Table 1) [10]. These data are also plotted in Fig. 1. A small right-shift in the projection plot is observed as the number of electronegative substitutents on the terminal carbons is reduced (Fig. 1), but global trends are conserved. We also examined $^2J_{C3,C5}$ in α- (**17**) and β- (**18**) D-xylopyranoses and α-D-arabinopyranose **19** for consistency with the proposed rule. In these cases, projection resultants of +1.5 were obtained, but observed couplings ranged from 0 to 2.0 Hz [10] (Table 1), suggesting that the ring oxygen exerts effects on $^2J_{CCC}$ that differ from those made by terminal hydroxyl groups, and that the projection resultant method does take these effects fully into account.

As mentioned above, $^2J_{C1,C5}$ is measureable in α-D-aldopyranosyl rings ($^4C_1$ forms; e.g., α-D-*gluco*, α-D-*manno*, α-D-*galacto*, α-D-*xylo*), but is not observed in the corresponding β anomers (Table 1) [9,19]. In α anomers, projections across the ring oxygen



Scheme 4.

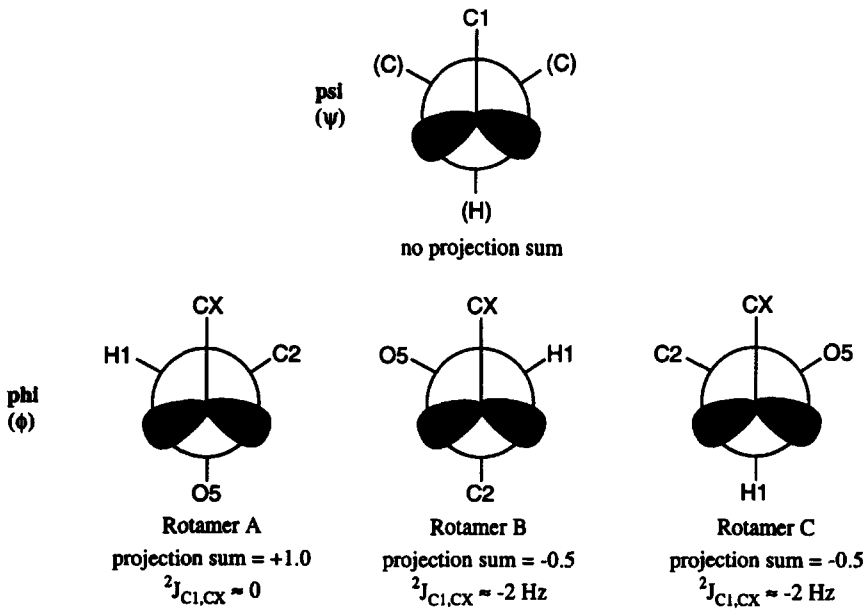


Scheme 5.

yield a projection resultant of −0.5, corresponding to an average observed absolute $^2J_{C1,C5}$ of ~2 Hz (Scheme 4). In β anomers, the projection resultant is +1.0, corresponding to no coupling (Scheme 4). It is generally held that geminal $^{13}C-^{13}C$ spin-coupling across oxygen is negative [26]. The less positive projection resultant (−0.5) for the α anomers is consistent with a more negative coupling, as was observed for $^2J_{CCC}$ (Fig. 1), although the relationship between projection resultant and observed $^2J_{CC}$ is different for $^2J_{CCC}$ and $^2J_{COC}$. Coupling constant calculations similar to those performed on **12** (see above) were conducted on the model compound, $HO-CH_2-O-CH_3$ (**20**), where the methylene carbon mimics the anomeric carbon. The computed $^2J_{COC}$ for **20** in a conformation similar to that observed in β anomers was +0.4 Hz, whereas a coupling of −2.8 Hz was obtained for **20** in a conformation found in α anomers. These results support the prediction that $^2J_{C1,C5}$ values become more negative in the conversion of β anomers to α anomers.

The above treatment of $^2J_{COC}$ leads to an interpretation of $^2J_{COC}$ values across the *O*-glycosidic linkage of oligosaccharides. $^3J_{COCC}$ and $^3J_{COCH}$ values are frequently used to examine linkage conformation due to their dependence on C–O–C–C and C–O–C–H dihedral angles, respectively [27–30]. Of interest here is whether $^2J_{COC}$ values across these linkages can be of use in such analyses. Consider a typical *O*-glycosidic linkage (Scheme 5) in which $^2J_{C1,CX}$ is under consideration. No projection sum is associated with ψ regardless of linkage conformation (since no electronegative substituents are found in this projection which can be related to C-1), and thus its contribution to the observed $^2J_{C1,CX}$ can be neglected (Scheme 6). Thus, the main contribution to the observed $^2J_{C1,CX}$ is predicted to originate from φ. Three staggered rotamers (rotamers A–C) can be envisioned about φ (Scheme 6). Rotamer A gives a projection sum of +1.0; in this rotamer $^2J_{C1,CX}$ is expected to be essentially zero based on the above discussion. In the remaining two rotamers, projection sums of −0.5 are obtained, and thus couplings of about −2 Hz are predicted in each. This conclusion differs from that made previously by Nunez and Barker [27], where a zero coupling was predicted for rotamer B.

The above predicted response of $^2J_{C1,CX}$ to φ can be partly tested by observing the complementarity between the two-bond coupling between C-1 and C-X and the three-bond coupling between C-2 and C-X (Scheme 6). Rotamer B is expected to dominate in simple aldopyranosides since the exoanomeric effect [31,32] is optimal in this geometry. In rotamer B, C-2 is anti to C-X, and thus $^3J_{C2,CX}$ is expected to be large (3–4 Hz) [9,10,19]. Thus a large $^3J_{C2,CX}$ should be accompanied by a $^2J_{C1,CX}$ of about −2 Hz. In methyl α-D-glucopyranoside, $^3J_{C2,CH_3} = 3.0$ Hz, and $^2J_{C1,CH_3} = 1.8$ Hz, in agreement with this prediction. Likewise, in methyl β-D-ribofuranoside, $^3J_{C2,CH_3} = 3.2$ Hz and $^2J_{C1,CH_3} = 2.1$ Hz, again as expected. Presumably, the $^2J_{COC}$ values are negative in these



Scheme 6.

cases. These results suggest that two-bond $^{13}C-^{13}C$ spin-coupling across an *O*-glycosidic linkage defined solely by ϕ and ψ values (Scheme 5) does not provide information about ψ, but can be used, in conjunction with $^3J_{COCC}$, to assess behavior about ϕ. This conclusion places earlier interpretations of *trans-O*-glycoside $^2J_{COC}$ in oligosaccharides [27,28] on firmer ground.

This report describes an empirical approach to the prediction of the magnitudes and signs of $^2J_{CCC}$ and $^2J_{COC}$ values in carbohydrates. Further verification of the method will derive from additional measurements of $^2J_{CC}$ in carbohydrates and from the application of NMR methods capable of establishing the signs of these couplings. Work on the latter problem is currently in progress.

3. Experimental

Labeled compounds.—D-[1-^{13}C]Glucose, D-[2-^{13}C]glucose, D-[1-^{13}C]mannose, D-[2-^{13}C]mannose, D-[1-^{13}C]allose, D-[1-^{13}C]altrose, D-[1-^{13}C]galactose, D-[2-^{13}C]galactose, D-[1-^{13}C]xylose, D-[5-^{13}C]xylose, D-[1-^{13}C]arabinose, methyl β-D-[1-^{13}C]allopyranoside, methyl β-D-[1-^{13}C]ribofuranoside, methyl β-D-[2-^{13}C]ribofuranoside, methyl α-D-[1-^{13}C]glucopyranoside and methyl α-D-[2-^{13}C]glucopyranoside were prepared as previously described [3,6,7,9,10]. 2-Deoxy-D-[1-^{13}C]*arabino*-hexopyranose (2-deoxy-D-[1-^{13}C]glucose) was obtained from Cambridge Isotope Laboratories.

NMR spectroscopy.—High-resolution ^{13}C NMR spectra of (^{13}C)-labeled compounds were obtained at 30 °C on a Varian Unity-Plus 600 MHz FT-NMR spectrometer

operating at 150.85 MHz for ^{13}C. Samples were dissolved in $^{2}H_2O$ (99 atom% ^{2}H, Cambridge Isotope Laboratories), and solutions were transferred to 5 mm NMR tubes. Spectra were processed with resolution enhancement in order to facilitate the detection of small J_{CC} values; details on the measurement of $^{13}C-^{13}C$ spin-coupling constants in carbohydrates have been published previously [12].

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