# Disaccharide Solution Stereochemistry from Vibrational Raman Optical Activity

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*Received December 27, 1993**

**Abstract:** The vibrational Raman optical activity (ROA) spectra of D-maltose, D-maltose-*O*-$d_8$, D-cellobiose, D-isomaltose, D-gentiobiose, D-trehalose, and α-D-cyclodextrin in aqueous solution between ~350 and 1500 $cm^{-1}$ measured in backscattering are reported, which show that ROA is an incisive new probe of disaccharide stereochemistry. Some of the ROA signals in the fingerprint region and two ROA couplets at ~430 ± 10 and ~917 $cm^{-1}$ have been identified to exhibit contributions from vibrational coordinates of the glycosidic link. The sign of the former couplet was found to depend on the configuration of the link, and its width was found to depend on the particular linkage type. The second ROA couplet was present only in the ROA spectra of D-maltose and D-isomaltose and was attributed from deuteration studies to a mode involving glycosidic C–O–C stretching coordinates and C–O–H deformations. Opposite signs for this couplet in D-maltose and D-isomaltose may reflect either the involvement of the $CH_2$ group in the linkage or conformational changes. Sign, intensity, and position of the ROA signals in the fingerprint region ~950–1200 $cm^{-1}$ appear to be characteristic for a particular disaccharide unit. Complementary information is obtained in the $CH_2$ and COH deformations region ~1200–1500 $cm^{-1}$, where the disaccharide ROA spectra were demonstrated to be superpositions of the corresponding ROA spectra of the constituent monosaccharides. Also in this region a positive ROA band at ~1260 $cm^{-1}$ was assigned to the β-anomeric form and used to estimate the anomeric populations in the reducing residues of some disaccharides.

## Introduction

Vibrational Raman optical activity (ROA) refers to a small differential Raman scattering of right and left circularly polarized light from chiral molecules.[1] Recent advances in instrumentation[2] have increased the sensitivity beyond the level necessary to record ROA spectra of aqueous solutions of biological molecules. Among these, carbohydrates[3–7] are particularly favorable, as the local vibrational coordinates are extensively coupled in the normal modes resulting in a rich ROA spectrum. The utility of ROA lies in the fact that it may provide new stereochemical information[8] inaccessible to other techniques: not only are the signs of ROA bands related to absolute configuration but their intensity also displays a great sensitivity to molecular conformation. In this paper we report some recent studies of disaccharides in aqueous solution which show that ROA is particularly sensitive to the type and stereochemistry of the glycosidic link.

Other chiroptical techniques such as electronic circular dichroism (ECD) have been applied to carbohydrates.[9,10] The main problem encountered in ECD studies of unsubstituted carbohydrates is that the structural features absorb in the vacuum UV region;[9] consequently, only the long wavelength tails[10] of ECD bands are accessible to commercial instruments. In contrast, each of the 3N-6 normal modes contributes accessible bands in the ROA spectrum. Vibrational circular dichroism (VCD), the extension of circular dichroism into the infrared, is a form of vibrational optical activity complementary to ROA, which has also been applied to the study of carbohydrates:[11,12] however, VCD suffers from low instrument sensitivity and from the strong infrared absorption of water, the natural solvent for biological activity.

NMR has provided most of the spectroscopic data on the solution stereochemistry of carbohydrates.[13,14] However, even for relatively simple carbohydrates band assignment can be problematic, as a large number of resonances fall within an extremely narrow chemical shift range. Furthermore, only an average conformation is revealed directly by NMR as a result of the relatively long time scale of the experiment. Usually, NMR data must be supplementdd by calculations of potential energy surfaces,[13] which serve to limit the amount of available conformational space and give meaning to the data collected from nuclear Overhauser effect (NOE) and spin-coupling experiments. The Raman effect on the other hand is essentially instantaneous so that distinct ROA signals can be observed from conformers that are exchanging fast on the NMR time scale.

The application of conventional vibrational spectroscopy to carbohydrates has been hindered by the complexity of their normal modes and the broad, overlapping nature of their bands.[15] As shown below, the interpretation of carbohydrate ROA spectra is far more incisive because only those few vibrational coordinates within a complicated normal mode which sample the skeletal chirality most directly make the largest contributions to the ROA intensity.

* Abstract published in *Advance ACS Abstracts,* May 1, 1994.

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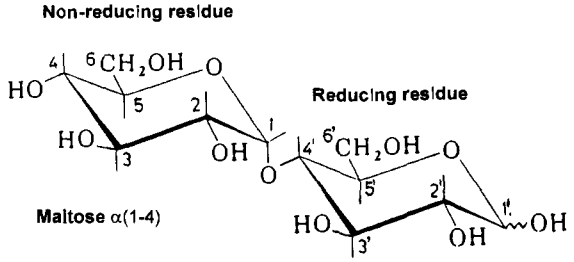


**Figure 1.** Structural formula and atomic numbering scheme of disaccharides.

Previous papers in this series were concerned with a preliminary survey of monosaccharide ROA spectra[5] followed by a comprehensive study of D-glucose,[6] the latter providing a cornerstone for the assignment of the ROA spectra of disaccharides containing D-glucose residues which are presented here. The example of D-maltose, an α(1–4)-linked disaccharide containing two D-glucose residues, is used in Figure 1 to illustrate the general structural features and the atomic numbering scheme employed.

## Experimental Section

Samples of D-cellobiose, D-gentiobiose, D-isomaltose, and α-D-cyclodextrin were supplied by Sigma, D-trehalose by Fluka, and D-maltose by Aldrich. A sample of D-maltose-*O*-$d_8$ was prepared by lyophilizing D-maltose from $D_2O$ solution twice to ensure at least 90% isotopic exchange before dissolving in $D_2O$ to a concentration of 3 M. All the other carbohydrate samples were dissolved in distilled $H_2O$ to a concentration of 3 M, except for D-cellobiose (1.2 M) and α-D-cyclodextrin (0.5 M), and allowed to equilibrate at room temperature for at least 24 h. The equilibrated samples were treated with charcoal to reduce fluorescence, filtered into quartz microfluorescence cells through 0.45-μm Millipore membrane filters to remove any dust particles that may cause spurious light scattering, and then centrifuged for at least 15 min.

The major features of the Glasgow ROA instrument GUROAS1, which is described in detail elsewhere,[2] are the backscattering geometry to maximize the signal-to-noise ratio, the cooled back-thinned CCD detector with its high quantum efficiency and low readout noise, the Lyot depolarizer, without which the spectra would be swamped with artifacts, and the new holographic super notch plus filter,[7] which suppresses stray light from the Rayleigh line and allows backscattered ROA to be measured down to ~350 $cm^{-1}$. During spectral acquisition the laser power was ~700 mW at the sample and the slit width was set for 120 μm, giving a spectral bandpass of ~12 $cm^{-1}$ using 514.5-nm laser excitation. The spectra were recorded over 2–4 h.

The ROA spectra are presented in the form of the circular intensity difference $I^R - I^L$, where $I^R$ and $I^L$ are the Raman scattered intensities in right and left circularly polarized incident light. The conventional Raman spectra are presented as the corresponding circular intensity sum $I^R + I^L$, and the dimensionless ratio $\Delta = (I^R - I^L)/(I^R + I^L)$ is defined as a suitable quantity for comparison with theory.

## Results and Discussion

The Raman and ROA spectra of D-maltose, D-maltose-*O*-$d_8$, D-cellobiose, D-isomaltose, D-gentiobiose, D-trehalose, and α-D-cyclodextrin in the range ~350–1500 $cm^{-1}$ are shown in Figures 2–8, respectively. All the spectra presented here, with the exception of α-D-cyclodextrin, which is the cyclic hexamer of D-glucose, are of disaccharides consisting of two D-glucose residues joined by an *O*-glycosidic linkage. This choice of samples made it possible to build upon the work on the D-glucose monomer[6] while at the same time investigating the influence of the glycosidic link on the ROA spectra. α-D-Cyclodextrin was included in this study to provide an example of an α(1–4)-linked oligosaccharide.

The glycosidic link can form between the anomeric carbon of one residue and either carbon 1, 2, 3, 4, or 6 of another. If the link involves the anomeric carbon of a residue, then it is trapped in a single anomeric form and is known as a nonreducing residue. If on the other hand the link does not involve the anomeric carbon of a residue, then it is free to undergo mutarotation producing

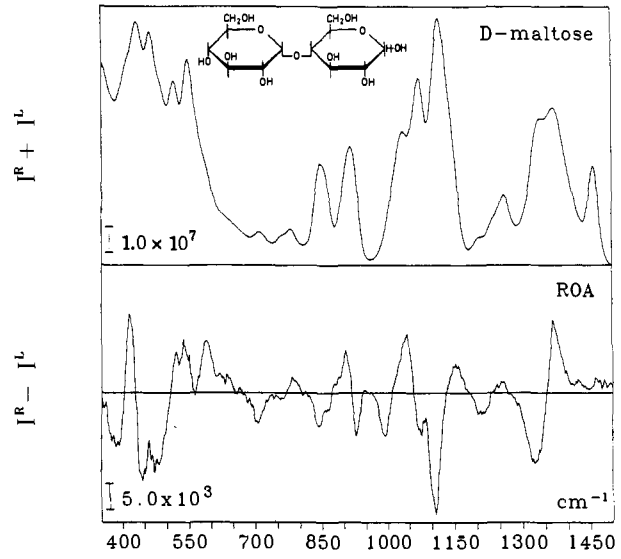


**Figure 2.** Backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of D-maltose.

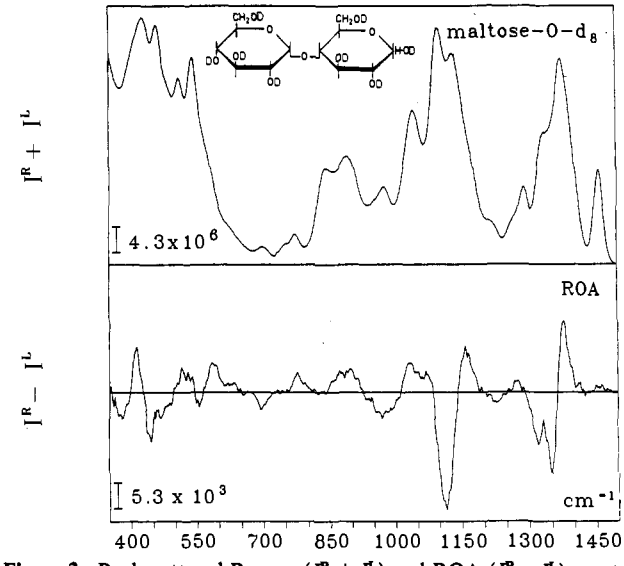


**Figure 3.** Backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of D-maltose *O*-$d_8$.

an equilibrium mixture of the two anomeric forms and is described as a reducing residue (Figure 1). The link may have either an α- or β-configuration depending on whether the C1–O1 bond involved in the link is in an axial or equatorial position, respectively, and the linkage type may be further classified according to which two carbon atoms are adjacent to the oxygen atom of the linkage.

Each disaccharide ROA spectrum can be conveniently subdivided into four distinct regions: the low-wavenumber region ~350–600 $cm^{-1}$; the anomeric region ~600–950 $cm^{-1}$; the fingerprint region ~950–1200 $cm^{-1}$; and the $CH_2$ and COH deformations region ~1200–1500 $cm^{-1}$. Each of these subdivisions will be discussed separately, although some correlations will be drawn from the complete spectra. In this study an empirical approach will be used, as at this time no *ab initio* ROA intensity calculations[16] have yet been performed on a carbohydrate molecule and the normal modes in disaccharides are too complex for the ROA intensities to be explained by simple models such as the two-group model or the bond polarizability model.[1]

**Low-Wavenumber Region (350-600 $cm^{-1}$).** Until recently, backscattered ROA measurements have been restricted to the spectral region above ~600 $cm^{-1}$ due to the severe stray light

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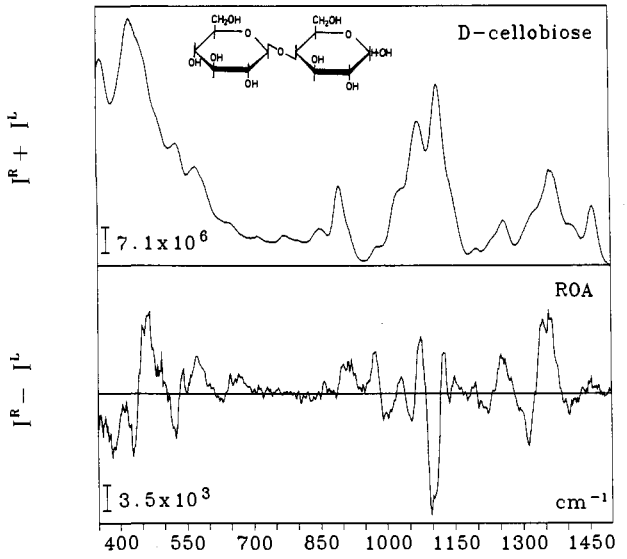


**Figure 4.** Backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of D-cellobiose.

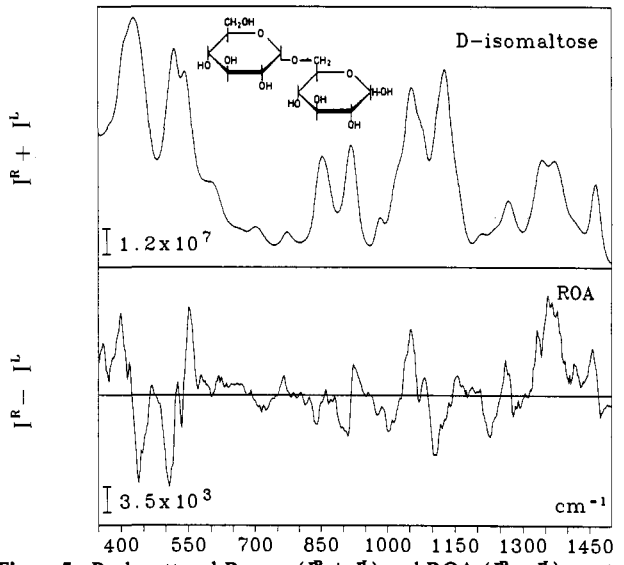


**Figure 5.** Backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of D-isomaltose.

problems encountered at lower wavenumber. However, with the advent of improved versions of holographic notch filters it has been demonstrated[7] that backscattered carbohydrate ROA spectra down to ~350 $cm^{-1}$ can be recorded. In this region normal coordinate analyses of monosaccharides[17,18] assign the Raman bands to vibrations involving exo- and endocyclic bending deformations of the C–C–O, C–C–C, C–O–C, and O–C–O groups coupled with associated exo- and endocyclic torsions about C–O bonds. It has also been suggested[19] that the intense Raman bands involving C–C–O deformations around the anomeric carbon which are found in this region are among the most sensitive to anomeric configuration in the entire monosaccharide spectrum. It has been calculated that the normal mode composition is similar in disaccharides[20,21] but that there is also the possibility of additional contributions arising from motions of the C–O–C group which forms the glycosidic link.

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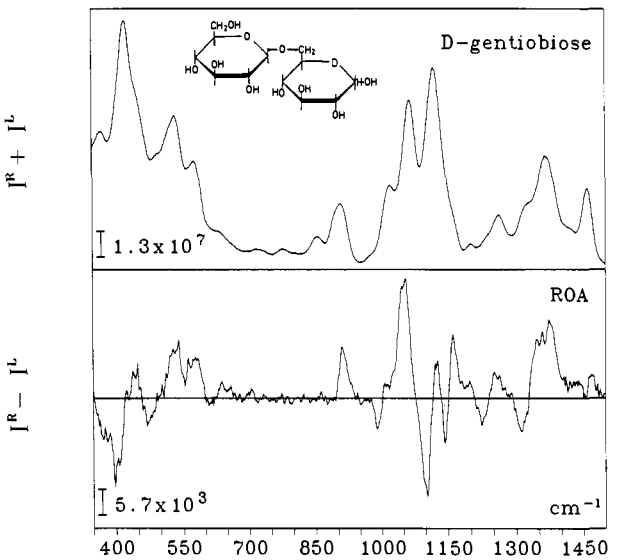


**Figure 6.** Backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of D-gentiobiose.

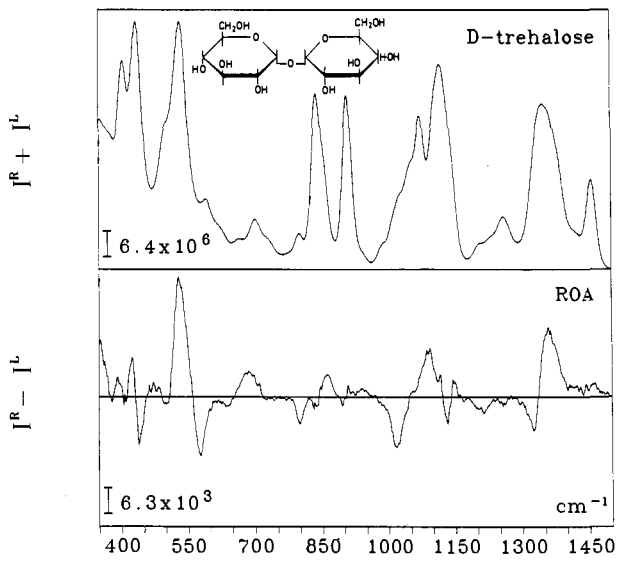


**Figure 7.** Backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of D-trehalose.

The complexity of the normal modes and the lack of specific conventional Raman assignments in this region hinder the assignment of ROA signals to particular vibrations. These difficulties are compounded by the fact that previous ROA studies[3–6] did not investigate below ~600 $cm^{-1}$. However, it is clear from comparison of the spectra of D-maltose (Figure 2) and D-maltose-*O*-$d_8$ (Figure 3) that C–O–H vibrations do not contribute significantly to either the Raman or ROA intensities in this region. Thus, it would appear that in common with the fingerprint region[3–6] the ROA signals in this region probe the backbone structure of carbohydrates and may prove even more sensitive to conformation.

One important correlation that can be made in this region is between the configuration of the glycosidic link and the sign of a ROA couplet centered at ~430 ± 10 $cm^{-1}$ found in all the disaccharides containing only D-glucose residues studied so far (Table 1). This couplet is positive at low and negative at high wavenumber for $\alpha$-linked species and negative at low and positive at high wavenumber for $\beta$-linked species. At present it is not clear whether the normal modes responsible for generating the ROA couplet are closely related, such as the in-phase and out-of-phase combinations of the same local vibrational coordinates, or are composed of quite different vibrational coordinates

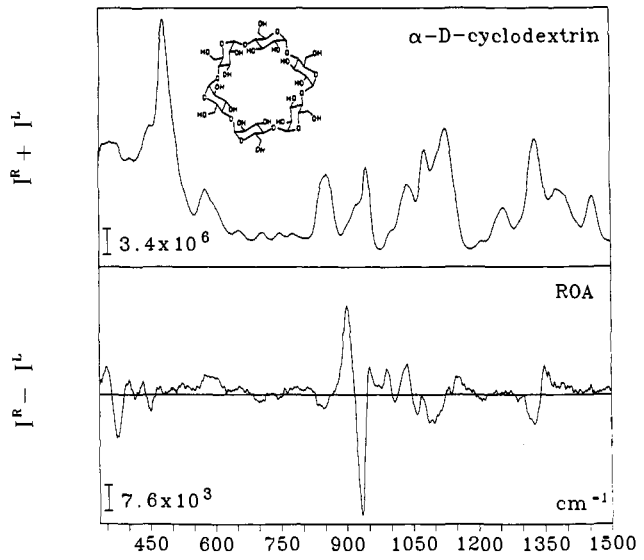


**Figure 8.** Backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of α-D-cyclodextrin.

**Table 1.** Position and Width (cm$^{-1}$) of Low-Wavenumber Glycosidic ROA Couplets

| oligosaccharide | linkage type and configuration | position and sign of ROA couplet | deconvoluted couplet width |
|---|---|---|---|
| D-maltose | α(1–4) ax.–eq. | 431 (+,–) | 31 |
| D-maltose-*O*-$d_8$ | α(1–4) ax.–eq. | 428 (+,–) | 34 |
| D-cellobiose | β(1–4) eq.–eq. | 440 (–,+) | 34 |
| D-isomaltose | α(1–6) ax.–eq. | 424 (+,–) | 39 |
| D-gentiobiose | β(1–6) eq.–eq. | 420 (–,+) | 41 |
| D-trehalose | α(1–1) ax.–ax. | 431 (+,–) | 19 |
| α-D-cyclodextrin | α(1–4) ax.–eq. | 362 (+,–) | 27 |

embracing different regions of the molecule. The fact that this couplet is centered within a ~20 cm$^{-1}$ range for all the disaccharides studied and that the signs of the ROA signals change on going from α-linked to β-linked species strongly suggests that it has a similar origin in all the disaccharides and that deformations around the anomeric carbon of the nonreducing residue play a crucial role in generating the ROA signals. (Recent work carried out in this laboratory on the ketose monosaccharides, unpublished results, has identified a ROA couplet in the same wavenumber range showing the same sign dependence to absolute configuration at the anomeric center as found in the disaccharides presented here.) However, since no such couplet is evident in the ROA spectra of D-glucose (not shown) or the methyl glycosides (not shown), it is likely that motions of the glycosidic link also make important contributions.

From inspection of Table 1, which lists the wavenumber at which this ROA couplet is centered together with its sign and width measured from the band maxima to the band minima of the deconvoluted ROA spectra for a selection of disaccharides together with α-D-cyclodextrin, it is clear that the width of this couplet displays a strong dependence on the linkage type. It was found by deconvolution of the Raman spectra presented here that this dependency is a function of the differences in the separation of the parent Raman bands together with the appearance of additional Raman bands contributing ROA signals of opposite sign for the different anomeric forms.

For the 1–4-linked species D-maltose, D-maltose-*O*-$d_8$, and D-cellobiose (Figures 2–4), the ROA signals of opposite sign for α- and β-linkages associated with three distinct Raman bands were found by deconvolution to have an overall separation of 31, 34, and 34 cm$^{-1}$, respectively. A similar band structure was also found for the α(1–1)-linked species D-trehalose (Figure 7) but with an overall separation of only 19 cm$^{-1}$. This results in a very narrow ROA couplet and may reflect the rigidity of this particular linkage type[22] or possibly the fact that both the anomeric centers are fixed in the α-anomeric form by the linkage in D-trehalose. The corresponding ROA signals in the 1–6-linked species, D-isomaltose and D-gentiobiose (Figures 5 and 6), associated with at least four and possibly five Raman bands were found by deconvolution to have an overall separation of 39 and 41 cm$^{-1}$, respectively, thereby showing a dependence on the linkage type. The presence of additional bands dependent on the configuration of the linkage serves to broaden this couplet relative to the other disaccharides studied here. This may reflect the fact that the 1–6-linked species have the $CH_2$ group of the reducing residue incorporated into the linkage, which results in an increase in the conformational freedom[23,24] and may also alter the composition of the normal modes in this wavenumber range. Furthermore, although α-D-cyclodextrin (Figure 8) has the same linkage type and configuration as D-maltose and also shows a ROA couplet with a band structure similar to those in the 1–4-linked disaccharides described above, the couplet changes sign at ~362 cm$^{-1}$. This wavenumber shift may reflect the strain imposed upon the linkage bonds on forming the cyclic structure.[25]

It would appear then that this couplet can differentiate not only between different linkage configurations but also between distinct linkage types in molecules containing D-glucose residues using the width of the deconvoluted ROA couplet. Furthermore, detailed analysis of the Δ-values of these ROA signals may provide valuable information on the conformation of these linkages.

**Anomeric Region (600-950 cm$^{-1}$).** It is clear from examination of the ROA spectra of the β-linked disaccharides, D-cellobiose (Figure 4) and D-gentiobiose (Figure 6), that they lack any ROA signals in the range from ~700 to 880 cm$^{-1}$. A similar result was also found for the β-anomeric form of the D-glucose monomer.[6] In contrast, the α-linked disaccharides, D-maltose (Figure 2) and D-isomaltose (Figure 5), exhibit three ROA signals in this range, as does the α-anomeric form of the D-glucose monomer. It is well established that the Raman band at ~845 cm$^{-1}$ is characteristic of the α-anomer,[17,26] and normal coordinate analyses[20,21] assign the bands in the range ~700–800 cm$^{-1}$ to bending coordinates of the heavy atoms involved in the glycosidic link or possibly of the hemiacetal fragment. Thus, the presence of ROA signals in the spectra of the α-linked species may reflect some chirality inherent in the linkage or the hemiacetal fragment when the C1–O1 group is in an axial position. This simple observation holds for all the di-, oligo-, and polysaccharides of D-glucose studied so far and provides an easy method of distinguishing between homogeneously α- and β-linked species of D-glucose.

One of the most intriguing and potentially useful features in this region is a ROA couplet in the spectrum of D-maltose centered at ~917 cm$^{-1}$, which is positive at low and negative at high wavenumber. Previous ROA studies[3,4] assigned this couplet to a vibration of the α(1–4) glycosidic link, as it was not present in the spectra of the monomer, D-glucose, nor either of the β-linked disaccharides studied here and was found to have approximately twice the intensity in the trimer, D-maltotriose, which contains two such links per molecule. (For α-D-cyclodextrin, Figure 8, the normalized intensity of this ROA couplet is 1 order of magnitude greater than the largest Δ-values usually encountered.) On the basis of the assignment of a band at 901 cm$^{-1}$ in the conventional IR spectrum of α-D-glucose to a C1–H deformation mode[26] this ROA couplet was attributed to interactions between the anomeric C1–H deformation and the glycosidic C–O–C stretch.[3,4] However, this assignment neglects the fact that a D-glucose band at 913 cm$^{-1}$ was demonstrated to be sensitive to

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O-deuteration and hence to contain a significant contribution from C–O–H deformations.[26] It is clear from a comparison of D-maltose (Figure 2) with its O-deuterated analogue (Figure 3) that the ROA couplet collapses and broadens and the associated Raman band is weakened and shifted to lower wavenumber upon deuteration. In a normal coordinate analysis of amylose,[27] for which D-maltose is the repeating unit, a conformation-sensitive mode at 946 $cm^{-1}$ was described as a skeletal vibration of the $\alpha(1-4)$ linkage and contributions were predicted not only from the linkage atoms but also from the ring atoms and the deformations of C2–O–H and C3–O–H groups. This leads us to the conclusion that it is probably more accurate to describe this couplet as originating in the C–O–C stretch coordinates of the glycosidic link interacting with ring stretching coordinates and C–O–H deformations and to suggest that the O2–H···O3′ intramolecular hydrogen bond found in the crystal structure of D-maltose[28] and known to persist to some degree in aqueous solution[29] may also be important in generating this ROA signal.

A similar couplet is present in the ROA spectrum of D-isomaltose (Figure 5) centered at ~918 $cm^{-1}$ but with the signs reversed relative to that found in D-maltose. Deconvolution of the parent Raman bands demonstrates that both these couplets are associated with two closely separated Raman bands yielding ROA signals of opposite sign. As these two molecules have the same configuration at all the chiral centers, the sign reversal of this glycosidic couplet must originate in the different linkage type in D-isomaltose with its characteristic conformations and distinct associated normal modes. Thus, this couplet not only provides a signature characteristic of certain types of $\alpha$-linked species but also carries conformational data.

**Fingerprint Region (950–1200 $cm^{-1}$).** This region of the vibrational spectrum is dominated by exo- and endocyclic C–O and C–C stretches with significant contributions from C–O–H deformations.[30,31] It was noted in a review of the vibrational spectra of carbohydrates[15] that this region is difficult to interpret on account of the widespread coupling of C–O and C–C stretches, the poor discrimination between endo- and exocyclic contributions, and the small differences between the configurational positions of each C–O group. It has been demonstrated that although C–O–H deformations contribute to the Raman intensity and position of bands in this region, they make no significant contribution to the associated ROA intensity[6] and that configurational changes have a comprehensive and characteristic effect on the ROA sign pattern.[3,5] This latter discovery means that detailed assignment of the individual Raman bands is unnecessary, as the ROA spectrum provides a "fingerprint" characteristic of the backbone structure.

The $\alpha$-linked disaccharides, D-maltose (Figure 2) and D-isomaltose (Figure 5), exhibit the same characteristic negative, positive, negative, and positive sign pattern as D-glucose in this region[5,6] but with an additional weak ROA signal, negative in D-maltose and positive in D-isomaltose, appearing at ~1077 and 1083 $cm^{-1}$, respectively. A normal coordinate analysis of D-maltose described a normal mode appearing at 1063 $cm^{-1}$ involving C–O stretching motions with a major contribution from the C1–O1 and C4′–O1 stretching coordinates of the glycosidic link.[21] Combining this with the fact that this signal is not present in D-glucose leads to the conclusion that this ROA signal is generated by motions of the glycosidic link. Furthermore, the sign change for D-maltose relative to D-isomaltose also reveals a conformational sensitivity. The other $\alpha$-linked species, D-trehalose (Figure 7), deviates significantly from the D-glucose signature presumably due to its unique, in this study, diaxial link.

In general, the $\beta$-linked species, D-cellobiose (Figure 4) and D-gentiobiose (Figure 6), exhibit more changes relative to the characteristic D-glucose signature than the $\alpha$-linked species. In particular, D-cellobiose shows an additional positive ROA signal at ~976 $cm^{-1}$, a negative ROA signal at ~1054 $cm^{-1}$, and a ROA couplet negative at low and positive at high wavenumber centered at ~1120 $cm^{-1}$, which is overlapping the negative ROA signal at lower wavenumber. By comparison D-gentiobiose (Figure 6) differs only in the presence of a couplet centered at ~1133 $cm^{-1}$ with the opposite sense to that observed in D-cellobiose. In addition, both exhibit changes in the relative intensities of the signals relative to D-glucose and the $\alpha$-linked disaccharides. Normal coordinate analysis[20] again seems to indicate that the glycosidic stretching coordinates contribute significantly to the normal modes responsible for the ROA couplets in the range ~1120–1140 $cm^{-1}$ for disaccharides with a $\beta$-linkage. Thus, it appears likely that the observed changes may be attributed to the glycosidic link and also that these signals have a sign dependence on conformation. Indeed, laminarin, a $\beta(1-3)$-linked polysaccharide of D-glucose, exhibits a dramatic increase in intensity for the ROA signals between ~1100 and 1150 $cm^{-1}$.[7] This boost may be reflecting the effect that a helical conformation has on this glycosidic mode.

The complex nature of vibrations in this region makes it difficult to assign accurately the vibrations involved in generating the ROA. However, it does mean that this region provides a "fingerprint" characteristic of the individual disaccharide units, not just a sum of the two constituent sugar residues, and as we shall, see, this is complementary to the type of information available from the $CH_2$ and C–O–H deformation region discussed later. Furthermore, the isolation of ROA signals originating in the link provides an additional perspective on their conformation to those already idenfified in the low-wavenumber and anomeric regions. Finally, it appears that the glycosidic link of the $\beta$-linked species has more influence in this region than the $\alpha$-linked species, which is in contrast to the anomeric region, where the opposite is true.

**$CH_2$ and COH Deformations Region (1200-1500 $cm^{-1}$).** Our recent study of the ROA spectra of D-glucose and several deuterated analogues produced a number of interesting results in this region.[6] The normal modes responsible for the negative and positive ROA signals at ~1220 and ~1260 $cm^{-1}$ in D-glucose were shown to involve coupled $CH_2$ and C–O–H deformations, and it was proposed that these two bands were associated with the gauche–gauche and gauche–trans rotamers of the exocyclic hydroxymethyl group, respectively. In addition, it was demonstrated that only the $\beta$-anomeric form of D-glucose would generate a ROA signal at ~1260 $cm^{-1}$. The results obtained from monosaccharides are expected to have a strong bearing on the interpretation of the disaccharide spectra presented here, as a normal coordinate analysis[32] of the dimer repeating unit of cellulose predicted that the majority of normal modes above 1200 $cm^{-1}$ are localized within the individual residues and are almost identical to those calculated for the monomer, $\beta$-D-glucose.

The positive ROA signal at ~1260 $cm^{-1}$, mentioned above, was assigned to the $\beta$-anomeric form because the ROA spectra of $\alpha$-D-methyl glucoside,[5] D-trehalose (Figure 7), and $\alpha$-D-cyclodextrin (Figure 8), all of which exist solely in the $\alpha$-anomeric form, exhibit no ROA signal at this wavenumber, whereas that of $\beta$-D-methyl glucoside,[5] which exists solely in the $\beta$-anomeric form, registers an increase in intensity relative to D-glucose. If we assume that the rotameric distribution of the exocyclic hydroxymethyl group in the molecules studied here is similar to that of D-glucose (or is not the decisive factor in determining the

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**Table 2.** Δ-Values for the 1260-cm$^{-1}$ ROA Signal and Calculated Anomeric Percentages

| saccharide | Δ-value (×10$^4$) | total % of β-anomer | % of β-anomer in the reducing residue |
|---|---|---|---|
| β-D-methyl glucoside[a] | +4.7 | 100 | |
| D-glucose[b] | +3.0 | 64 | 64 |
| D-glucosamine[b] | +1.4 | 30 | 30 |
| D-maltose[c] | +1.8 | 38 | 76 |
| D-isomaltose[c] | +1.8 | 38 | 76 |
| D-cellobiose[c] | +3.7 | 78 | 56 |
| D-gentiobiose[c] | +3.7 | 78 | 56 |

[a] Data from ref 5. [b] Data from ref 6. [c] This work.

ROA intensity of this band), then it may be used to estimate the anomeric proportions of D-glucose residues.

Table 2 lists the experimental Δ-values for the ~1260 cm$^{-1}$ band for a number of mono- and disaccharides which were calculated by dividing the intensity of the ROA signal by the intensity of the deconvoluted parent Raman band. The Δ-value of $4.7 \times 10^{-4}$ for β-D-methyl glucoside was taken as a standard representing a molecule which has a population of 100% β-anomer, and the populations of the others were calculated relative to this standard. For the disaccharides there are two anomeric centers contributing to the total value; one in the nonreducing residue and one in the reducing residue. For α-linked species the nonreducing residue is trapped in the α-anomeric form by the linkage, so it makes no contribution to the ROA intensity of the 1260-cm$^{-1}$ band. Thus, the total β-anomeric percentage is equal to half the β-anomeric percentage of the reducing residue. However, for β-linked species the anomeric center of the nonreducing residue is trapped by the linkage in the β-anomeric form. Therefore, to find the population of the anomeric center of the reducing residue, it is necessary to remove the contribution from the nonreducing residue. This is achieved by considering the two residues separately. The nonreducing residue is 100% in the β-anomeric form, so it makes a contribution of 50% to the total, as there are two residues contributing. The reducing residue makes up the remainder, so by subtracting 50% from the total percentage and multiplying by 2, we can find the anomeric population of the reducing residue.

The percentage of β-anomer present for the two monosaccharides D-glucose and D-glucosamine[6] listed in Table 2 have been well characterized by NMR spectroscopy.[33,34] For D-glucose the value of 64% obtained is in agreement with the accepted value of 64% obtained from NMR experiments,[33] and our value of 30% for D-glucosamine hydrochloride is not too far removed from the recognized value of 37%.[34] These figures demonstrate that although our technique does not at present offer the precision of other methods (due to the weak intensity of this ROA signal, the uncertainty as to where exactly the base line should lie, and the difficulty of deconvoluting the parent Raman band accurately), it can still provide a reasonable guide to the anomeric populations.

Nonetheless, it is clear from inspection of Table 2 that the two α-linked species, D-maltose and D-isomaltose, have identical Δ-values for the positive ROA signal at 1260 cm$^{-1}$, as have the two β-linked species, D-cellobiose and D-gentiobiose. Furthermore, comparison of the ROA spectra of D-maltose (Figure 2) with D-cellobiose (Figure 4) reveals that the β-linked species exhibits a much stronger ROA signal at ~1260 cm$^{-1}$. This difference is reflected in the Δ-values presented in Table 2 and can be ascribed to the fact that the anomeric center of the nonreducing residue of D-maltose is trapped in the α-anomeric form by the linkage. When the contribution from the nonreducing residue is removed, the α- and β-linked disaccharides are found to have 76% and 56% β-anomer present in the reducing residue, respectively. NMR studies on maltose and cellobiose[35] and gentiobiose[36] indicated that the reducing residue of these molecules had approximately the same proportions as the D-glucose monomer, i.e. 64% β-anomer. Our results seem to indicate that there is a strong correlation between the intensity of the ~1260 cm$^{-1}$ ROA signal and the β-anomeric population; however, due to the problems mentioned above it is difficult at this stage to decide whether the differences found between α- and β-linked species are genuine or simply reflect the inaccuracies currently inherent in our method.

Between ~1300 and ~1400 cm$^{-1}$ the two β-linked disaccharides, D-cellobiose and D-gentiobiose (Figures 4 and 6), have Raman and ROA spectra almost identical to D-glucose, which can be attributed to the preponderance of the β-anomeric form in these molecules. It would appear then that the ROA signals in D-cellobiose and D-gentiobiose are localized in the individual residues and may be assigned to the same normal modes as in D-glucose. The situation is somewhat different for the α-linked species, as the Raman band at ~1332 cm$^{-1}$ increases in intensity in these spectra relative to that in D-glucose with concomitant changes in the ROA spectra. This intensity increase was ascribed to the presence of an additional band in the conventional Raman spectrum of the α-anomeric form of D-glucose, which was assigned by deuteration studies[26] to a normal mode involving C–O–H-bending and $CH_2$-twisting motions. It is clear from inspection of the Raman and ROA spectra of maltose-*O*-$d_8$ (Figure 3) that this band is indeed sensitive to O-deuteration, as there is a drop in intensity in both the Raman and ROA bands.

Further evidence for the ROA being localized in the individual residues in this region comes from the ROA spectra of D-lactose (not shown here), which displays a remarkable similarity to that of the sum of its two constituent monomers, β-D-galactose and D-glucose. The only major difference is the appearance of a weak negative ROA signal at ~1407 cm$^{-1}$ in the spectrum of D-lactose, which is also present in the ROA spectrum of D-cellobiose. Dauchez *et al.* assigned disaccharide Raman bands in the range ~1400–1500 cm$^{-1}$ to angle-bending vibrations around the carbons involved in the glycosidic link.[20,21] Thus, it is possible that this ROA signal is associated with the β(1–4) link found in both D-lactose and D-cellobiose.

## Concluding Remarks

We have demonstrated that the ROA spectra of disaccharides based on D-glucose contain a number of new signals sensitive to the glycosidic link in addition to many signals similar to those found in D-glucose itself. These signals can have quite different intensities, or even opposite signs, for different linkage conformations. This suggests that ROA could be particularly useful for studying oligo- and polysaccharides where the solution conformations can be difficult to assign using existing physical methods on account of the flexibility of the glycosidic links, which results in multiple conformations coexisting in solution.[37]

It is apparent that there are four distinct regions in our disaccharide ROA spectra, each providing complementary information. The anomeric region specifies the nature of the link. The fingerprint region provides a sign pattern characteristic of the entire disaccharide unit, whereas in the $CH_2$ and COH deformations region the ROA signals are localized in the individual residues. These three regions could provide estimates of the residue content and linkage types present in oligosaccharides provided a sufficiently large database of model mono- and disaccharides could be built up. Future development of the assignments in the fourth region at low wavenumber could provide further information of this nature.

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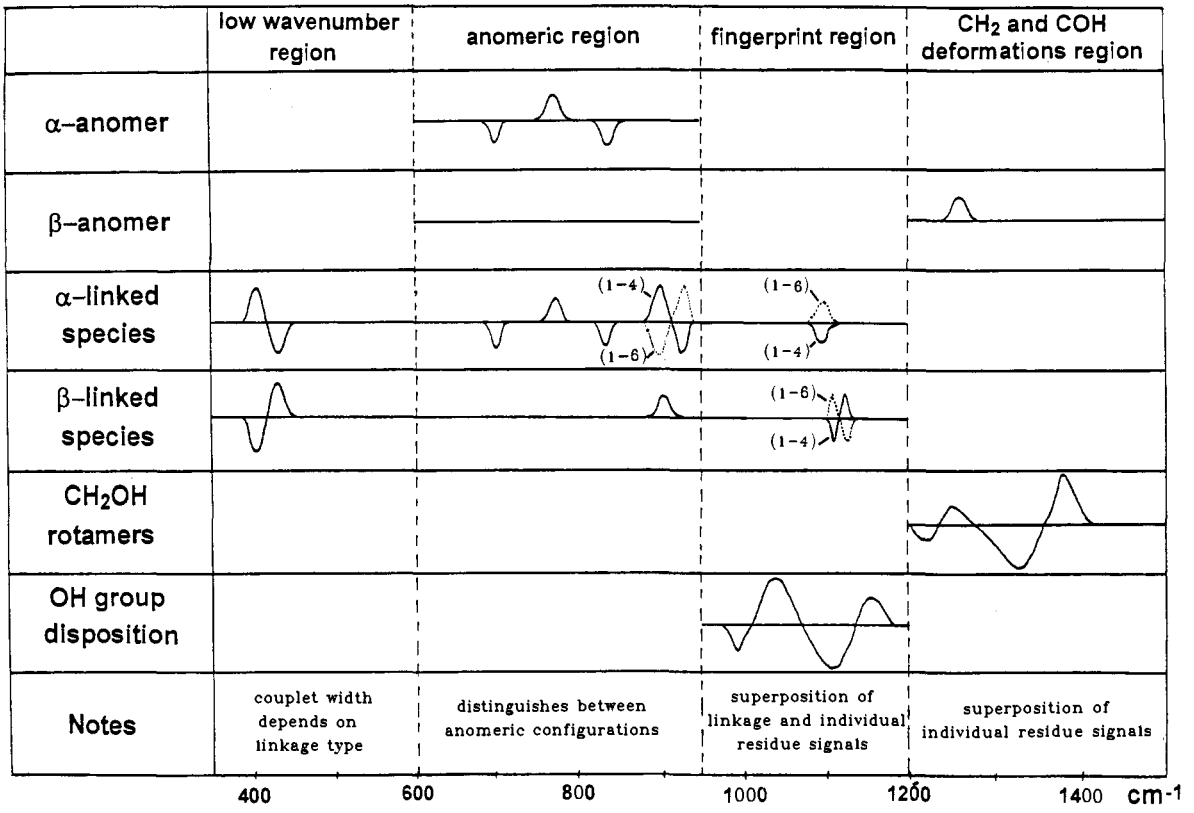


**Figure 9.** Assignment map for ROA signatures of carbohydrates containing D-glucose residues.

We have also tried to extend the number of parameters used to extract information from the ROA spectrum. Until now we have concentrated mainly on the frequency and sign, and in general terms the intensity, of the ROA signals. For the ROA couplet at $\sim$430 $\pm$ 10 cm$^{-1}$ we have used the shape of this signal to extract information on the linkage type, and for the positive ROA band at $\sim$1260 cm$^{-1}$ we have accurately calculated the $\Delta$-value and used it to determine the anomeric population of the reducing residue in disaccharides. This shows that a consideration of shape and intensity of the ROA signals can significantly increase the information extracted from ROA spectra.

Finally, by combining the assignments on D-glucose and its isotopomers[6] with the assignments presented here, we have created an assignment "map" for carbohydrates containing D-glucose residues (Figure 9). These assignments do not necessarily hold for carbohydrates not belonging to the gluco homomorphic series, as changes in the orientation of ring substituents lead to comprehensive changes in the ROA.

**Acknowledgment.** We thank the Science and Engineering Research Council for a research grant for L.D.B. and for a Research Studentship for A.F.B., and the Deutsche Forschungsgemeinschaft for a Research Fellowship for L.H. (Habilitandenstipendium II C1-He 1588/3-1 + 3-2). We also thank Dr. Z. Q. Wen for many useful discussions.