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# Solid-state NMR and FTIR studies on bilayer membranes from 1,2-dioctadec-(14-ynoyl)-*sn*-glycero-3-phosphatidylcholine

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**Abstract**

Multilamellar dispersions from a new model phospholipid, 1,2-dioctadec-(14-ynoyl)-*sn*-glycero-3-phosphatidylcholine (DO(14-yne)PC), bearing a triple bond in the fatty acid chains are studied by $^{2}H$, $^{31}P$ NMR and Fourier transform infrared (FTIR) spectroscopy. The investigations are focused on the evaluation of the molecular properties of the lipid molecules as function of temperature and sample composition. Information about the fatty acid chain conformations are obtained from FTIR measurements by analysing the $CH_2$ wagging and stretching modes. $^{2}H$ NMR studies are performed on two selectively deuterated compounds that provide further insights into the molecular characteristics at two specific positions along the fatty acid chains. These studies demonstrate that the introduction of the triple bond is accompanied by a reduction of fatty acid chain order which holds for both the conformational and the orientational order. Likewise, $^{31}P$ NMR spectroscopy is used for the determination of the dynamics and ordering in the head group region. Here, particular emphasis is given to the evaluation of the lipid lateral motions that are quantified over a large temperature range within the liquid crystalline phase. It is found that the lateral mobility of the lipid molecules is almost unaffected by the triple bond in the fatty acid chains. The addition of cholesterol gives rise to a reduction in lateral mobility for DO(14-yne)PC, as can be followed by spin echo, 2D-exchange NMR and stimulated echo experiments. 

*Keywords:* Phospholipids; $^{2}H$ NMR; $^{31}P$ NMR; FTIR; Membrane conformation; Membrane dynamics

## 1. Introduction

During recent years solid-state nuclear magnetic resonance (NMR) spectroscopy has provided detailed information about the molecular behaviour of lipid molecules in model and biological membranes [1–5]. In this connection, dynamic NMR techniques have demonstrated their particular potential for the evaluation of both the specific molecular organization and the types and time scales of the molecular motions occurring in such membrane components. $^{2}H$ NMR spectroscopy [1–3] of selectively deuterated compounds is able to monitor the molecular features of the hydrophobic fatty acid chains, whereas $^{31}P$ NMR spectroscopy can be used to study the behaviour of the polar phospholipid headgroup [6–8]. By performing lineshape, relaxation and 2D-

Abbreviations: DO(14-yne)PC, 1,2-dioctadec-(14-ynoyl)-*sn*-glycero-3-phosphatidylcholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine

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exchange NMR experiments [9–12], molecular motions can be probed with correlation times between about $10^0$ and $10^{-11}$ s. On this basis, it could be shown that membranes possess a high degree of flexibility which is expressed by the presence of various internal (conformational) and overall reorientational motions. At the same time, it has been demonstrated that external parameters like temperature, pressure or sample composition might have a strong impact on the structural as well as on the dynamical features in such membrane systems.

In this contribution we present $^2$H and $^{31}$P NMR studies on a new model membrane that is based on 1,2-dioctadec-(14-ynoyl)-*sn*-glycero-3-phosphatidylcholine (DO(14-yne)PC, see Fig. 1), a phospholipid bearing an acetylenic group at carbon 14 of the fatty acid chains [13,14]. This phospholipid should be distinguished from the well-known lipids with diacetylenic groups that are of potential interest in the field of drug delivery [15]. In fact, the present synthetic phospholipid with a single acetylenic group has gained considerable interest due to its potential use for the stabilization and reconstitution of membrane proteins [13,14]. It should be mentioned that so far no NMR study has been reported on phospholipids containing a triple bond in the hydrophobic chain region.

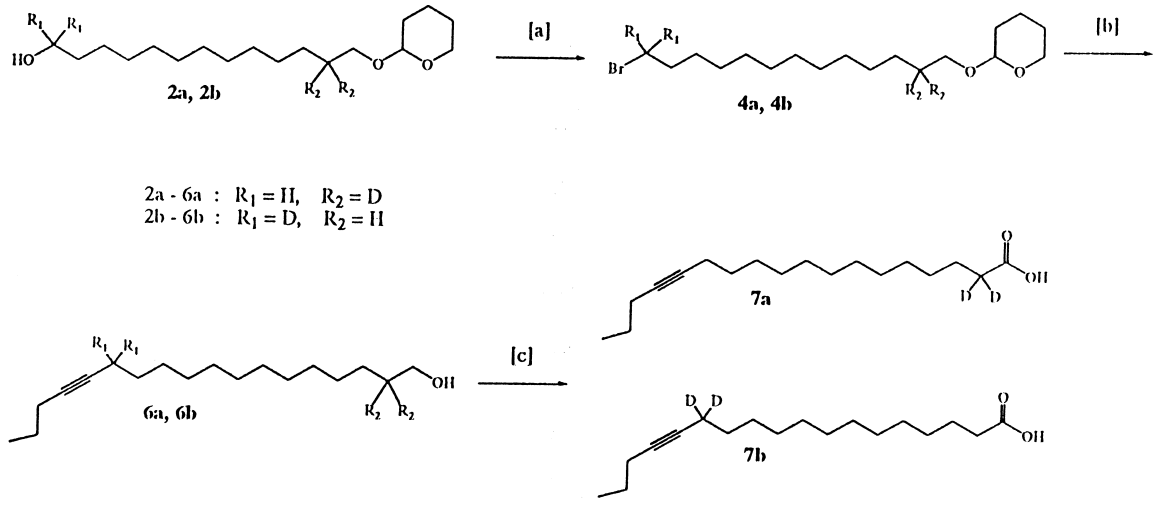
For the present $^2$H NMR studies on fully hydrated bilayers from DO(14-yne)PC selectively deuterated compounds have been synthesized that should monitor the behaviour of the fatty acid chains in the vicinity of the glycerol backbone as well as of the C–C triple bond. Two samples have been studied – pure hydrated phospholipids and a membrane containing 40 mol% cholesterol – that could provide a first insight into the molecular behaviour of this new class of unsaturated phospholipids. Fourier transform infrared (FTIR) spectroscopy measurements, performed on the same non-oriented samples, address to the specific conformational properties in the fatty acid chain regions [16–18]. In this connection $CH_2$ stretching and wagging bands have been analysed to evaluate the conformational disorder as function of temperature and sample composition [19,20]. To our knowledge this is the first report about FTIR measurements on phospholipids bearing an isolated acetylenic unit in the aliphatic chains. Moreover, we will compare the data derived for DO(14-yne)PC primarily with those reported from earlier studies on DMPC. Such a comparison appears to be reasonable because both compounds only differ at C-13 in the fatty acid chains. That is, DMPC is terminated at this position by a methyl group, whereas DO(14-yne)PC bears at C-13 an additional *n*-pentyne fragment, $-C\equiv C-(CH_2)_2-CH_3$. The following investigation should demonstrate whether this structural difference in the fatty acid chains has an impact on the molecular behaviour, i.e., the molecular order and dynamics.

## 2. Materials and methods

### 2.1. Materials

The starting materials for the synthesis were purchased either from Aldrich Chemicals (Steinheim, Germany) or from Fluka Chemie (Buchs, Switzerland). Cholesterol and DMPC were obtained from Sigma Chemicals (Deisenhofen, Germany), and GPC and *sn*-glycero-3-phosphatidylcholine from Lukas Meyer (Hamburg, Germany). Specifically deuterated samples of DO(14-yne)PC (**1a** and **1b**, see Fig. 1) were obtained, as described elsewhere [21]. To do so, the deuterated 14-octadecynoic acids **7a** and **7b** have been prepared via two multiple step synthetic routes which required a variation of the earlier published procedures [13,14]. In particular, it was necessary to avoid strong acidic or basic reaction conditions after the introduction of deuterium. Therefore, the specifically deuterated 13-tetrahydropyrane-2-yloxytridecanoles **2a** and **2b** (see Scheme 1) have been used during the synthesis, that were obtained by reduction of suitable carboxylic acid methyl esters with $LiAlD_4$ and successive chain extensions [21] From the coupling of the bromides **4a** and **4b** with 1-pentyne, the $C_{18}$ chains could be obtained. Removing of the protecting group and oxidation with chromium reagents led to the desired deuterated acids. Acid **7a** lost less than 5% of its deuterium content in the final oxidation step while acid **7b** is formed with 100% deuterium at position C-13.

For the preparation of the phospholipid, 1,2-dioctadec-(14-ynoyl)-*sn*-glycero-3-phosphatidylcholine, DO(14-yne)PC, a previously published preparation method was used [13]. 14-Octadecynoic acid (1 g,




[a] i) TosCl, pyridine, DCM, 3a: 96%, 3b: 93%; ii) LiBr, abs. DMF, 4a: 94%, 4b: 97%;
[b] i) $LiNH_2$, $NH_3$ liq., DMSO, THF, 1-pentyne, 5a: 83%, 5b: 93%; ii) MeOH, TosOH, 6a: 99%, 6b: 99%;
[c] $CrO_3$, acetone, $H_2SO_4$, 7a: 84%; PDC, abs. DMF, 7b: 72%.

TosCl : p-toluenesulfonyl chloride; TosOH : p-toluenesulfonic acid; PDC : pyridinium dichromate

Scheme 1.

3.57 mmol), dissolved in 40 ml of dry chloroform, was first transformed into its imidazolide by addition of *N,N*-carbonyldiimidazole (630 mg, 3.89 mmol) and stirring at room temperature for about 45 min. Afterwards, carefully dried *sn*-glycero-3-phosphatidylcholine (335 mg, 1.3 mmol) and 1,8-diaza-bicyclo-[5.4.0]undec-7-ene (543 mg, 3.57 mmol) were added. After further stirring at room temperature for at least 24 h, the solvent was removed. DO(14-yne)PC of high purity was obtained from the residue by recrystallization in acetone.

## 2.2. NMR spectroscopy

### 2.2.1. Sample preparation

Multilamellar dispersions (steroid-free sample) were prepared by hydrating 100 mg of dry DO(14-yne)PC in the same amount of deuterium-depleted water. A homogeneous multilamellar dispersion was obtained by repeated freeze–thawing, centrifuging and vortexing of the sample. The homogenized material was transferred into a 5-mm NMR sample tube and sealed under vacuum. DO(14-yne)PC/cholesterol samples were prepared by dissolving DO(14-yne)PC and cholesterol (3:2 molar ratio) in a small amount of freshly distilled chloroform. The solvent was partially removed by a stream of nitrogen gas, and the residual solvent was evaporated under vacuum for at least 5 h. After this procedure the dry mixture was dispersed in deuterium-depleted water in the same way, as described above.

### 2.2.2. NMR measurements

The $^2H$ and $^{31}P$ NMR experiments were performed on a Bruker CXP 300 NMR spectrometer (Rheinstetten, Germany) at 46.07 MHz ($^2H$) and 121.5 MHz ($^{31}P$), interfaced to a MacSpect station (Tecmag, Houston, USA), with a broadline 5-mm double tuned probe. $^2H$ NMR spectra and spin–spin relaxation times ($T_2$) were obtained employing the quadrupole echo sequence, $(\pi/2)_x-\tau_e-(\pi/2)_y-\tau_e-$acq. The $\pi/2$ pulse width typically was 2.0 μs. For the acquisition of the spectra the delay between the $\pi/2$ pulses was fixed to 20 μs. The recycle delay varied between 0.3 s (liquid crystalline phase) and 6 s (gel phase). Spin–lattice relaxation times ($T_1$) were obtained by a modified inversion–recovery sequence, $(\pi)-\tau_r-(\pi/2)_x-\Delta-(\pi/2)_y-\Delta-$acq. Here, the initial $\pi$ pulse was replaced by a composite pulse, as described elsewhere [22].

All $^{31}P$ NMR experiments were performed in the presence of broadband proton decoupling (decoupling power: 20 W in the liquid crystalline phase and 55 W in the gel phase). $^{31}P$ NMR spectra were obtained employing a single pulse sequence ($\pi/2$ pulse width: 3 μs). Spin–spin relaxation times were measured using the spin-echo sequence (echo delay: $\tau_e$) while for the determination of the spin–lattice relaxation times a saturation–recovery sequence has been employed. The recycle delays were between 6 s (liquid crystalline phase) and 100 s (gel phase). Depending on the particular experiment the number of scans varied between 256 and 1024. For the 2D-exchange NMR experiments two sets of experiments – referring to the cos- and sin-component – have been performed employing a basic three-pulse sequence, $(\pi)$-$\tau_r$–$(\pi/2)$-$t_1$–$(\pi/2)$-$\tau_m$–$(\pi/2)$-$t_2$ with appropriate phase cycling schemes. The two data sets were combined as described elsewhere in order to obtain a 2D-exchange spectrum in pure absorptive mode [23,24]. For each experiment 64 $t_1$ values have been used ($t_1$-increment: 50 μs). The number of scans was 64. The above mentioned three-pulse sequence was also used for the stimulated echo experiments [25]. Here, signals were recorded at fixed $t_1$ and $t_2$ intervals of 200 μs and variable mixing times $\tau_m$. The sample temperature during the variable temperature experiments was controlled with a Bruker BVT 1000 temperature control unit. Generally, the temperature stability was found to be ± 1 K.

#### 2.2.3. Data processing and simulations

FORTRAN programs have been developed which describe the behaviour of an $I = 1/2$ spin system during the corresponding lineshape and 2D-exchange experiments. The theoretical lineshapes and relaxation times are obtained by a numerical diagonalization of the corresponding relaxation matrices using standard software packages [26]. Further details about the isotropic rotational diffusion model – used for the description of the $^{31}P$ NMR spectra in the liquid crystalline phase – can be found in [27,28]. The fitting of the experimental spectra has been done by superimposing the experimental and theoretical spectra. The final (best fit) spectra were obtained by taking into account the overall lineshape as well as the relative amplitudes in case of partially relaxed spectra. Both the simulations and data processing of the experiments were performed on SUN workstations using the NMR1, NMR2 and Sybyl/Triad software packages (Tripos, St. Louis, USA).

### 2.3. FTIR measurements

#### 2.3.1. Sample preparation

Multilamellar lipid dispersions were prepared as previously described for the NMR measurements but the amount of water was increased to about 70–80 wt.%. The construction of the IR cell excluded a loss of water and thus incomplete hydration. Liquid alkynes were taken without further treatment and 14-octadecynoic acid was dissolved in a small amount of tetrachloromethane.

#### 2.3.2. IR measurements

Lipid dispersions or reference alkynes were placed in 50-μm thick infrared cells with $CaF_2$ windows. The IR cell was thermostated with a variable temperature unit from LOT/Oriel (Langenberg, Germany). IR spectra were recorded with Perkin Elmer Paragon 1000 (Überlingen, Germany) and Bruker IFS 66 FTIR (Karlsruhe, Germany) spectrometers. Typically 128 to 512 interferograms were collected, apodized with a triangular function and Fourier transformed with one level of zero-filling. The spectral resolution was 2 $cm^{-1}$. For each membrane system three samples of the same composition have been prepared. The whole series of variable temperature spectra was measured twice for each sample. The data analysis thus was based on an average over six measurements.

#### 2.3.3. Data analysis

For the analysis of the $CH_2$ wagging band region the Bruker IFS 66 spectrometer software (Opus 3.0) was used. After a linear baseline correction (1330–1390 $cm^{-1}$ for DMPC and 1315–1400 $cm^{-1}$ for DO(14-yne)PC) the spectra were iterated with four (DMPC) or five (DO(14-yne)PC) vibration bands with Gaussian and Lorentzian contributions of variable amount. Their initial positions were 1378 $cm^{-1}$ (symmetric methyl deformation mode), 1368 $cm^{-1}$ (kink and gtg sequences), 1353 $cm^{-1}$ (double *gauche* sequences), 1342 $cm^{-1}$ (end *gauche* sequences) and 1330 $cm^{-1}$ (special band in DO(14-yne)PC). During the least square fit analysis the band intensities, po-

sitions and widths were varied. The integral intensities of the $CH_2$ wagging bands were then normalized with respect to the methyl deformation band. The amount of specific *gauche* sequences were calculated according to the procedure given by Senak et al. [20]. The estimated uncertainty for the various conformers is 10% to 15%. For the analysis of the $CH_2$ wagging progressions [29,30], subtraction of the underlying $PO_2^-$ antisymmetric stretching band near 1230 $cm^{-1}$ was required. For this purpose an IR spectrum, recorded at the highest available temperature in the liquid crystalline phase (typically at 353 K), was subtracted from the spectrum acquired at the other temperatures. Afterwards, a flattened baseline was generated in the spectral region of interest by selecting appropriate spectral minima. All progression intensities – which are a sum of the $k=1$ to $k=4$ components – were further normalized with respect to the progression intensity obtained for pure DO(14-yne)PC at 278 K. Further details about the actual procedures can be found in [29,30]. The wagging progression intensities are only given for temperatures above 273 K. At lower temperatures the above procedure failed due to a change of the $PO_2^-$ antisymmetric stretching bandshape. The frequencies of the $CH_2$ symmetric stretching vibrations were determined from the interpolated zero crossing in the first derivative spectra.

### 2.4. Differential scanning calorimetry (DSC)

The phase behaviour of the various samples was established by differential scanning calorimetry. Typically a temperature range of 200 K to 350 K at heating rates of 2 K/min or 5 K/min was examined using a Netzsch DSC 200 calorimeter (Selb, Germany).

## 3. Results

In the present work multilamellar (non-oriented) dispersions of a new synthetic phospholipid, [13,14] DO(14-yne)PC (see Fig. 1), were investigated by means of dynamic $^2H$, $^{31}P$ NMR and FTIR spectroscopy between 200 K to 350 K. $^2H$ NMR experiments were performed on two phospholipids which were deuterated either at C-2 or at C-13 in the *sn*-1 and

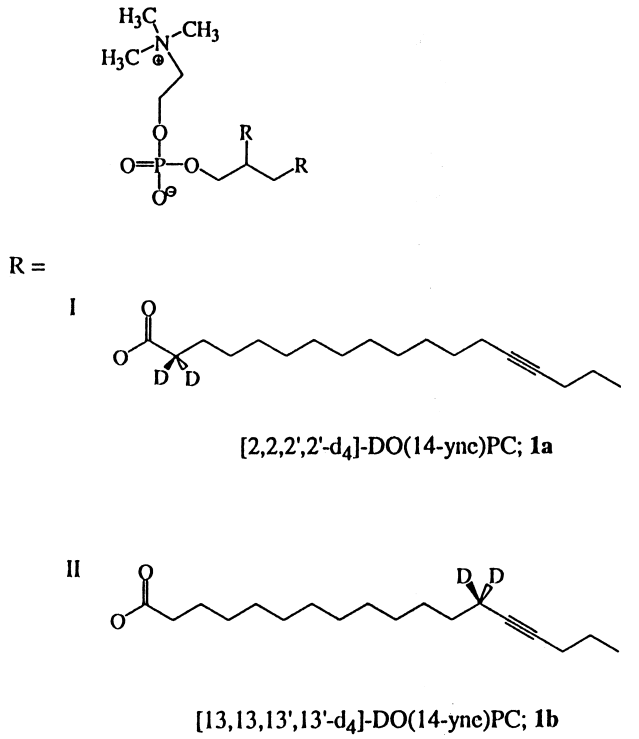



Fig. 1. Chemical structure of the phospholipid DO(14-yne)PC used in the present study.

*sn*-2 chains. Two different membrane systems have been examined during the present spectroscopic studies: (i) pure hydrated lipids with a water content of 50 wt.% and (ii) a hydrated lipid/cholesterol mixture with a steroid content of 40 mol%. Both systems exhibit a liquid crystalline and a gel phase, as established by differential scanning calorimetry. The transition temperature of pure, hydrated DO(14-yne)PC was found to 296 K [13]. Cholesterol lowers this transition. For the DO(14-yne)PC/cholesterol sample, thus a transition at about 273 K is observed that also is visible during the $^2H$ and $^{31}P$ NMR studies presented below. However, during the calorimetric studies this transition is obscured by an additional peak due to the freezing of excess water.

### 3.1. FTIR studies

In Fig. 2 (top) representative FTIR spectra are given for DO(14-yne)PC and DO(14-yne)PC/cholesterol in the liquid crystalline phase. The spectral range covers the conformation sensitive wagging band region of the $CH_2$ groups, for which five ab-

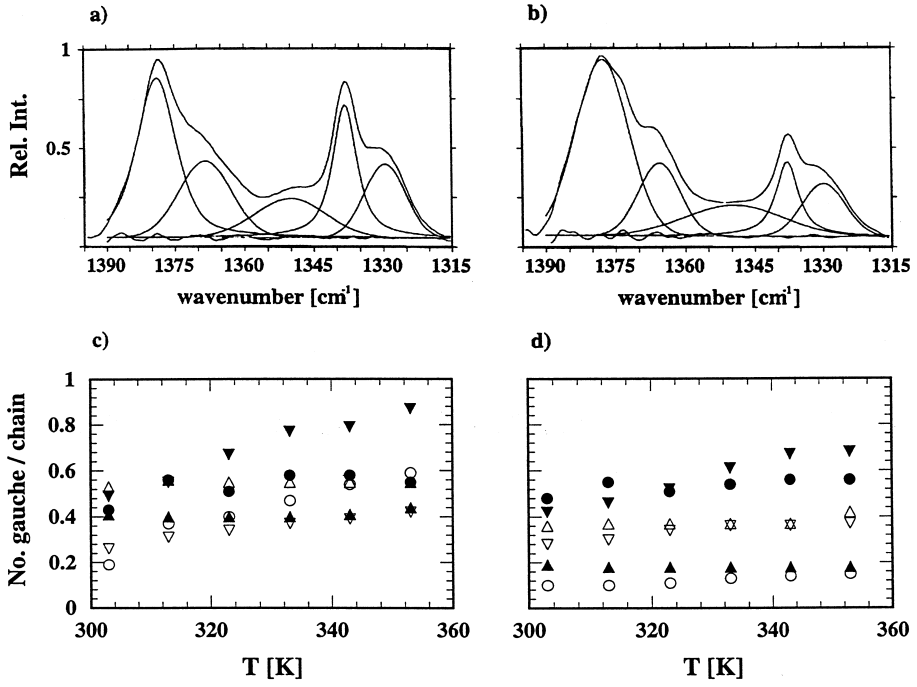



Fig. 2. FTIR spectra (wagging band region) for pure DO(14-yne)PC (a) and DO(14-yne)PC/cholesterol (3:2 molar ratio) (b). The spectra also contain the theoretical wagging bands due to the various conformers (see text) and the difference spectra between experiment and simulation. Number of kink/gtg, gg and eg conformers per chain for bilayers from pure DO(14-yne)PC, pure DMPC (c), and DO(14-yne)PC/cholesterol (3:2 molar ratio), DMPC/cholesterol (3:2 molar ratio) (d). Full and open symbols refer to the data for DO(14-yne)PC and DMPC, respectively. ▽,▼: kink/gtg conformers; ○,●: gg conformers; △,▲: eg conformers.

sorption bands are found. The intense band at 1378 $cm^{-1}$ refers to the methyl group 'umbrella' deformation state while the absorption bands at 1368, 1356 and 1342 $cm^{-1}$ reflect kink(gtg′)/gtg, double *gauche* (gg), and end *gauche* (eg) sequences in the fatty acid chains, as known from FTIR studies on lipids with saturated acyl chains [20]. A comparison with the FTIR spectra for DMPC (not shown) reveals two special features in the DO(14-yne)PC spectra, namely the additional band at 1328 $cm^{-1}$ and the unusual intense eg band at 1341 $cm^{-1}$.

Measurements [21] on other alkynes and on DO(14-yne)PC deuterated at C-13 have demonstrated that both effects are directly connected with the incorporation of a C–C triple bond. Thus, the band at 1328 $cm^{-1}$ is caused by the two $CH_2$ groups (C-13 and C-16) next to the C≡C unit. Due to their rigidity they are not able to form gg, kink/gtg or eg sequences. Therefore, during the curve fitting procedure, [20,31] that yields the amount of the various *gauche* sequences (see Section 2), they had to be treated separately. Complementary measurements on 14-octadecynoic acid and other alkynes, dissolved in tetrachloromethane, revealed the need for a new scaling factor for the intense eg band [21]. At the same time, the band intensities for gg and kink/gtg sequences were not altered, when compared with those for the corresponding n-alkane (octadecane). With the assumption that the RIS model [20,32] is still valid for the conformational behaviour of 14-octadecynoic acid, a new scaling factor of 1.11 (instead of 0.69 for DMPC) has been derived for the eg band at 313 K. This assumption appears to be justified since the amount of eg conformers is independent from the chain length within the framework of the RIS model. Obviously, the signal increase stems from the influence of the triple bond on the molecular dipole moment, although at present no further information about this topic is available. The final results for gg, kink/gtg and eg sequences for DO(14-yne)PC with and without cholesterol are summarized in Fig. 2 along with the corresponding data for DMPC.

The conformational behaviour at the transition to the gel state again can be followed by the wagging modes in the IR spectra, as demonstrated for other

bilayer systems. It has been shown previously that a high conformational order in the gel phase (all-*trans* chains) causes a coupling of the methylene wagging motions to form a progression of bands [28,29] between 1180 $cm^{-1}$ and 1380 $cm^{-1}$. In Fig. 3 we show the IR difference spectra (see experimental section) for DO(14-yne)PC and DO(14-yne)PC/cholesterol samples at 278 K. The temperature variation of the amount of all-*trans* conformation can be followed by plotting the intensity of the progression bands as function of temperature, as given in Fig. 4 for the $k=1$ to $k=4$ progression bands. For pure DO(14-yne)PC the progression band intensity drops down to zero at the calorimetric main transition. For DO(14-yne)PC/cholesterol the progression band intensity is reduced by 50% at 273 K, i.e., at the corresponding main transition. The remaining progression band intensity is reduced gradually until it vanishes at about the main transition of the pure lipid. This is also confirmed by the temperature dependent frequency shifts of the symmetric $CH_2$ stretching band for DO(14-yne)PC and DO(14-yne)PC/cholesterol, which are given in Fig. 4. The graph for pure DO(14-yne)PC demonstrates a pronounced shift of this vibration mode to higher frequencies in the vicinity of the main transition. The centre frequency thus changes from about 2849 $cm^{-1}$ in the gel phase – being typical for an all-*trans* chain – to 2854 $cm^{-1}$ in the conformationally disordered liquid crystalline phase. At the main transition of the DO(14-yne)PC/cholesterol mixture this frequency shift is much less pronounced. Interestingly enough, for this sample a second (greater) shift is obtained at the main transition of the pure model system which is in line with the above-mentioned behaviour of the wagging band progressions.

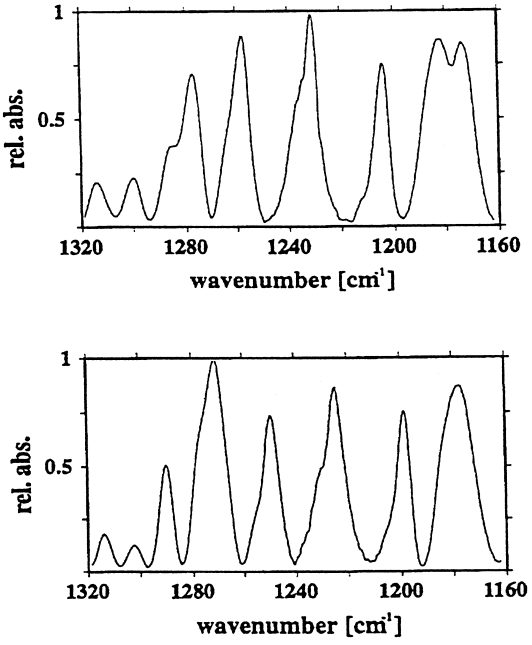



Fig. 3. Wagging band progression for pure DO(14-yne)PC (top) and DO(14-yne)PC/cholesterol (3:2 molar ratio) (bottom), recorded at 278 K.

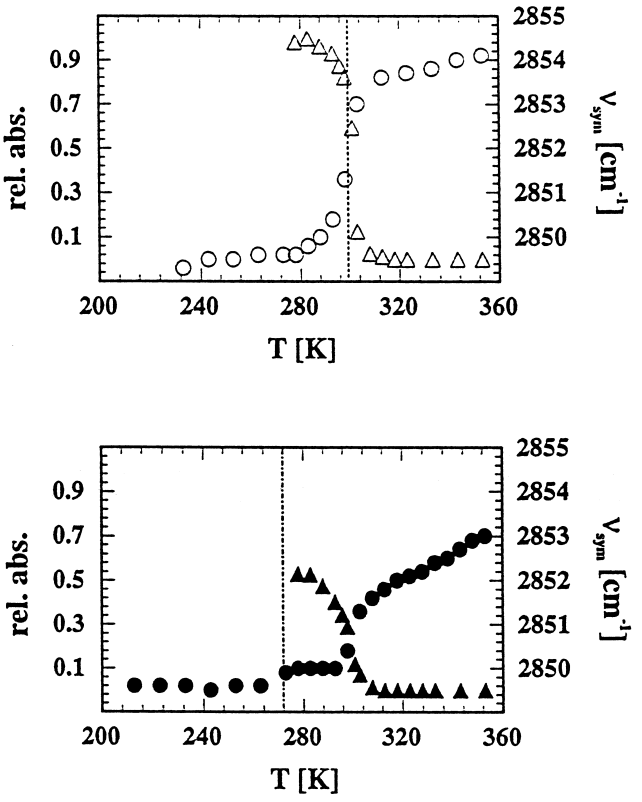



Fig. 4. Wagging band progression intensities (△,▲, left scale) and $CH_2$ stretching frequency (○,●, right scale) for pure DO(14-yne)PC (top) and DO(14-yne)PC/cholesterol (3:2 molar ratio) (bottom). Dashed lines indicate the calorimetric phase transitions.

### 3.2. $^{2}H$ and $^{31}P$ NMR studies

In Fig. 5 representative $^{2}H$ NMR spectra are given for multilamellar dispersions of DO(14-yne)PC (left column) and DO(14-yne)PC/cholesterol (right column), deuterated in the fatty acid chains either at

C-2 or at C-13. The liquid crystalline spectra generally are characterized by rather narrow, axially symmetric powder lineshapes which reflect highly mobile phospholipid molecules. In the case of [2,2,2′,2′-$d_4$]-DO(14-yne)PC three quadrupolar splittings can be distinguished. They stem from two signals for the *sn*-2-chain deuterons (11 kHz and 14 kHz at 350 K for DO(14-yne)PC) and from a further signal for the *sn*-1-chain deuterons (20 kHz). This inequivalence of the deuterons in the *sn*-2 chain has been reported earlier for other phospholipids [33] and can be related to the specific orientation of the *sn*-2 chain that starts out perpendicular to the bilayer normal and turns down at C-2 by a *gauche* conformation. As a result, the deuterons at C-2 in the *sn*-2 chain become inequivalent. At the same time, the *sn*-1 chain is extended directly down into the bilayer which gives rise to a further splitting [34]. The addition of cholesterol to the DO(14-yne)PC membrane is manifested by a distinct increase of the experimental quadrupolar splittings (about 30%) in the corresponding $^2$H NMR spectra of [2,2,2′,2′-$d_4$]-DO(14-yne)PC/cholesterol.

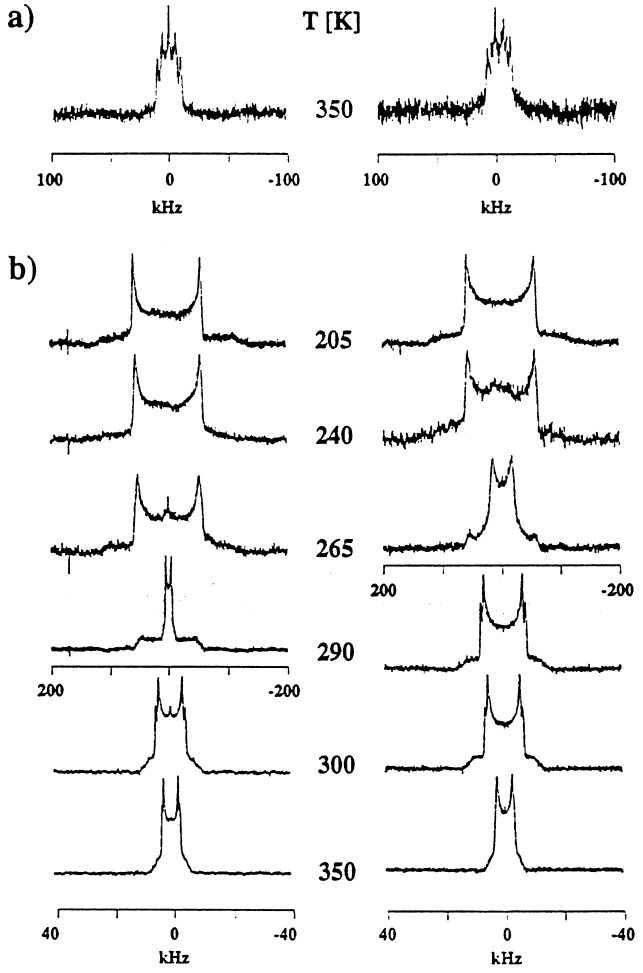



Fig. 5. Experimental $^2$H NMR spectra of (a) [2,2,2′,2′-$d_4$]-DO(14-yne)PC at 350 K and (b) [13,13,13′,13′-$d_4$]-DO(14-yne)PC as function of temperature. The left and right columns refer to bilayers from pure DO(14-yne)PC and from DO(14-yne)PC/cholesterol (3:2 molar ratio).

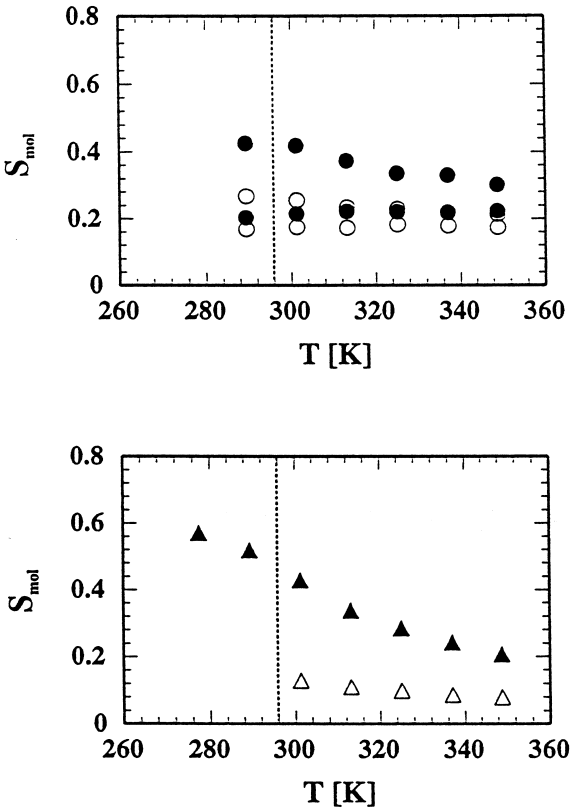



Fig. 6. $S_{mol}$ curves for [2,2,2′,2′-$d_4$]-DO(14-yne)PC (top) and [13,13,13′,13′-$d_4$]-DO(14-yne)PC (bottom) derived from the quadrupolar splittings of the *sn*-2 chains. For [2,2,2′,2′-$d_4$]-DO(14-yne)PC, two data sets are given that reflect the two inequivalent deuterons (see text). Open symbols, pure DO(14-yne)PC; full symbols, DO(14-yne)PC/cholesterol (3:2 molar ratio).

The $^2$H NMR spectra of [13,13,13′,13′-$d_4$]-DO(14-yne)PC behave very similar. In that case only two splittings (5 and 6 kHz) are observed which again reflect the inequivalence of the deuterons due to the *sn*-1 and *sn*-2 chains. A comparison with the data for other model lipids reveals that the bigger splitting belongs to the deuterons of the *sn*-1 chain [33]. The

addition of cholesterol is accompanied by significant larger quadrupolar splittings (up to a factor of 2.5). An increase of the spectral splittings also can be achieved by lowering the sample temperature towards the main transition. At the transition to the gel phase the $^2$H NMR spectra of all samples undergo a discontinuous change. They become significantly broader and display a superposition of two subspectra. For instance, the spectrum of [13,13,13′,13′-d$_4$]-DO(14-yne)PC at 290 K contains a broad spectral component (gel phase component) with a quadrupolar splitting of 100 kHz that is typical for motionally hindered lipid molecules being almost rigid on the NMR time scale. The second spectral component is much narrower and can be attributed to a coexisting liquid crystalline phase. The sample containing cholesterol behaves quite similar, as two spectral components are observed below the main transition. It is found that all DO(14-yne)PC samples possess such a temperature range of coexisting liquid crystalline and gel phases which lasts for about 35 to 40 K. Eventually, at sufficient low temperatures only the gel component remains. The observed quadrupolar splitting of 113 kHz implies that the fatty acid chains still are not completely rigid and presumably undergo some fast librational motions.

From the experimental $^2$H NMR spectra we can derive the segmental order parameter $S_{mol} = -2 \bullet S_{CD}$ that expresses the amount of chain order in the liquid crystalline phase. $S_{CD}$ is related to the experimental splitting $\Delta\nu_Q$ between the perpendicular singularities by [1]

$$\Delta\nu_Q = (3/4)(e^2qQ/h)S_{CD} \quad (1)$$

($e^2qQ/h$) is the static quadrupolar coupling constant with a typical value of 167 kHz for an aliphatic C–$^2$H bond. Fig. 6 summarizes the temperature dependent order parameters $S_{mol}$ evaluated from the smaller splittings of the *sn*-2 chains of the present systems. In the case of [2,2,2′,2′-d$_4$]-DO(14-yne)PC, two values are given which reflect the inequivalence of the two deuterons at this position (see above) and which are distinguished by their absolute values and by their temperature dependence, in agreement with other phospholipid membranes [33,35–37]. $S_{mol}$ is found to increase from C-13 to C-2, reflecting a rise in conformational order towards the lipid head group, and grows continuously upon lowering the sample temperature. The actual temperature dependence varies with both the chain position and the sample composition. The influence of the latter parameter, i.e., the presence of cholesterol, is manifested by a stronger temperature dependence.

$^2$H spin–lattice and spin–spin relaxation times have been measured for the DO(14-yne)PC and DO(14-yne)PC/cholesterol samples deuterated at positions C-2 and C-13 (data not shown). In the liquid crystalline phase $T_1$ is found to increase with temperature ranging from 12 ms (300 K) to 30 ms (349 K) for [2,2,2′,2′-d$_4$]-DO(14-yne)PC and from 22 ms to 60 ms for [13,13,13′,13′-d$_4$]-DO(14-yne)PC, respectively. The cholesterol content doesn't show any remarkable influence on the spin–lattice relaxation for both deuterated positions. The derived $^2$H spin–spin relaxation times exhibit a slight decrease upon sample heating within the liquid crystalline phase. For example, for [2,2,2′,2′-d$_4$]-DO(14-yne)PC the values

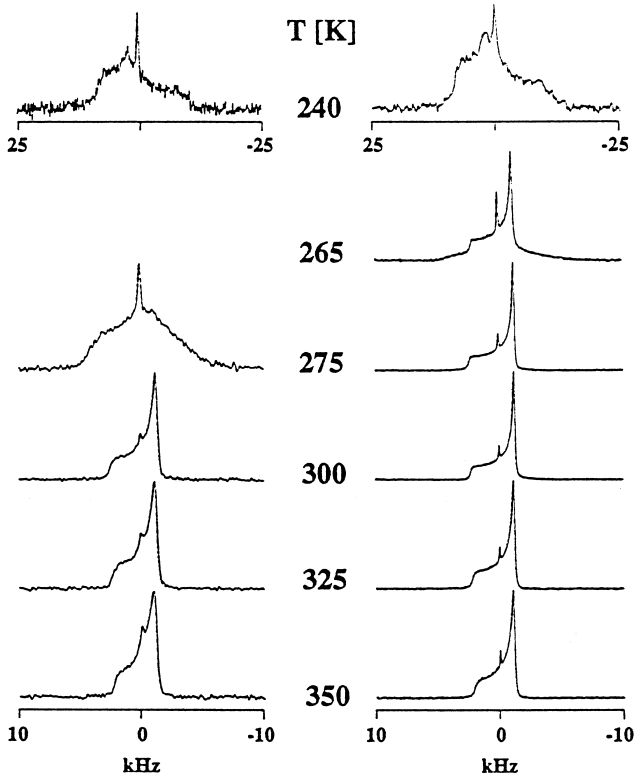



Fig. 7. Experimental $^{31}$P NMR spectra of DO(14-yne)PC (left column) and DO(14-yne)PC/cholesterol (3:2 molar ratio) (right column) as function of temperature.

range from 420 μs (300 K) to 320 μs (349 K). Upon addition of cholesterol the $T_2$ data are reduced by a factor of 0.7, preserving the actual slopes of the curves. Cooling of the DO(14-yne)PC samples below the main transition gives rise to discontinuous changes of the relaxation times, implying a sudden change in lipid mobility. A further inspection of the data in the gel phase has been omitted due to the problems with coexisting phases, as mentioned earlier.

In Fig. 7 representative $^{31}$P NMR lineshapes are shown that cover a temperature range from 240 K to 350 K. The left column refers to the pure DO(14-yne)PC/water dispersion, the right column contains the spectra for the sample with 40 mol% cholesterol. Again, the liquid crystalline phase is characterized by the presence of axially symmetric powder spectra. The addition of cholesterol has only a minor influence on the widths of the experimental $^{31}$P NMR spectra, as can be deduced from the experimental chemical shift anisotropies $\Delta\sigma$ in the liquid crystalline phase. They vary from 4.4 kHz to 4.7 kHz (36.2 to 38.6 ppm, pure DO(14-yne)PC) and from 4.6 kHz to 4.9 kHz (37.9 to 40.3 ppm, DO(14-yne)PC/cholesterol), respectively. Such rather low values reflect the presence of fast motional processes that cause a substantial reduction of the static chemical shift anisotropy.

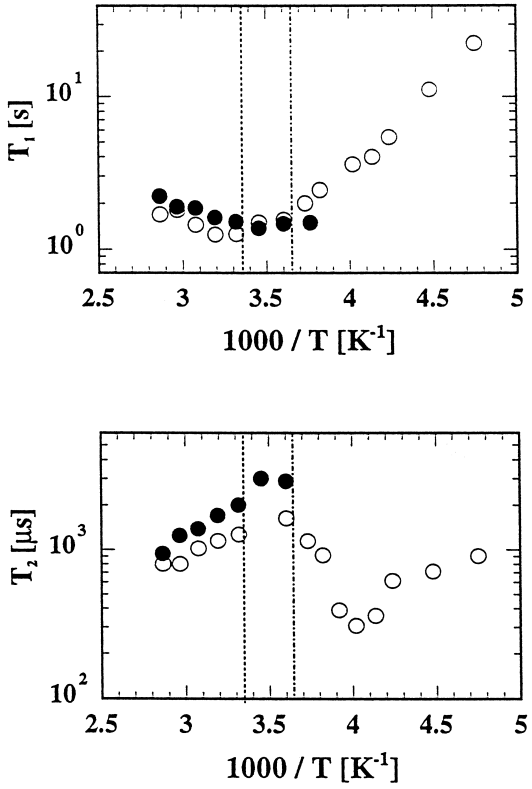



Fig. 8. Experimental $^{31}$P $T_1$ (top) and $T_2$ data (bottom) for DO(14-yne)PC bilayers. The data for pure DO(14-yne)PC and DO(14-yne)PC/cholesterol (3:2 molar ratio) samples are distinguished by open and full symbols, respectively. Dashed lines indicate the phase transitions.

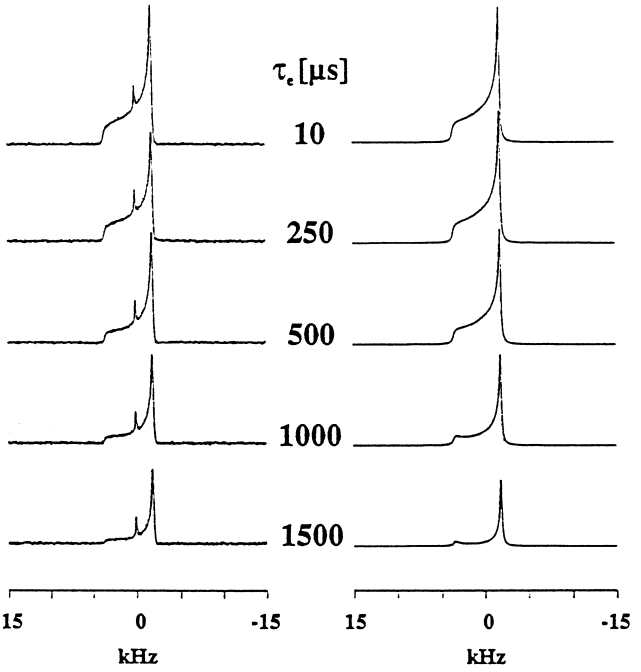



Fig. 9. Experimental (left column) and theoretical (right column) partially relaxed $^{31}$P NMR spectra (spin echo experiment) as function of the pulse delay $\tau_e$, recorded for DO(14-yne)PC/cholesterol (3:2 molar ratio) at 280 K. The theoretical spectra were obtained assuming isotropic rotational diffusion (correlation time $\tau = 3.3 \times 10^{-2}$ s) and a chemical shift anisotropy of $\Delta\sigma = 4.5$ kHz. In addition, a residual linewidth of the form $LW = A + B \times (3\cos^2\theta - 1)/2$ with $A = 110$ Hz, $B = 110$ Hz has been used ($\theta$: angle between chemical shift principle axis system and magnetic field direction).

At the main transition the situation changes completely, as again two coexisting phases are observed. In the corresponding $^{31}$P NMR lineshapes a second spectral component shows up, as visible for example in the spectra at 275 K (left column) or at 265 K (right column). Eventually, at about 240 K the molecular motions affecting the $^{31}$P NMR spectra are too slow and do not cause any lineshape effects. Now, an axially asymmetric $^{31}$P NMR lineshape is

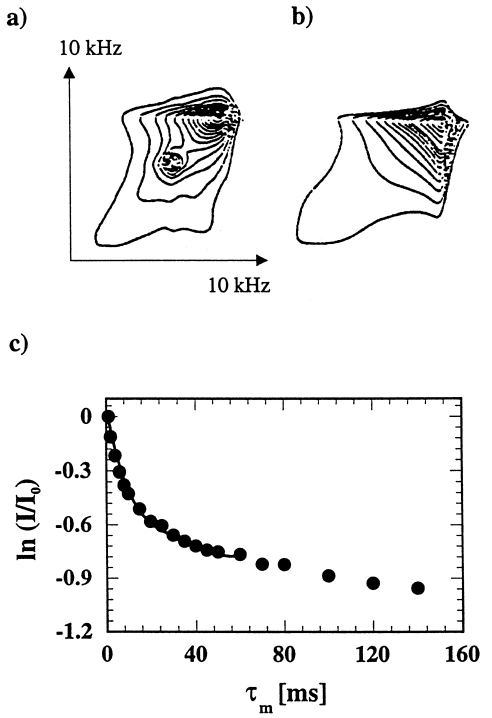



Fig. 10. Experimental (a) and theoretical (b) $^{31}$P 2D-exchange NMR spectra recorded for DO(14-yne)PC/cholesterol (40 mol% cholesterol) at 301 K at a mixing time $\tau_m = 50$ ms. The theoretical spectra were obtained assuming isotropic rotational diffusion (correlation times $\tau = 2.3 \times 10^{-2}$ s) and a chemical shift anisotropy of $\Delta\sigma = 4.5$ kHz. In addition, a residual linewidth of the form $LW = A + B \times (3\cos^2\theta - 1)/2$ with $A = 110$ Hz, $B = 110$ Hz has been used ($\theta$: angle between chemical shift principle axis system and magnetic field direction). (c) $^{31}$P NMR stimulated echo intensities DO(14-yne)PC/cholesterol bilayers at 300 K with $t_1 = t_2 = 200$ μs and variable $\tau_m$. For the simulation of the theoretical curve (solid line) the isotropic rotational diffusion approach with a correlation times of $\tau = 1.7 \times 10^{-2}$ s has been used.

detected with chemical shift tensor components of $\sigma_{11} = 85$ ppm, $\sigma_{22} = 21$ ppm and $\sigma_{33} = -105$ ppm. These values are very close to those reported for barium diethylphosphate ($\sigma_{11} = 80$ ppm, $\sigma_{22} = 19$ ppm and $\sigma_{33} = -113$ ppm) which frequently is used as a reference system for the $^{31}$P NMR studies in phospholipids [38]. The sharp isotropic signal, observed at about 0 ppm, has been attributed to the presence of small micelles in the lipid/water dispersion. It is known that for such species very fast overall tumbling, induced by translational diffusion along the curved surface, averages all anisotropic magnetic interactions [39].

In Fig. 8 $^{31}$P spin–lattice and spin–spin relaxation data are given that were obtained from variable temperature relaxation experiments for the DO(14-yne)PC and DO(14-yne)PC/cholesterol samples. In the liquid crystalline phase $T_1$ decreases on lowering the sample temperature until a lower limit is reached at the main transition with $T_1 = 1.25$ s for the pure DO(14-yne)PC membrane. Further cooling within the gel phase results in a continuous increase of $T_1$. The addition of cholesterol has a negligible effect on spin–lattice relaxation. The $^{31}$P spin–spin relaxation data for both DO(14-yne)PC samples, given in the lower part of Fig. 8, again display some characteristic features. In the liquid crystalline phase $T_2$ is found to decrease with increasing temperature which holds for both samples. The presence of cholesterol affects the absolute $T_2$ values but does not alter the slope of the $T_2$ curve. The $T_2$ data of DO(14-yne)PC/cholesterol therefore are just shifted to higher absolute values. With respect to the spin–spin relaxation in the gel phase, we refer to the previous remarks that the observed variations primarily are due to changes of the amounts of the two coexisting phases. For the same reason, only the spin–spin relaxation data for pure DO(14-yne)PC have been measured in the gel phase.

Analysis of the $^{31}$P spin echo experiments in the liquid crystalline phase has been achieved by assuming that lipid lateral diffusion dominates $^{31}$P spin–spin relaxation. This is demonstrated in Fig. 9 where partially relaxed $^{31}$P NMR spectra for DO(14-

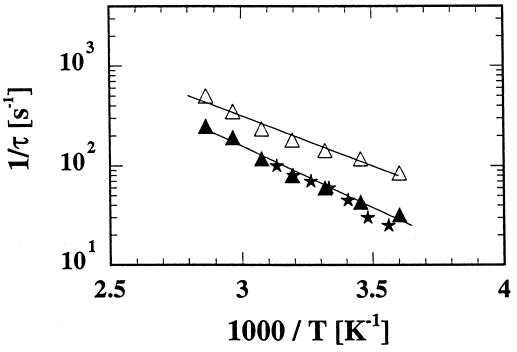



Fig. 11. Arrhenius representation of the correlation times for isotropic rotational diffusion (reflecting lipid lateral diffusion) obtained from the analysis of the partially relaxed $^{31}$P NMR spectra of DO(14-yne)PC (open symbols) and DO(14-yne)PC/cholesterol (full symbols) bilayers. Triangles, data from partially relaxed spectra (spin echo experiment); asterisks, data from stimulated echo experiments.

yne)PC/cholesterol ($T = 280$ K) are given along with their theoretical counterparts. The theoretical spectra have been calculated using the model of isotropic rotational diffusion that also has been used earlier in this context [26]. Obviously, the experimental $^{31}P$ NMR lineshapes possess a $T_2$ anisotropy that can be reproduced by the theoretical spectra and which is expressed by longer relaxation times for the perpendicular or parallel singularities (spectral edge and shoulder) than for the central spectral region. In a similar way, we have analysed the partially relaxed spectra recorded at other temperatures within the liquid crystalline phase of both DO(14-yne)PC samples.

The presence of lateral diffusive motions has been established independently by $^{31}P$ 2D-exchange and stimulated echo experiments. A representative 2D-exchange spectrum for DO(14-yne)PC/cholesterol at 301 K is shown in Fig. 10, exhibiting additional spectral intensity in the off-diagonal regions due to a motionally induced exchange of magnetization. It should be noted that the uniform distribution of the spectral intensity is highly indicative for the presence of a diffusive process, [40] as confirmed by the theoretical spectrum given in the same figure. In Fig. 10 (bottom) a representative stimulated echo decay curve [25] along with the best fit simulation (using the isotropic rotational diffusion approach) is given. The results from the analysis of the stimulated echo experiments and the above-mentioned spin-echo studies are summarized in Fig. 11. It can be seen that the lateral mobility is slowed down by the addition of cholesterol. The temperature dependence, however, remains almost unaffected, as reflected by the activation energies of 21 and 24 kJ/mol for DO(14-yne)PC and DO(14-yne)PC/cholesterol, respectively. We have estimated the lateral diffusion coefficient $D_t$ which can be derived from the relationship $D_t = R^2/6\tau$. With the assumption of an average vesicle radius of $R = 1$ μm a diffusion coefficient of $D_t = 1 \times 10^{-7}$ cm$^2$/s is obtained for DO(14-yne)PC/cholesterol at 301 K.

## 4. Discussion

In the following we will discuss the experimental results from the present variable temperature FTIR and NMR investigations on a new phospholipid that bears a triple bond at C-14 of the hydrophobic fatty acid chains. In the past, both spectroscopic techniques have shown their suitability to exploit the molecular features in biological membranes. FTIR spectroscopy is applied to probe the conformational behaviour in the fatty acid region [16–18]. From an analysis of the stretching and wagging bands, information of the conformational disorder in the liquid crystalline and gel phase can be achieved. In a similar way, dynamic solid-state NMR techniques can be used for the evaluation of the molecular ordering and the motional processes occurring in such systems. The present $^2H$ and $^{31}P$ NMR investigations thus address to the molecular behaviour of the fatty acid chains as well as of the head group region. Although investigations have been reported on lipids with both saturated and unsaturated fatty acid chains, so far phospholipids bearing isolated acetylenic groups have not been considered. In the following we will discuss the structural and dynamic properties of the DO(14-yne)PC samples separately by comprising the available data on other membrane systems.

### 4.1. Phospholipid order

As mentioned previously, information about the chain order can be gathered from FTIR as well as from NMR investigations. The former method is suitable for the evaluation of the local conformational properties. The experimental NMR spectra inherently contain conformational and orientational chain order contributions which generally are difficult to separate. In the following, the conformational properties will be mainly discussed on the basis of the present FTIR measurements that have been performed on both DO(14-yne)PC and DMPC samples.

For the liquid crystalline phase the direct comparison with the data from DMPC reveals that in the DO(14-yne)PC sample, the amount of gg sequences is doubled at 303 K being almost constant over the whole experimental temperature range. At the same time, a significant increase is registered for the amount of kink/gtg sequences along with a strong temperature dependence, while the amount of eg conformers is lowered with respect to DMPC. This behaviour might be understood by a less dense chain

packing in the liquid crystalline phase due to the sterically more hindered terminal pentyne group. The larger amount of free volume in the aliphatic chain region thus facilitates the formation of chain disorder. The kink/gtg sequences are assumed to occur over the whole fatty acid chains and therefore exhibit a pronounced temperature dependence. The gg sequence is expected to be sterically more hindered and most likely located in the lower part of aliphatic chains, i.e., towards the C–C triple bond. In this connection, it might be speculated whether their increase in DO(14-yne)PC relative to DMPC is due to the fact that the further bend of the acetylenic unit to some extent stabilizes the sterically demanding gg sequence. However, it should be mentioned that the determination of the gg conformers is accompanied with the largest error since the corresponding absorption band is rather broad and to some extent obscured by the kink/gtg and eg bands. Finally, we attribute the lower amount of eg conformers for DO(14-yne)PC to an ordering effect by the adjacent acetylenic group.

The above interpretations are further supported by investigations on the DO(14-yne)PC samples containing cholesterol. Here, only a minor influence on the amount of gg conformers is registered while the amount of kink/gtg sequences is reduced. Thus, the rigid part of cholesterol is located in the upper part of the aliphatic chain region and primarily affects the amount of kink/gtg sequences. The reduction of the amount of eg conformers upon addition of cholesterol might be traced back to stronger interactions between the two phospholipid layers that constitute a bilayer, i.e., a mutual penetration of the hydrophobic regions of adjacent layers along with a better bilayer packing. All in all, the ordering effect on DO(14-yne)PC membranes by the addition of a steroid, however, is much less pronounced than for DMPC. For instance, at 333 K the calculated total number of *gauche* conformers in DO(14-yne)PC model membranes are 3.1 (pure) and 2.5 (with cholesterol) whereas in DMPC a decrease from 2.4 (pure) to 1.3 (with cholesterol) is obtained. DO(14-yne)PC with cholesterol also exhibits a greater amount of kink/gtg sequences than found for pure DMPC. This again supports the assumption of a large free volume that is created by the incorporation of the acetylenic unit. The increase in conformational order upon addition of cholesterol can also be deduced from the $S_{mol}$ curves obtained from the $^2H$ NMR experiments. Thus, the relative increase of $S_{mol}$ upon addition of cholesterol was found to be stronger for C-13 than for C-2. For the latter segment $S_{mol}$ is assumed to be mainly affected by a change of the orientational chain order (see below). As a consequence, the stronger rise of $S_{mol}$ at position C-13 reflects the additional reduction of conformational disorder due to the presence of cholesterol.

At 296 K – the main transition of the pure lipid/water dispersion – both DO(14-yne)PC samples experience a substantial increase in conformational order. This is manifested in the sudden increase of the $CH_2$ progression band intensity and by the frequency shift of the $CH_2$ stretching bands. The gel phases for pure DO(14-yne)PC and DO(14-yne)PC/cholesterol thus are characterized by phospholipids that prevail the all-*trans* chain conformation. In this respect DO(14-yne)PC bilayers without cholesterol resemble conventional phospholipids, like DMPC or DPPC, where similar progression bands have been reported for the gel phase [29,30].

The presence of cholesterol in DO(14-yne)PC membranes is accompanied by a reduction of the progression band intensity in the liquid crystalline phase by about 50% at 280 K. Such a reduction corresponds to about 5% *gauche* probability at each position or 0.6 *gauche* conformation per chain [29]. Surprisingly, the finite progression band intensity persists for about 30 K in the liquid crystalline phase until it drops down at about 296 K. A similar trend is found for the $CH_2$ stretching bands, where again a pronounced temperature dependence of the absorption frequency is registered at 296 K. In fact, a very similar behaviour has been reported for other membrane systems with unsaturated phospholipids (containing double bonds) and cholesterol [30]. The latter findings for DO(14-yne)PC/cholesterol is further supported by the $S_{mol}$ data for position C-13. Here, the temperature dependence below 303 K is much more pronounced, reflecting the strong rise in conformational order.

Above 300 K the methylene wagging band range can then be analysed in terms of the kink/gtg, gg and eg conformers, as outlined above. In this respect, DO(14-yne)PC/cholesterol bilayers can be well distinguished from DMPC or DPPC samples with choles-

terol. In those saturated lipids progression bands as well as normal wagging bands can be detected at even higher temperatures in the liquid crystalline phase which has been related to coexisting conformationally ordered ('liquid-ordered') and conformationally disordered ('liquid-disordered') phases, respectively [30,41]. The fact that the number of *gauche* defects is significantly reduced in the DMPC/cholesterol sample again confirms the model of a denser packing in DMPC membranes than for DO(14-yne)PC bilayers. In the former case the sterically unfavourable *gauche* conformers therefore are suppressed to a large extent.

The segmental order parameters $S_{mol}$ for the bilayers from DO(14-yne)PC also were found to be lower than those reported for DMPC membranes [36,42]. In fact, $S_{mol}$ is a product of two contributions: (i) the conformational order parameter $S_{conf}$, and (ii) the orientational order parameter $S_{ZZ}$ [43,44]. The former quantity reflects the various conformers that contribute to a particular chain segment and that are interconverted due to fast *trans-gauche* isomerizations. The derived conformers from FTIR experiments only represent integral values for the complete fatty acid chain and therefore cannot be used directly for the calculation of $S_{conf}$ at a specific chain segment. The chain order parameter $S_{ZZ}$ refers to the average overall chain orientation with respect to a local preferential axis (director) [45]. It has been shown that by a comprehensive analysis of the NMR lineshape and relaxation data both the conformational and orientational order parameters can be separated in such membrane systems [11,46,47]. In the present case a similar analysis is not possible, since the accessible temperature range – during which the dynamical properties could be analysed – is very limited. Moreover, only two selectively deuterated compounds were available.

For a qualitative discussion we thus make the assumption that at position C-2 $S_{mol}$ is dominated by the contribution from overall chain disorder. Conformational changes in the glycerol backbone – that also might occur by the introduction of the acetylenic unit or the incorporation of cholesterol – are neglected to first approximation. With this assumption we can estimate the relative changes in overall chain order for DO(14-yne)PC and DMPC membranes. For DMPC deuterated at position C-2 of the *sn*-2 chain, a value of $S_{mol} = 0.32$ (at 296 K) was reported (see [36]) that has to be compared with $S_{mol} = 0.25$ for DO(14-yne)PC. This reduction in overall chain order by about 25% for DO(14-yne)PC again can be related to the introduction of the pentyne segment that causes a less dense packing of the molecules along with a larger freedom for overall lipid reorientation or fluctuation.

In the same way we can consider the relative change in overall chain order by the addition of cholesterol. In the case of DMPC an increase by a factor 1.7 has been derived from the published literature values which is close to the value of 1.6 found for DO(14-yne)PC. On the basis of this simple approach it therefore can be concluded that the relative chain ordering enhancement by the incorporation of cholesterol is similar for DMPC and DO(14-yne)PC bilayers. Nevertheless, DO(14-yne)PC membranes are less ordered than DMPC membranes, for the reasons outlined above.

### 4.2. Phospholipid dynamics

Previous investigations on membrane systems of quite different structure have shown that the lipid molecules can undergo various local and overall motions that are affected by parameters like temperature, phase and sample composition. At present, the following types of motions are discussed for phospholipids [1–5,11,48,49]: (i) *trans-gauche* isomerizations of the fatty acid chains, (ii) conformational motions of the glycerol backbone, (iii) rotations around bonds linking the phosphate head group with the glycerol backbone, (iv) phospholipid long axis rotation and fluctuation, (v) collective order fluctuation and (vi) lateral diffusion of the lipid molecules within the layers. In this connection it has been shown that the complete determination of all these processes only is possible by a detailed analysis of a comprehensive dynamic NMR study, comprising both $^{31}P$ and $^{2}H$ NMR experiments. Before we start the discussion of the present $^{2}H$ and $^{31}P$ work, we should recall that only two selectively deuterated compounds were available since the synthetic effort for other labelled compounds was too high. Likewise, we were limited by the temperature range that could be analysed since – as shown below – in the solid gel phase all molecular motions immediately

are quenched on the NMR time scale. For this reason we will discuss the conformational and reorientational dynamics of DO(14-yne)PC membranes on a qualitative basis by comparing the lineshape and relaxation data with those reported for DMPC. However, the lateral lipid motion in the liquid crystalline phase has been established by the analysis of $^{31}P$ spin-echo, 2D-exchange NMR and stimulated echo experiments.

We will start with the $^{2}H$ NMR studies on DO(14-yne)PC bilayers that have been selectively deuterated either at position C-2 or C-13, i.e., next to the carbonyl group or to the C–C triple bond, respectively. In the liquid crystalline phase these compounds show motionally averaged, axially symmetric $^{2}H$ NMR spectra which resemble those from other typical model lipids, such as DMPC or DPPC. This implies that the underlying motional processes occur in the fast exchange limit, despite the introduction of the bulky pentyne segment at position C-13. Earlier studies on DMPC have shown that the experimental NMR data adequately can be described by fast overall chain rotation and fluctuation as well as fast local *trans-gauche* isomerizations [10,11,47]. Due to the resemblance of the chemical structure it appears reasonable that the same motions also occur in DO(14-yne)PC bilayers.

At the transition to the gel phase the situation changes completely, as the spectra for DO(14-yne)PC show the presence of two coexisting phases. One component refers to the liquid crystalline phase with a high chain mobility. The other component can be ascribed to a solid (gel) phase where all molecular motions are frozen in on the NMR time scale. The fraction of the latter component grows continuously on lowering the sample temperature. As a result, at about 40 K below the phase transition the liquid crystalline component completely has vanished. The sudden slow-down in mobility for the gel component most probably originates from the large sterical hindrance due to the acetylenic unit. Thus, a density increase is expected at the main transition along with the build-up of fatty acid chains in the all-*trans* conformation (see previous chapter). Due to the bend in the lower part of the chains the lipid molecules become interconnected, i.e., they are unable to undergo overall motions. This situation is different from the gel phase in DMPC samples where such a hindrance in the lipid chain region does not exist and the lipid motions therefore are slowed down gradually upon cooling [10,11,47]. It is noted that the above observations hold for the DO(14-yne)PC/cholesterol sample as well. Apparently, cholesterol has a minor effect on the DO(14-yne)PC dynamics which would be consistent with the presence of a large free volume, as discussed above. Finally, it should be emphasized that unlike the behaviour of the chain conformations (see above discussion of $CH_2$ progression bands), the discontinuities of the lipid dynamics are in line with the corresponding calorimetric phase transitions of pure DO(14-yne)PC and DO(14-yne)PC/cholesterol, respectively.

It might be suspected that the observation of a two-component system is related to the fact that the deuterated *sn*-1 and the *sn*-2 chains freeze in at different temperatures, as mentioned previously for DMPC [42]. This argument, however, can be rejected since the motionally narrowed sub-spectrum, assigned to the liquid crystalline phase, still contains two separate splittings due to the inequivalent *sn*-1 and *sn*-2 chain deuterons.

Further information about the underlying motional mechanisms can be obtained from the $^{2}H$ relaxation data. During the studies on DMPC bilayers [11] it has been shown that in the liquid crystalline phase $T_1$ is dominated by fast local conformational changes. The present $T_1$ data for DO(14-yne)PC, labelled at C-2, were found to be identical with those reported for DMPC over the whole liquid crystalline phase. It therefore is very likely that the same conformational motions – being on a similar time scale – are responsible for spin–lattice relaxation in DO(14-yne)PC and DMPC membranes. The observation of smaller $T_1$ values for DO(14-yne)PC, labelled at C-13, than for DMPC might be related to slower *trans-gauche* isomerizations for DO(14-yne)PC due to the attachment of the bulkier pentyne segment. In the gel phase a discontinuous rise of the $T_1$ values for all DO(14-yne)PC samples has been registered which support the previous statement that at the main transition the lipid motions suddenly are quenched. In the present case, the $^{2}H$ spin–spin relaxation data, which are sensitive to motions in the intermediate range, are less striking. In the liquid crystalline phase the slight decrease of $T_2$ gives evidence to the presence of a molecular process that is slow on the NMR

time scale. A further analysis of the $^2H$ spin–spin relaxation data has been omitted since the spectral effects during the $^{31}P$ NMR measurements were found to be much more remarkable.

The $^{31}P$ NMR studies focus on the mobility of the phospholipid head group region [7,8,50] which again comprises local and overall types of motion. Surprisingly enough, the experimental $^{31}P$ spin–lattice relaxation data for both DO(14-yne)PC samples, i.e., with and without cholesterol, fall together and are almost undistinguishable from an earlier $^{31}P$ NMR studies on pure DMPC and egg yolk phosphatidylcholine [50,51]. The coincidence of the $^{31}P$ $T_1$ data for DMPC and DO(14-yne)PC again suggests that they are caused by the same molecular processes. Since a pronounced discontinuity in $T_1$ is missing at the main transition, such head group motions apparently are decoupled from the motions of the hydrocarbon chains and are unaffected by the chemical modification in that region. Due to this conformity of the available $^{31}P$ NMR spin–lattice relaxation data and since the former study [51] on DMPC has provided a comprehensive and convincing analysis we refrained from a further analysis for DO(14-yne)PC. In that study [52] it has been shown that in the liquid crystalline phase $^{31}P$ spin–lattice relaxation is determined by head group rotation about the P–$O_{11}$ bond and overall rotational diffusion, while in the gel phase fast single bond liberations are dominant. Such restricted local processes also account for the fact that $^{31}P$ spin–lattice relaxation changes almost continuously at the main transition while the overall lipid motions are slowed down abruptly.

The analysis of the partially relaxed $^{31}P$ spin-echo NMR spectra for DO(14-yne)PC has shown that the spin–spin relaxation effects can be understood on the basis of a lateral diffusive motion of the lipid molecules. This process has been described by an isotropic rotational diffusion model, that has been successfully applied during an earlier analysis on non-oriented DMPC and DPPC membranes [27]. The consistent analysis of the partially relaxed $^{31}P$ NMR spectra, stimulated echo data and 2D-exchange spectra for the samples with non-oriented DO(14-yne)PC membranes has shown that the lateral lipid motions occur on a slow time scale. The derived lateral diffusion coefficient of $D_t = 1 \times 10^{-7}$ cm$^2$/s for DO(14-yne)PC/cholesterol is also consistent with the literature data that were obtained for other phospholipid membranes employing various experimental techniques [48,52–56]. The most frequently used FRAP (fluorescence recovery after photobleaching) [55,56] and field gradient NMR techniques [52–56] showed values in the order of $10^{-8}$ to $10^{-7}$ cm$^2$/s which vary with sample composition and temperature. Nevertheless, it should be mentioned that for the present derivation of the diffusion coefficient a simple model with a single averaged vesicle radius of 1 μm has been used.

The influence of the sample temperature has been examined for the DO(14-yne)PC samples by performing variable temperature spin echo and stimulated echo experiments. With a simple Arrhenius representation activation energies of 21 (DO(14-yne)PC) and 24 kJ/mol (DO(14-yne)PC/cholesterol) have been derived which match the results from previous temperature dependent lateral diffusion measurements [48,55] and the theoretical prediction on the basis of cooperative movements of adjacent molecules [57].

The addition of cholesterol to the DO(14-yne)PC membrane is accompanied by a reduction of lateral diffusion. The activation energy, however, remains almost unchanged. The reduction of lateral lipid motion would be consistent with the proposed stronger interactions between the hydrophobic regions of the layers (see above discussion of phospholipid order). Former studies on other phospholipid membranes revealed a similar reduced lateral mobility [48,58,59]. Those studies have further demonstrated that the actual changes in lateral diffusion depends on temperature, cholesterol content and lipid structure [48].

Finally, it should be noted that there is some ongoing debate about the origin of slow motional contributions during $^{31}P$ and $^2H$ NMR experiments in the liquid crystalline phases of such membrane systems [60]. On the one hand, the experimental data – comprising spin echo, Carr-Purcell-Meiboom-Gill and field cycling experiments [47,51,61] – have been completely ascribed to collective order fluctuations. On the other hand, the model of lateral diffusion has been found to be the method of choice to account for the description of spin echo or 2D-exchange NMR experiments [12,26,62–64]. More recent work indicated that both processes might contribute [65]. The actual contribution, however, might depend on the chemical structure, vesicle size, etc. In the present

work we have shown that for the DO(14-yne)PC membranes three types of independent experiments adequately could be described in the frame of lipid lateral diffusion. The validity of the present model also is strongly supported by the derived diffusion coefficients and the temperature dependence. Both of them were found to be in good agreement with former studies on lateral diffusion in phospholipid membranes [48,52–56].

## 5. Conclusions

In the present work a new synthetic phospholipid (DO(14-yne)PC), bearing an acetylenic group in the fatty acid chains, has been investigated by solid-state NMR and FTIR spectroscopy. The study addressed to the evaluation of the molecular properties of the lipid molecules comprising both the chain ordering and lipid mobility in terms of various motional contributions. It has been shown that in the liquid crystalline phase of DO(14-yne)PC highly mobile lipid molecules exist. They generally are less ordered than reported for DMPC membranes which holds for both the conformational and the overall chain order. Cooling below the main transition is accompanied by the formation of a biphasic region: (i) a liquid crystalline phase with a high lipid mobility, and (ii) a gel phase with immobile and highly ordered fatty acid chains in the all-*trans* conformation. These results demonstrate a behaviour of DO(14-yne)PC that is different from most conventional model membranes and which is caused by the large sterical hindrance in the fatty acid chains due to the C–C triple bond. From the $^{2}H$ and $^{31}P$ NMR experiments it was further derived that in the liquid crystalline phase conformational and overall types of motion are present, being on a similar time scale as reported for the well-known DMPC. The same is true for lipid lateral diffusion which has been studied in detail during the present work by $^{31}P$ spin-echo, 2D-exchange and stimulated echo experiments. Apparently, the phospholipid mobility in the liquid crystalline phase remains almost unaffected by the introduction of a C–C triple bond in the fatty acid chain region. The present study also dealt with the impact of cholesterol on the molecular properties of DO(14-yne)PC membranes. It was found that an influence of this component becomes apparent in the lipid chain order and lipid lateral diffusion. Further work along this line, which addresses to the specific molecular features in other unusual phospholipids, is in progress.

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