for most of the splitting. Excellent agreement is also observed in the galactose series.

The accuracy of these additive calculations indicates the general validity of the additivity principle and thus justifies the use of a simple retro-additive approach to the interpretation of complex CD spectra. Application of selective bichromophoric derivatization to complex stereochemical problems can provide for interpretable information-rich CD spectra of nanomolar quantities. Such an approach is currently being applied to oligosaccharide microanalysis, wherein the free hydroxyls are derivatized with one type of chromophore and the linkage positions are tagged with a second chromophore. Resulting subunits are then fully characterized by UV (to determine chromophoric ratio) and CD measurements.

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Determination of Dipole–Dipole Couplings in Oriented n-Hexane by Two-Dimensional NMR

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NMR spectra of molecules partially oriented in liquid crystals contain information about the anisotropic dipole–dipole interactions of the spins. It is of interest to recover the intramolecular dipolar coupling constants $D_{ij}$ between each pair of spins since $D_{ij}$ is proportional to $(r_{ij}^{-3})$ and quantitatively describes the molecular structure and the motions affecting the structure. However, in conventional NMR spectroscopy the number of detectable transitions becomes intractably large as the number of distinct transitions, even taking into account the symmetry of the molecule, can be as large as 60,000, concealing the 16 unique dipolar couplings between protons.

Several techniques have been proposed to overcome this problem. One useful approach is selective isotopic substitution, e.g., selective positioning of protons in otherwise deuterated molecules and measurement of the NMR spectrum under conditions of deuterium decoupling. This allows an arbitrary reduction of the number of active spins but requires demanding synthetic effort. Another possible solution is the observation of high-order multiple-quantum spectra in order to reduce the redundant information in single-quantum NMR spectra.

We give here a preliminary report of an alternative method to analyze spectra and determine structures of molecules dissolved in liquid crystals. The basic idea is the use of randomly deuterated samples (a synthetically facile undertaking) which contain a mixture of all possible isotomers of a molecule. This approach has been used before in the analysis of some cyclic compounds in nematic liquid crystals. A highly deuterated sample will contain to complex stereochemical problems can provide for interpretable CD spectra. Application of selective bichromophoric derivatization has been used before in the analysis of some cyclic compounds.

Figure 1. Two-quantum filtered COSY-type spectrum of 81% randomly deuterated n-hexane, 23 mol % in EK 11650, taken with the pulse sequence described in the text. Deuterium-decoupled FIDs, 128 × 1024 points, were collected on a 360-MHz spectrometer (nonquadrature in $\tau_1$) with a spectral width of 16667 Hz in both dimensions and a recycle delay of 5 s. For each $\tau_1$ point, 200 FIDs were accumulated while the double-quantum mixing time $\tau_1$ was incremented from 0 to 9800 µs in 200-µs steps after every fourth FID. For $\tau_2$, 4 ms was used. The data set was zero-filled to 512 × 2048 points prior to Fourier transformation. The squared patterns which give the dipole coupling constants are illustrated for four of the 16 proton pairs.

Table I. List of Dipole Coupling Constants Obtained for the Eight AB and Eight AC Coupling Patterns

<table>
<thead>
<tr>
<th>Sites</th>
<th>$D_{AB}^{xy}$</th>
<th>$D_{AC}^{xy}$</th>
<th>Sites</th>
<th>$D_{AB}^{xy}$</th>
<th>$D_{AC}^{xy}$</th>
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<td>3968</td>
<td>E1E6</td>
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<td>713</td>
<td>706</td>
<td>E1E7</td>
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<td>612</td>
<td>E2E4</td>
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<td>1086</td>
<td>1106</td>
<td>E5E6</td>
<td>598</td>
<td>591</td>
</tr>
</tbody>
</table>

The COSY-type experiment. The INADEQUATE-type experiment.

The dipole coupling constants $D_{ij}$ are defined by the equation $H_2 = \sum_{ij} D_{ij}(3I_i I_j - I_i I_j)/2$, interpreting one complex spectrum is reduced to one of analyzing a large number of simple overlapping spectra. The crucial point is the ability to recognize the individual signals which derive from the same spin system (arise from one isotopomer). This can be achieved by the combined application of two-dimensional NMR and multiple-quantum NMR techniques as is shown below for the case of n-hexane.

An 81% randomly deuterated sample of n-hexane was synthesized by exchange of n-hexane in the gaseous phase with D$_2$ over Pd on charcoal at 190 °C. It was determined by mass spectroscopy to have a statistical distribution of isotomers. Most molecules have between 0 to 5 protons on the chain. Figure 1 shows a two-quantum filtered COSY-type spectrum of the mixture, taken with the pulse sequence $(\pi/2)^{-1} - (\pi/2)^{-1} - \tau_1/2 - \pi - \tau_1/2 - (\pi/2)^{-2} - \pi_2 - \tau_2/2$-sample.

The phase $\phi$ is incremented in 90° steps, alternating the receiver.


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three EIE, (two shown), three E2E2 (one shown), and four EIEz were produced parallel to the one-quantum axis, corresponding to their group type; thus there are two MM, two ME, two ME, protons, E2.

The spectrum thus has 16 subspectra of the type A, or AB. Signals in occurrence and a wide range of isomers. The COSY-type spec-trum records signals from one and three proton isotopomers are eliminated by effectively contains only signals from two proton isotopomers, since any contribution to the signal from the liquid crystal. The doubledipole couplings could be read off this map and from a similar experiment in which a pulse was applied in

Here two- and one-quantum signals were correlated on 0 and 180'. Here two- and one-quantum signals were correlated where $\tau_2$ is the two-quantum preparation time result in different relative intensities to any of the six double-quantum frequencies. Different two-quantum preparation times result in different relative intensities of the subspectra. Table I includes a list of dipole coupling constants obtained with two preparation times, 250 s and 2.5 s. The values of $D_2$ from the COSY-type and INADEQUATE-type experiments agree rather well. It remains to assign the couplings constants to specific pairs of protons on the molecule. These couplings can be used to test various theoretical models of conformational motions for hydrocarbon chains in anisotropic environments. The fact that $D_2$'s for a molecule with 14 protons can be determined bodes well for the application of two-dimensional and multiple-quantum NMR to structure and motions of oriented molecules.

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Assignment of Secondary Amide 15N Resonances of Bleomycin $A_2$ by Two-Dimensional Multiple-Quantum $^1H$-15N Shift-Correlation NMR Spectroscopy

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As part of our program to delineate the solution conformation of metal and nucleic acid complexes of the bleomycins (Bleo),1 Figure 1a, we have assigned the $^{15}$N resonances of Bleo $A_2$, the most abundant congener of these antineoplastic antibi-otics.8 Because isotopic enrichment of this antibiotic cannot readily be achieved, our experiments were performed at natural abundance in aqueous solution by using the recently introduced two-dimensional multiple-quantum $^1H$-$^{15}$N shift-correlation NMR spectroscopic method.4-10 This method is several orders of

\[ D_2 = \frac{1}{2} \operatorname{det}(\mathbf{A}) \]

where $\phi$ is incremented by 90° while the receiver oscillates between 0 and 180°. Here two- and one-quantum signals were correlated in a two-dimensional map as shown in Figure 2. Six vertical lines were produced parallel to the one-quantum axis, corresponding to the six possible double-quantum frequencies

\[ 2\pi \nu_m + 2\pi \nu_e + 2\pi \nu_e \]

These couplings can be used to test various theoretical models of conformational motions for hydrocarbon chains in anisotropic environments. The fact that $D_2$'s for a molecule with 14 protons can be determined bodes well for the application of two-dimensional and multiple-quantum NMR to structure and motions of oriented molecules.

\[ \tau_2/2 \text{-sample} \]


