The separated analysis of the flip-flop exchange can be monitored by dithionite treatment of vesicles loaded with lipopeptide but without quencher. The addition of sodium dithionite resulted in fast reduction of all accessible NBD fluorophors at the outer face of the vesicles. This first loss of emission signal was followed by a slower decay, indicating the flip-flop of the intravesicular lipopeptides to the outside (Figure 2c).

The apparent rate constant for the intervesicle transfer of tetrapeptide **20** and heptapeptide **23** between POPC vesicles can be compared with data for lipopeptides with a single farnesyl modification or two hydrophobic residues (farnesyl thioether and palmitoyl thioester).[19] Here half-times of 21 s for a peptide with the sequence NBD-GCMGLPC(Far)-OMe and 155 h for the peptide NBD-GC(Pal)MGLPC(Far)-OMe were calculated for experiments at 37 °C, while tetrapeptide **20** has a half-time of about 9 min at 20 °C. Thus, in comparison to a farnesyl thioether, a single palmitoyl group confers a significantly enhanced stability of membrane insertion, however desorption from the model membrane still occurs at a relatively fast rate. Combination of a farnesyl thioether with a palmitic acid thioester results in a very slow but still detectable intervesicle transfer, but in the presence of two palmitoyl groups desorption of membrane-bound lipidated peptides cannot be detected at all. Thus, a bis-palmitoyl membrane anchor will lead to quasi irreversible membrane-anchoring of doubly palmitoylated proteins which can only be reversed by hydrolysis of the palmitic acid thioester bonds.

These values correspond well to data recorded for model peptides, which are derived from other lipidated proteins (such as Rho) and which have been used to predict the membrane binding properties of their parent proteins.[19, 20] Thus, determining a full set of data for several different hemagglutinin-derived lipopeptides in a detailed biophysical analysis should allow for an extrapolation to the membrane binding properties of this lipoglycoprotein as well.

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First Use of a Mineral Liquid Crystal for Measurement of Residual Dipolar Couplings of a Nonlabeled Biomolecule**

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Dedicated to Professor Fred Wudl on the occasion of his 60th birthday

The classic NMR strategy for determining the conformation of a biomolecule[1] involves exploiting the combination of the scalar coupling ^3J, to obtain dihedral angle information, and the ^1H–^1H dipolar cross-relaxation rate, which has a $(1/r^6)$ dependence where r is the internuclear distance. The limitation of this approach is that only short range structural information is obtained (a few bonds for the scalar coupling

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Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author.

and distances of less than 5 Å for the relaxation). The consequence is that only a few constraints per nucleus can be detected so the accumulation of errors (for example, in multidomain proteins, oligosaccharides, or oligonucleotides) can lead to an imprecise overall structure. In order to circumvent this problem, a different approach has recently been proposed in which structural data that are defined relative to an absolute molecular frame are also used.[2, 3] Methods that exploit residual dipolar couplings are particularly interesting.[3, 4] However, as dipolar couplings are almost averaged to zero (values of tenths of Hertz) because of the almost isotropic overall tumbling of the solute molecule, it is necessary to render this tumbling more anisotropic. This can be achieved by replacement of the aqueous phase with an anisotropic medium such as a magnetically oriented mesophase.[4] As a consequence new couplings, *D*, add to the usual scalar couplings, *J*, to split the signals by a few Hertz.[5] By studying pairs of spins separated by well-known distances (any C–H or N–H bond) one can finally extract more precise and longer range structural information.[5]

There are currently four types of organic/biologic liquid crystals (LCs) used successfully for this purpose: surfactant-based bicelles,[4, 6, 7] purple membranes,[8] phages,[9] and cellulose microcrystals.[10] Here, we propose the use of mineral liquid crystals (MLCs)[11] to generate an anisotropic medium. The inherent advantages of this approach are numerous: (1) some MLCs can be well-aligned when a magnetic field is applied,[12] even with only very small amounts of mineral materials (1–3% w/w, to be compared with 5–30% w/w for bicelles, 10% w/w for purple membranes, 5% w/w for phages, and 8% w/w for cellulose); (2) these MLCs, which are sometimes formed using polar solvents other than water, present nematic phases that are stable on a very long timescale (few years) and over a wide temperature range (the liquid-state temperature range of the solvent), in stark contrast with the organic LC systems whose stability is often an experimental problem for this type of NMR spectroscopic method;[15] (3) the dissolved biomolecule can simply be recovered after the experiment by flocculation of the mineral colloid; and finally, (4) whereas, in the case of organic LCs, the NMR signal is strongly dominated by the liquid-crystal resonances due to the high ratio of LC molecules to biomolecules, in MLCs the absence of ^{1}H and ^{13}C nuclei renders isotope labeling of the studied molecule unnecessary. This is obviously a major improvement especially for systems such as oligosaccharides or oligonucleotides for which labeling is chemically challenging and particularly expensive. In this communication, the potential of such MLCs for NMR spectroscopic structural characterization is demonstrated by reporting the residual C–H dipolar couplings observed for a nonlabeled pentasaccharide containing the Lewis^X motif (Figure 1), dissolved in a readily synthesized and handled aqueous suspension of V_2O_5.

Davidson and co-workers, in their study of the behavior of V_2O_5 aqueous suspensions in a magnetic field, have shown that the phase diagram of such suspensions presents a domain of concentration ($0.13 < c < 0.2$ M) in which the nematic phase flows and is easily oriented in a magnetic field.[14, 16] We therefore decided to work at a V_2O_5 concentration just slightly above 0.13 M, and at a pH value of 2.5. Once dissolved in a V_2O_5 aqueous suspension, the ^{1}H and ^{13}C NMR shifts of the pentasaccharide were found to be identical to those in pure D_2O. The dynamic properties of the sugar in the nematic medium are affected in the expected fashion. The increase of the local correlation times, which is deduced from off-resonance proton dipolar cross-relaxation rates,[17] results from the higher viscosity of the solution and this leads to the expected increase of the proton longitudinal and transverse self-relaxation rates; this indicates that the presence of paramagnetic impurities in V_2O_5 (V^{IV}) is of little importance. The differences between the ^{1}H,^{13}C couplings ($J + D$) of the pentasaccharide dissolved in the MLC and the corresponding J values observed in pure D_2O at the same pH value are illustrated in Figure 1; they range between -4.4 Hz and

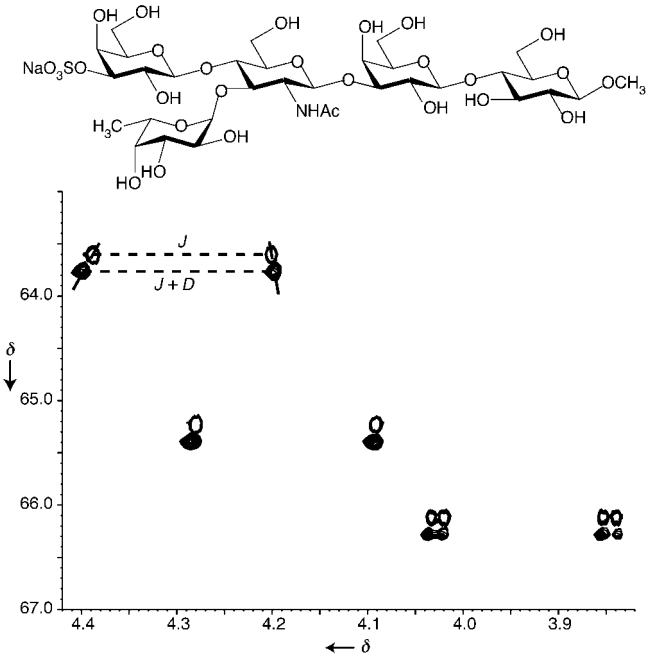

Figure 1. Overlay of a region of the ^{13}C–^{1}H correlation maps of the pentasaccharide acquired in pure D_2O (light cross-peaks; this spectrum is shifted along the ^{13}C dimension by 0.15 ppm for clarity) and in the MLC medium (dark cross-peaks). Since no decoupling is applied during the acquisition time, the D values can be extracted. For the three C–H pairs shown, which correspond to the C4 of the sulfated galactose unit, the C4 of the galactose unit, and the C3 of the fucose unit (from top left to bottom right), the D values are equal to 8.3, 2.0, and -0.4 Hz, respectively.

15.7 Hz.[18] These D values are much larger than the experimental uncertainties in the measurement.[19] They cannot be assigned to a relaxation mechanism, since this would also affect the natural linewidth of the peaks by at least the same order of magnitude.[20, 21] We can therefore safely assign these additional splittings to the presence of residual dipolar couplings. This interpretation is substantiated by the similar D values found for all axial C–H bonds of each pyranoside unit.[18, 22]

It is remarkable that at this low MLC concentration (< 2.4% w/w) a relatively large variation in the peak splittings is observed for such a small molecule in both the ^{1}H and

  1433-7851/01/4002-0374 $ 17.50+.50/0

^{13}C NMR spectra. This can be compared with the systems of Bolon and Prestegard[23a] or Martin-Pastor and Bush,[23b] who used about 20% w/w of DHPC/DMPC in water to detect the orientation of oligosaccharides of similar size (DHPC and DMPC = 1,2-dihexanoyl- and 1,2-ditetradecanoyl-*sn*-glycero-3-phosphacholine, respectively). In these cases, the orientation of the biomolecule is induced by an excluded volume effect,[24] because, since the anisotropy of the biomolecule is usually small, it requires a large volume fraction of bicelles to induce its partial orientation.[24, 25] For V_2O_5, the mechanism responsible for its orientation is expected to be more similar to that of the purple membrane, namely a highly charged surface that orients the biomolecule predominantly by electrostatic interactions, together here with numerous possible hydrogen bonds. The orientation tensors of the biomolecules in these two mediums (V_2O_5 and bicelles) are consequently expected to be different, which represents another reason for the development of new anisotropic media with the aim of ultimately obtaining more accurate structures.[7]

We have shown that the use of MLCs at low concentrations ($\sim$2–3% w/w), which are easy to handle, opens the way to the measurement of residual dipolar couplings of biomolecules without requiring isotopic labeling. This represents, to our knowledge, the first application of the mesogenic properties of an MLC. Finally, since V_2O_5 ribbons are stable in the pH range between 1 and 3, it is important to find other MLCs that could be used at more basic pH values. We are currently developing new MLCs that work at higher pH values as well as investigating known MLCs, such as clays of the montmorillonite (smectite) family,[26] imogolite nanotubes (aluminosilicate),[27] boehmite rods (γ-AlOOH),[28] and gibbsite disks ($Al(OH)_3$),[29] either in their pure forms or doped with traces of paramagnetic metals in order to favor magnetic alignment.

Experimental Section

Synthesis: Various pathways for synthesizing V_2O_5 aqueous suspensions exist and have recently been described.[30] However, for our NMR experiments, where we have studied nonexchangeable protons, it was relevant to use deuterated water ($D_2O > 95\%$). To limit the volume of D_2O needed, we scaled down the ion-exchange synthesis.[30, 31] In a typical synthesis, sodium metavanadate (6.5 mL) dissolved in pure D_2O (99.90% D, Euriso-Top; $[NaVO_3] = 1.0$ M) was passed through a column (inner diameter 1.1 cm, height 19.5 cm) filled with proton-exchanging resin (Dowex 50 W-X2, 50–100 mesh, 6.15 g) and eluted with pure D_2O (ca. 40 mL).

The resin was previously prepared by successively passing the following solutions through the column: 1) an aqueous NaOH solution (1.0 M, 10 mL); 2) pure H_2O (Millipore (18.2 MΩ cm), ca. 100 mL) until pH 7 was achieved; 3) an aqueous HCl solution (1.0 M, 10 mL); and finally, 4) pure H_2O (ca. 100 mL) until the pH value returned to 7. This treatment was repeated three times, with pure D_2O (ca. 100 mL) being used for the final rinse.

Once the eluent became colored, it was collected in fractions of 2.0 mL. After 24 h of aging, fractions 5 and 6 were homogeneous, viscous, birefringent, dark red gels of concentration, $V_2O_5 \cdot 128\,D_2O$ (6.64% w/w, 0.43 M) and $V_2O_5 \cdot 320\,D_2O$ (2.77% w/w, 0.17 M), respectively (measured by thermogravimetric analysis (TGA) up to 180 °C). The NMR sample is obtained by dilution of fractions 5 or 6 with D_2O, until the concentration of $V_2O_5 \cdot 380\,D_2O$ (2.34% w/w, 0.15 M) was reached, which is very close to the boundary of the biphasic phase (2.22% w/w, 0.13 M).[16] From proton NMR spectra, we could estimate the enrichment to be approximately 95–96% D_2O. Since V_2O_5 suspensions have been reported to take about two weeks to reach chemical equilibrium,[30] they were allowed to age for about a month prior to use in the NMR experiments. The ^2H splitting of the bulk water due to the orientation of the MLC was 91 Hz, in agreement with ref. [14a].

NMR experiments were performed at 293 K on a Bruker DRX spectrometer (18.6 T; 800 MHz for ^1H spectra) with an inverse 5 mm triple resonance probehead. Two samples containing 0.31 mg of the pentasaccharide[32] were used, one dissolved in 400 µL D_2O and the other in 400 µL of the MLC medium (0.8 mM). $^1H,^{13}C$ 2D maps were acquired using the HSQC sequence with sensitivity enhancement and gradient selection.[34] No decoupling was applied during the 0.95 s acquisition time (resolution 0.53 Hz). The precision on the $^1H,^{13}C$ dipolar splitting, D, was estimated to be in the order of 1 Hz.

The recovery of the biomolecule from the MLC can be readily achieved since most MLCs are based upon suspensions of charged colloids. Their flocculation can be induced, as predicted by the DLVO theory,[35] by increasing the ionic force of the medium.[36] The flocculus can be removed by centrifugation or filtration, and the biomolecule can then be extracted from the supernatant by methods such as chromatography, dialysis, or by extraction with organic solvent.

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$$D_{ij} = S\frac{\gamma_i\gamma_j\hbar}{r_{ij}^3}[A_a(\tfrac{3}{2}\cos^2\theta - \tfrac{1}{2}) + A_r\tfrac{3}{4}\sin^2\theta\cos^2\phi] \quad (1)$$

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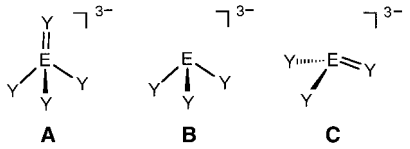
Novel Approach to Mixed Group 15/16 Element Ligands—Formation of Unusual Trichalcogenophosphonato Ligands in Mixed Fe/Cr Clusters**

Manfred Scheer,* Shubhangi B. Umbarkar, Saurav Chatterjee, Rajiv Trivedi, and Pradeep Mathur*

Dedicated to Professor Henri Brunner on the occasion of his 65th birthday

Complex chemistry of "naked" main group element ligands has been a fascinating area of research over the last few decades. Within this field, ligands consisting of different main group elements are scarce and are only well established between Group 15 and Group 16 elements.[1] Known synthetic approaches for obtaining such complexes make use of E_4Y_n cages (E = element from Group 15, Y = element from Group 16, $n = 3$, 4) or their metal salts, such as $M^I_3AsS_3$; another method is the chalcogenation of pnicogenido complexes. Recently Fenske et al. have used $SP(SSiMe_3)_3$ as a precursor to $(PS_4)^{3-}$ and $(P_2S_6)^{4-}$ ligands.[2]

While EY_4^{3-} (type **A**; E = P, As; Y = S, Se) is established as a ligand in some complexes, the molecular structure of the complexed EY_3^{3-} ligand (type **B**) has only been observed for E = As in $[Cp'_3Ti_2(\mu\text{-}O)(\mu\text{-}AsS_3)]$ ($Cp' = \eta^5\text{-}C_5H_4Me$)[3] and for E = P in $[Pd_3(PS_4)(PS_3)(PEt_3)_4]$.[2b] So far complexes of type **C** ligands are unknown.



We report here a novel synthetic approach to mixed Group 15/16 element ligands by using the reaction of Group 15 element complexes with those of Group 16. This method leads to novel trichalcogenophosphonato ligands of type **C** (Y = S, Se). Our initial attempts to prepare mixed-metal clusters containing mixed chalcogenide/phosphorus bridging ligands centered on thermolysis and photolysis reactions between $[Fe_3(CO)_9(\mu_3\text{-}Y)_2]$ (Y = S (**1a**) or Se (**1b**)) and $[CpCr(CO)_2(\eta^3\text{-}P_3)]$ (**2**). Under such conditions, no new product was obtained; either the starting materials were

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