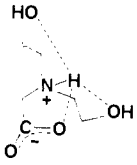
larger than α(N-H···X). The magnitudes of d(N···O) and d(N···X) are usually comparable in three-center bonds, e.g., in our sample, there are 165 bonds in which d(N···X) ≥ d(N···O) and 139 in which d(N···O) ≥ d(N···X). Thus, the major and minor components of a three-center bond cannot be distinguished reliably without a knowledge of the proton position.

Figure 1g shows the distribution of Δ (the deviation of the proton from the N, O, X plane, ignoring sign) for all three-center bonds in our sample.[15] The mean value of Δ is 0.137 (6) Å. The mean Δ value of the 18 three-center bonds determined by neutron diffraction is somewhat smaller [0.104 (19) Å], but the difference is not statistically significant. We conclude that the proton in three-center bonds usually lies within about 0.2 Å of the N, O, X plane. Surprisingly, Δ shows a small tendency to increase as the three-center bond becomes more symmetrical. Thus, the Spearman rank correlation coefficient of Δ and [d(H···O) − d(H···X)] is -0.148, which is significantly different from zero at the 99% level. Essentially the same result is obtained if the analysis is confined to three-center bonds in which both contacts are intermolecular.

Four-Center Bonds

We define a four-center bond as one in which the proton forms three contacts to hydrogen-bond-acceptor atoms. Each contact must be in the "forward" direction [α(N-H···O), α(N-H··X), α(N-H···Y) ≥ 90°] and shorter than the sum of the van der Waals radii of the atoms involved [d(H···O), d(H···X), d(H···Y) > 0]. This arrangement is very uncommon: there are only six four-center bonds in our sample (Table IV). Presumably, this is because of the unfavorable repulsions between N, O, X, and Y in **2**. All of the bonds involve positively charged donor nitrogen atoms. In contrast, only 395 of the 1199 two-center bonds involve N^+-H groups. These proportions are significantly different at the >99.9% level (χ^2 test). We conclude that a positively charged N^+–H group is far more likely to form a four-center bond than an uncharged N–H group.

Bonds 2 and 3 in Table IV are completely intramolecular. The molecules involved (**5** and **6**) are probably ideally suited to



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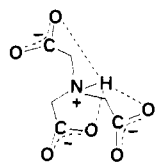

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four-center bonding. Each contains a positively charged N^+–H group, surrounded by three acceptor atoms which "shield" the proton and prevent it from forming a normal, intermolecular two-center bond.

Summary

Three-center hydrogen bonds, as defined in this study, are relatively common in organic crystal structures. However, many of the bonds involve intramolecular N–H···X contacts with α(N–H···X) < 110°. The energy associated with such interactions may well be small, and it is debatable whether they should be described as hydrogen-bonding contacts. We note that the definition of three-center bonding used above was an experimental convenience and is not necessarily recommended as the "best" definition.

Approximately 78% of the three-center bonds in which both contacts are intermolecular involve positively charged N^+–H groups. This suggests that intermolecular three-center bonds are unlikely to be a favorable packing arrangement in organic crystal structures unless the hydrogen atom carries an appreciable net positive charge. A similar result was found for four-center bonds, which are very rare and invariably involve N^+–H groups. Many aspects of the geometry of three-center bonds can be rationalized in terms of the N···O, N···X, and O···X nonbonded repulsions.

(15) For an earlier investigation of the planarity of three-center bonds, see: Parthasarathy, R. *Acta Crystallogr., Sect. B* **1969**, *B25*, 509-518.

Communications to the Editor

Long-Range ^1H-^1H Spin-Spin Couplings through the Interglycosidic Oxygen and the Primary Structure of Oligosaccharides as Studied by 2D-NMR

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The potential of modern high-field two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy in sequencing organic oligomers has been clearly demonstrated in recent 2D NOE studies on small proteins,[1] oligopeptides,[2,3] and oligosaccharides.[4] In these works, sequence information was inferred from spectral correlations established via dipolar interactions (cross relaxations) between protons in contigous oligomeric units. Other, interresidual spin–spin interactions such as indirect homo- and/or heteronuclear long-range couplings may, under favorable conditions, also furnish the requested sequence information.[2] In this communication, we show that 2D correlations established via four-bond interglycosidic $^4J_{HCOCH}$ couplings may represent a convenient, alternative route for the sequencing of small to medium-sized oligosaccharides.

Available literature data show $^4J_{HCOCH}$ couplings to occur across ether linkages in simple organic molecules and assume values in the range between 0 and 1.5 Hz with moderate stereoselectivity.[5,6] The presence of the same type of couplings across glycosidic bonds in oligosaccharides, however, has not been analyzed yet. As shown in the sequel, the actual values of integlycosidic $^4J_{HCOCH}$ couplings in a typical oligosaccharide are estimated to be lower than 0.2 Hz, still they are detectable by suitable experimental techniques. The latter are readily available from the so-called delayed COSY method[7] in which magnetization transfer between resonances

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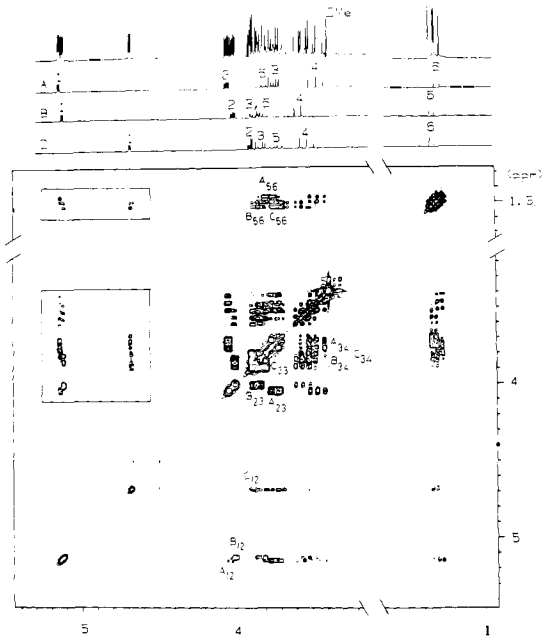
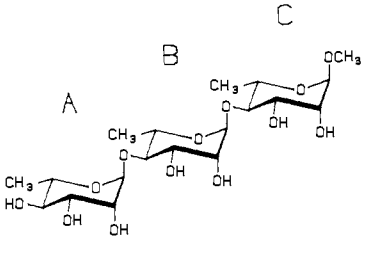


Figure 1. Contour plot of an absolute-value 200-MHz ^1H delayed COSY spectrum (BRUKER WP-200 SY) of 0.3 M **1** in D_2O, T = 323 K. (The cross peaks of the anomeric protons are also shown on an expanded scale in Figures 2 and 3.) The corresponding region of the one-dimensional ^1H NMR spectrum is shown at the top while the assignments of the protons are given at the bottom. The two-dimensional map is composed of 512 × 1K data point spectra, each incremented by 1 ms. A 2-s recycle delay T_w was allowed between each pulse sequence, and an extra delay, equal to 0.4 s (Δ), was inserted before the evolution and the 0.512-s detection period. Quadrature detection was applied in both directions using a 16-step phase cycling for N-type peak selection. Data were multiplied with a sine-bell shaping function, zero filled to 1K × 2K, and then Fourier transformed and symmetrized. (The strong HDO signal was irradiated all the time except the acquisition.)

correlated via long-range couplings becomes enhanced through the introduction of fixed delayes into both evolution (t_1) and detection (t_2) periods. Accordingly, sequencing of oligosaccharides by means of interresidual long-range couplings may, conveniently, be devided into two consecutive steps. First, proton–proton connectivities within the individual sugar residues are established via vicinal interproton couplings by conventional COSY experiment. This is then followed by one (or more) delayed COSY measurement(s) adjusted for optimum visualization of correlations due to small couplings.[7]

The trisaccharide, Me α-L-Rhap-(1–4)-α-L-Rhap-(1–4)-α-L-Rhap (**1**), used in this work consists of three identical, six-mem-



bered, rhamnopyranosyl units arranged in a uniform (1–4) α-glycosidic sequence. Its 200-MHz ^1H NMR spectrum recorded in D_2O is displayed in Figure 1. Proton–proton connectivities within the three individual spin systems (denoted for brevity as $A_1 \cdots A_6$, $B_1 \cdots B_6$, and $C_1 \cdots C_6$) were established as follows. First, a COSY-90 experiment was carried out without fixed delays, using 0.5-s acquisition time. Evaluation of the resulting correlation map (not shown) yielded a partial assignment of the three "independent" spin systems involving protons A_1–A_4, B_1–B_4, and C_1–C_5. Failure to assign all intraunit connectivities via $^3J^1H$–^1H couplings in one single experiment was due to the strong overlap, at 200 MHz, of cross peaks arising from interactions between

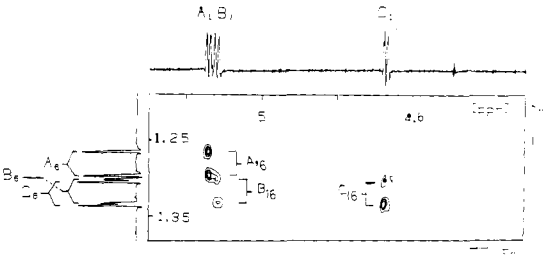


Figure 2. Expanded anomeric region of the COSY spectrum in Figure 1. Only cross peaks with methyl signals are displayed.

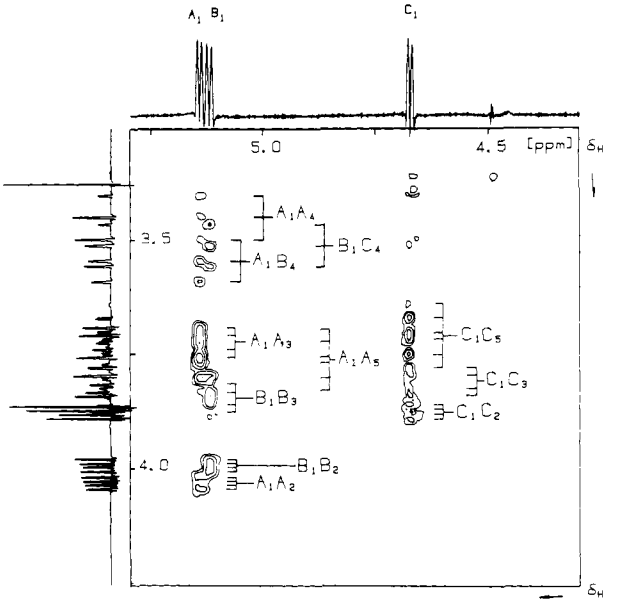


Figure 3. Expanded anomeric region of the COSY spectrum in Figure 1. All cross peaks are assigned except the B_1–B_5 cross peak, which is strongly overlapped with B_1–B_3.

protons in nearly identical chemical environments. To overcome the shortcomings of the limited spectral dispersion, the missing connectivities, in the present study, were inferred from the second, delayed, COSY-90 experiment. Inspection of pertinent 2D correlation maps, shown in Figures 1 and 2., immediately reveals the occurence of cross peaks between anomeric proton resonances and respective ring-methyl signals: A_1–A_6, B_1–B_6, and C_1–C_6. This piece of intraunit long-range coupling information combined with ^3J connectivities for A_5–A_6, B_5–B_6, and C_5–C_6 completed the assignment of the three individual spin systems. Correctness of the assignment was further supported by conventional (1D) ^1H–^1H double-resonance experiments, selective ^1H NOE measurements, proton T_1 data, and 2D cross-correlations between ^1H and ^{13}C chemical shifts.[8,9]

From the enlarged portion of the correlation map obtained in the delayed COSY experiment (Figure 3) it can be seen that the anomeric proton resonances A_1 and B_1 show additional cross peaks with B_4 and C_4, respectively. From the proton network of the molecule **1** it is evident that the latter correlations may only arise through interresidual spin–spin interactions, which immediately settles the sequence of the sugar units in **1** as A–B–C. While for the trisaccharide studied in this work the observed interresidual spin–spin interactions also define the sites of glycosidic linkages, a similar statement may not necessarily be valid for other oligosaccharides. Examination of Figure 3 shows that cross peaks attributable to five-bond interunit spin–spin interactions ($^5J_{HCCOCH}$) A_1–B_3, A_1–B_5 and B_1–C_3, B_1–C_5, if existent, remained below detection limit. In other systems, however, stereospecific

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$^5J_{HCCOCH}$ couplings may assume larger values[6] and, accordingly, may result in the appearance of respective cross peaks rendering thereby the localization of the glycosidic linkages less straightforward. Ambiguities as to the site of glycosylation can presumably be removed either by the determination of the relative signs of interresidual long-range couplings ($^4J_{HOCH}$ and $^5J_{HCOCCH}$ are supposed respectively to be of negative and positive signs[6,10]) or by proton–carbon chemical-shift correlations. It may be noted that in our sequencing experiments[11] with other di- and trisaccharides including Bzl β-D-Xylp-(1→4)-α-L-Rhap, Bzl β-L-Xylp-(1-4)-α-L-Rhap, Me α-L-Rhap-(1→4)-α-L-Rhap-(1→2)-α-L-Rhap, and Me α-L-Rhap-(1→4)-α-L-Rhap-(1→3)-α-L-Rhap we failed to detect cross peaks due to five-bond interresidual interactions whereas the observation of four-bound interunit couplings caused no particular difficulties.

It can be expected that the experimental approach outlined above may be successfully employed for the sequencing of oligosaccharides containing a larger number of sugar units, provided the spectral dispersion is increased accordingly. It should, however, be emphasized that the detectability of magnetization transfer in a COSY experiment is governed primarily by the actual values of pertinent proton T_2 relaxation times.[7] Thus, in low-viscosity solutions, internal flexibility of the oligomer may be necessary to take full advantage of the higher magnetic fields.

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Supplementary Material Available: ^{1}H NMR parameters of **1** and spectrum simulation compared with the experimental spectrum is presented (3 pages). Ordering information is given on any current masthead page.

(10) Relative signs of intraresidual long-range couplings were determined in a separate COSY-45 experiment performed with the methyl glycoside of the repeating unit, Me α-L-Rhap. It was found that $^4J_{HCOCH} < 0$ while $^5J_{HCOCCH} > 0$. (See also supplementary material.)

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pH-Dependent Ion Transport across Polymer Membrane. pH-Induced Reversible Conformational Change of Transmembrane Poly(L-aspartic acid) Domain in Polymer Membrane

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Biological membranes consist of a continuous nonpolar hydrocarbon matrix from the phospholipid bilayer substantially impermeable to most polar substances and protein molecules capable of reversibly binding specific substrates and of transporting them across the membrane even against a gradient of concentration. The protein molecules may undergo reversible conformational changes to create a "hole" or a "channel" in the membrane for the specific substrate transported.[1]

In order to mimic the function of biomembrane, i.e., specific, facilitated, and/or active transports, numerous studies on model systems have been reported, most of which have examined the transport through fluid membranes or lipid-bilayer membranes by the aid of naturally occurring and synthetic ionophores.[2]

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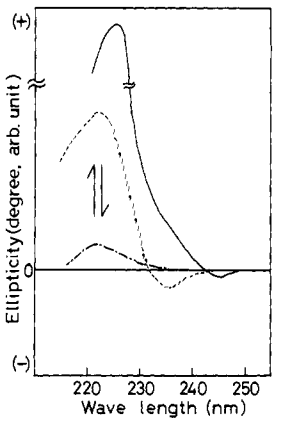

Figure 1. Circular dichroism spectra of the graft copolymer film measured by using a JASCO J-500A spectropolarimeter: (—) before hydrolysis; (---) after hydrolysis, at pH 3; (-·-) after hydrolysis, at pH 9.

However, such mobile carrier-mediated transports appear not to be very common in natural systems, and neither liquid membranes nor lipid-bilayer membranes are satisfactory as model systems and for possible practical applications because of their low stability. As for carrier immobilized on stable polymer membranes, various kinds of polymers have been examined.[3-6] However, they may not be regarded as a biomembrane model since the morphology of these membranes is different from that of biomembrane, where the most important feature is its mosaic structure.[7]

Our primary purpose is to compose the biomembrane model having transmembrane permeating pathway from synthetic macromolecules. In the present paper will be described the first example of the pH-induced reversible conformational change in the transmembrane polypeptide domain of the synthetic membrane prepared from butyl methacrylate (backbone)–L-aspartic acid (branch) graft copolymer.

The design of membrane with microdomains of poly(amino acid) in the matrix of vinyl polymers is based on the synthesis of vinyl polymers with poly(amino acid) branches,[8,9] some of which were found to have microdomains of poly(amino acid) similar to biomembrane.[8] In the present study, butyl methacrylate (backbone)–β-benzyl L-aspartate (branch) comb-type graft copolymer was synthesized by a similar procedure to that reported previously[9] and hydrolyzed in the form of membrane to prepare the membrane of butyl methacrylate–L-aspartic acid graft copolymer.

Figure 1 shows the circular dichroism (CD) spectra of the film cast on a quartz plate (5 mm thick) from the chloroform solution of the graft copolymer[10] before and after hydrolysis. Before

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(10) The graft copolymer used in the CD measurement was that having the degree of branching of 2 mol % and the number-average degree of the polymerization ($\overline{DP_n}$) of the branch of 17. Molecular weight of the trunk was estimated as ca. 10 000 by gel permeation chromatography.

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