Molecular and Crystal Structure of the Lipid-Model Amphiphile, Dioctadecyldimethylammonium Bromide Monohydrate

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Dioctadecyldimethylammonium bromide monohydrate ($2C_{18}N^+2C_1Br^-\cdot H_2O$), $C_{38}H_{80}NBr\cdot H_2O$, was crystallized from its solution in chloroform and hexane. The crystals are triclinic with the space group $P\bar{1}$, $Z=2$, a (normal to the bilayer surface)=3.811(2), b=0.7890(2), c=0.7418(2) nm, α=104.37(3), β=103.06(4), and γ=74.93(3)°. The structure was solved by the direct method and refined by the block-diagonal least-squares procedure: R=0.08 for 3121 observed reflections. The crystal structure consists of bimolecular layers stacking regularly along the a-direction. The two amphiphile molecules in the unit cell are related by centrosymmetry and pack tail to tail in a bilayer structure with tilting hydrocarbon chains by about 45° to the bilayer surface. The packing cross section per molecule in the layer plane is 0.567 nm². The hydrocarbon chains pack with an triclinic ($T//$) subcell with dimensions of a_s=0.42, b_s=0.49, c_s=0.26 nm, α_s=88, β_s=104, and γ_s=111°. One of the two alkyl chains bends near the nitrogen atom and conforms a part of a head region. Two water oxygen atoms with half occupancies were found in an asymmetric unit and accomodated statistically in a unit cell. These water molecules are linked with bromide anions by hydrogen bonds with the distance of about 0.34 nm. Bromide anions and ammonium cations are on the same plane parallel to the bilayer surface.

Dialkyldimethylammonium bromide, $2C_nN^+2C_1Br^-$, is a first totally synthetic amphiphile which conforms the biological membrane-like bilayer structure.[1,2] Their bilayer structures have been studied by electron microscopy,[1,2] differential scanning calorimetry and wide- and small-angle X-ray analysis,[3] and it has shown that the similarity of the molecular assembly of $2C_nN^+2C_1Br^-$ to those of phospholipids found in biological membranes. In the case of $2C_{18}N^+2C_1Br^-$, the electron micrograph showed the multilamellar liposome with mean thickness of 4.2 nm when the amphiphile solution was sonicated in the presence of uranyl acetate. The small-angle X-ray diffraction pattern taken at 25 °C (the crystal liquid-crystal transition temperature, T_c=51 °C) showed the long period of about 3.6 nm, independent of the water concentration.[3] This long period seems to correspond to the lamellar periodicity of the electron-dense striations in the electron micrograph. Since the extended bimolecular length of $2C_{18}N^+2C_1Br^-$ is about 5.0 nm on the assumption of the planar zigzag conformation of two dioctadecyl groups, it was speculated that the hydrocarbon chains of $2C_{18}N^+2C_1Br^-$ inclined at 47° to the lamellar surface in the bimolecular structure.[3]

Recently, the totally synthetic amphiphiles become important because of their ability to conform desired bilayer assemblies in the form of the single crystals, casting films and Langumuir-Blogett films. For a proper understanding of the physicochemical properties of these molecular assemblies, detailed structural information on an atomic level is required. In the cases of $2C_nN^+2C_1Br^-$ (n=18 and 16), single crystals suitable for X-ray analysis were obtained as the first example for the totally synthetic amphiphiles which could conform bimolecular layer.[4] In this paper, we discuss the structure of $2C_{18}N^+2C_1Br^-$ determined by X-ray single crystal analysis. Since this compound is chemically simple as a lipid-model able to conform the biological membrane-like bilayer structure, the precise structure on the atomic level will be useful for the better understanding of the physicochemical properties for more complicated amphiphilic compounds.

Experimental and Structure Determination

Dioctadecyldimethylammonium bromide, $2C_{18}N^+2C_1Br^-$, was prepared by stepwise alkylation of dimethylamine.[1] $2C_{18}N^+2C_1Br^-$ (50 mg) was dissolved in 2 ml of chloroform. After complete dissolution, 8 ml of hexane was added. Transparent, plate-like crystals formed at the bottom of the vessel by keeping the solution for several days at room temperature (ca. 20 °C). The size of the large crystals reached 5 mm×5 mm×0.1 mm. Many of them were usually multiple crystals with the common axis normal to the plate surface. Large plate-like crystals were cut into small pieces in order to obtain those with suitable dimensions for X-ray analysis. After many trials of X-ray investigation of such crystals, single crystals available for X-ray diffraction study were found. Approximate dimensions of the crystal used in this analysis were 0.8 mm×0.5 mm×0.01 mm.

Lattice parameters and diffraction intensities were measured on a Rigaku four-circle diffractometer with Ni-filtered Cu $K\alpha$ radiation (λ=0.154184 nm). The lattice parameters were refined by the least-squares fit using 25 reflections in the 2θ range of 19°—65°. By using the ω-scan mode at a scan rate of 10° min^{-1}, intensities of 3919 reflections were measured up to 100° in 2θ. The scan width was $\Delta\omega=(1.8+0.15\tan\theta)°$. Three reference reflections monitored every 50 reflections showed no significant intensity deterioration during the data collection. Corrections were made for the Lorentz and polarization factors, but not for absorption and extinction effects. However, the (h00) reflections with $h<12$ were excluded from the data set

because of an absorption effect on these reflections as shown in the later. A total of 3121 reflections greater than $3\sigma(F_o)$ were used in the following analysis.

Density was measured by flotation method using aqueous solution of sodium chloride. On the basis of the measured density and a unit cell volume, the unit cell contains two $2C_{18}N^+2C_1Br^-$ molecules and seems to contain one or two water molecules. While the elemental analysis showed the monohydrated structure of this amphiphile (Calcd for $C_{38}H_{80}NBr\cdot H_2O$: C, 70.33; H, 12.74; N, 2.16%. Found: C, 70.18; H, 12.75; N, 2.12%.). Further, the result of X-ray structure analysis also supported monohydrated structure as shown below.

Crystal Data: $C_{38}H_{80}NBr\cdot H_2O$, F.W.=648, triclinic, space group $P\bar{1}$, Z=2, a=3.811(2), b=0.7890(2), c=0.7418(2) nm, α=104.37(3), β=103.06(4), γ=74.93(3)°, V=2.055(2) nm^3, D_x=1.047, D_m=1.04 g cm^{-3}, ρ(Cu $K\alpha$)=10.2 cm^{-1}.

Determination and Refinement of the Structure. The direct method with the MULTAN 78 program[5] was applied for $P1$ and $P\bar{1}$ space groups. Only for the latter space group, two planar zigzag alkyl chains could be readily discerned. The vector between two bromide anions related by the centrosymmetric operation for the $P\bar{1}$ space group was clearly appeared in the three-dimensional Patterson map, which also supported this space group for the $2C_{18}N^+2C_1Br^-\cdot H_2O$ crystal. After several cycles of block-diagonal least-squares refinement starting from the coordinates of all the non-hydrogen atoms of the amphiphile obtained by the direct method, difference Fourier syntheses revealed the several peaks, which was assumed to be oxygen atoms of water molecules. After several refinement cycles including these newly found water positions, it was found that two water molecules with half occupancies were able to be accomodated statistically in an asymmetric unit in terms of

Table 1. Fractional Coordinates and Equivalent Isotropic Temperature Factors[16] for Non-Hydrogen Atoms of $2C_{18}N^+2C_1Br^-\cdot H_2O$ with Estimated Standard Deviations in Parentheses

Atom	x	y	z	$B_{eq}\times10^4$/nm^2
Br	0.0537(0)	0.2000(1)	0.3716(2)	541
N	0.0606(2)	0.8896(10)	0.8116(10)	388
C(1)	0.0280(2)	1.0379(13)	0.7951(14)	520
C(2)	0.0530(3)	0.7640(14)	0.9164(14)	536
C(31)	0.0671(2)	0.7940(12)	0.6139(12)	384
C(32)	0.0987(3)	0.6286(12)	0.6019(13)	449
C(33)	0.1101(3)	0.5701(13)	0.4083(13)	501
C(34)	0.1258(3)	0.7049(12)	0.3528(12)	430
C(35)	0.1620(2)	0.7456(12)	0.4770(12)	436
C(36)	0.1730(2)	0.8926(12)	0.4184(13)	428
C(37)	0.2100(3)	0.9350(12)	0.5337(13)	440
C(38)	0.2193(2)	1.0903)12)	0.4773(13)	458
C(39)	0.2564(3)	1.1309(12)	0.5850(13)	455
C(310)	0.2651(3)	1.2877(13)	0.5286(13)	470
C(311)	0.3026(3)	1.3286(12)	0.6334(13)	465
C(312)	0.3109(3)	1.4847(13)	0.5760(13)	476
C(313)	0.3486(3)	1.5243(13)	0.6811(14)	491
C(314)	0.3573(3)	1.6808(13)	0.6246(14)	512
C(315)	0.3949(3)	1.7189(13)	0.7317(15)	533
C(316)	0.4037(3)	1.8762(14)	0.6752(15)	578
C(317)	0.4415(3)	1.9117(15)	0.7801(17)	701
C(318)	0.4513(4)	2.0678(19)	0.7225(21)	990
C(41)	0.0944(2)	0.9560(12)	0.9324(12)	404
C(42)	0.1045(2)	1.0986(12)	0.8587(13)	433
C(43)	0.1406(2)	1.1406(12)	0.9728(12)	422
C(44)	0.1507(2)	1.2888(12)	0.9121(13)	435
C(45)	0.1879(2)	1.3315(12)	1.0158(12)	413
C(46)	0.1966(2)	1.4851(12)	0.9564(13)	410
C(47)	0.2339(2)	1.5271(12)	1.0601(13)	434
C(48)	0.2421(2)	1.6843(12)	1.0026(13)	440
C(49)	0.2793(2)	1.7253(12)	1.1079(13)	442
C(410)	0.2873(2)	1.8844(12)	1.0516(13)	458
C(411)	0.3250(2)	1.9237(12)	1.1561(13)	460
C(412)	0.3332(3)	2.0818(13)	1.1003(14)	478
C(413)	0.3706(3)	2.1212(13)	1.2039(14)	497
C(414)	0.3790(3)	2.2785(13)	1.1475(14)	514
C(415)	0.4169(3)	2.3136(13)	1.2482(15)	560
C(416)	0.4254(3)	2.4722(15)	1.1906(15)	611
C(417)	0.4631(3)	2.5045(16)	1.2873(19)	789
C(418)	0.4723(4)	2.6601(20)	1.2295(21)	994
OW(1)	−0.0004(5)	0.4159(18)	0.6982(23)	838
OW(2)	0.0251(6)	0.5303(23)	0.1079(29)	1187

stereochemistry and hydrogen bonding scheme. After an anisotropic refinement of non-hydrogen atoms, hydrogen atoms located on their calculated positions with isotropic temperature factors were included in the refinement. The quantity minimized was $\Sigma w(|F_o|-|F_c|)^2$ with $w=1.0$ for reflections with $F_o \leqq 50.0$ or with $w=0.5$ for other reflections. After several cycles some hydrogen atoms with unacceptable geometry were excluded from further calculations.

At this stage, all the magnitudes of the observed structure factors for the (h00) reflections with $h<12$ were consistenly smaller than those of the calculated ones. Especially, the pronounced discrepancy was observed for (100) reflection. The main reason of these systematic disagreement between the observed and calculated structure factors was most likely due to the absorption effect originated from the plate-like crystal shape and its long lattice constant a (3.811 nm) directed normal to the plate surface of the crystal. Therefore, these reflections were excluded from the reflection data set in the further refinement, which decreased the R-value by about 1%. The final R value was 0.070 (R_w=0.084) for all non-hydrogen atoms and 71 hydrogen atoms. The final atomic parameters for non-hydrogen atoms are given in Table 1.[6]

The atomic scattering factors were taken from International Tables for X-Ray Crystallography, Vol. IV.[7] Computations were done on an ACOS 700S computer at the Crystallographic Research Center, Institute for Protein Research, Osaka University and on a HITAC M-280H computer at the Computer Center, University of Tokyo.

Results and Discussion

Molecular Conformation of $2C_{18}N^+2C_1Br^-$. The molecular structure is shown in Fig. 1 with bond lengths, bond angles and dihedral angles together with atomic labelling for non-hydrogen atoms. One of the two hydrocarbon chains is bent at almost right angles at the C(33) atom. After this bending both chains have a trans zigzag conformation with their zigzag plains parallel to each other. Dihedral angles of the folding part, C(42)-C(41)-N-C(31), C(41)-N-C(31)-C(32), N-C(31)-C(32)-C(33), C(31)-C(32)-C(33)-C(34), and C(32)-C(33)-C(34)-C(35) are 64, 60, −165, 64, and 64°, respectively (Figs. 1 and 4). This folding pattern (G, G, T, G, G) has been known as the short folding model on the lamella surface of the polyethylene single crystal. To make stable packing of hydrocarbon chains in the bilayer structure, the $2C_{18}N^+2C_1Br^-$ molecule adopts the most simple and compact folding pattern. In the rest of the molecule, all the dihedral angles are within the range of 180±3°, with exception of N-C(41)-C(42)-C(43) (−173°) and C(33)-C(34)-C(35)-C(36) (−175°). The two zigzag planes are arranged in parallel fashion (Fig. 1 (b) and (c)) with about 0.40—0.43 nm separation. One zigzag chain (C(41), C(42), C(43), ···, C(418))

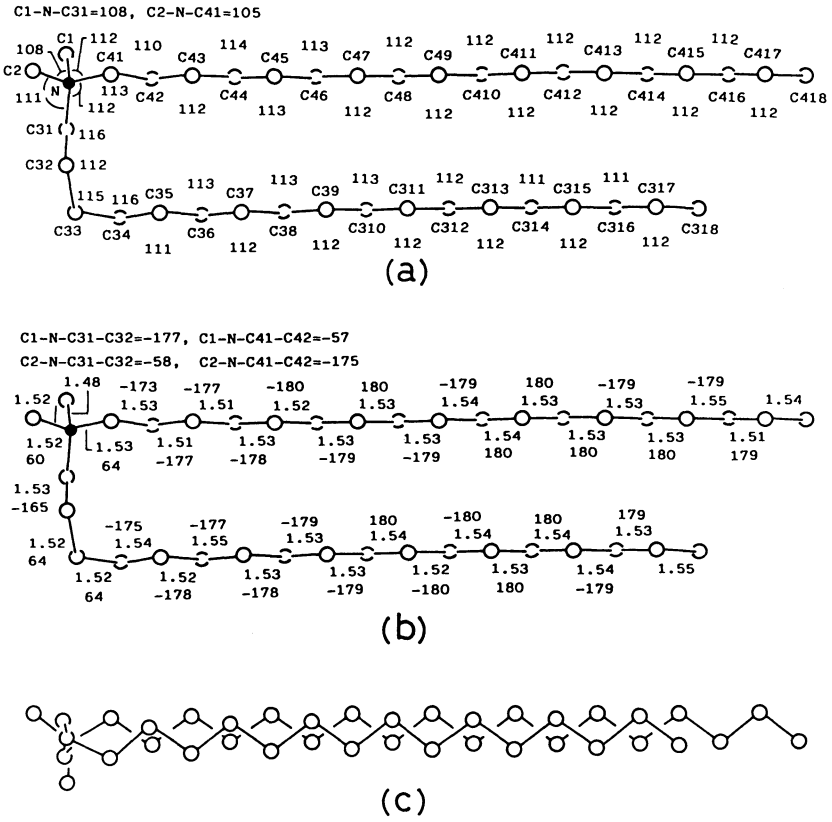

Fig. 1. (a) Atomic labelling and bond angles (ϕ/°), and (b) bond lengths (l×10/nm) and dihedral angles (ϕ/°) in $2C_{18}N^+2C_1Br^-$. Maximum estimated standard deviations for bond lengths, bond angles and dihedral angles are 0.002 nm, 1° and 1°, respectively. (c) Molecular conformation viewed from the direction normal to the zigzag plane.

is translated by three methylene unit along the chain axis to the other (C(33), C(34), C(35), ···, C(318)) (Fig. 1(c)). The average C–C bond length and C–C–C bond angle in the molecule are 0.153(1)nm and 112(1)°, respectively. There are in good agreement with values previously found in the long hydrocarbon chains of the other related amphiphilic compounds.[8,9]

Crystal Structure of $2C_{18}N^+2C_1Br^-$. The two $2C_{18}N^+2C_1Br^-$ molecules of the unit cell are related by centrosymmetry and pack tail to tail in a bimolecular layer with tilting long hydrocarbon chains (Fig. 2). The packing cross section per molecule in the layer plain (0.567 nm²) is determined by the space requirements of the hydrophilic part of the molecule, bromide anion and water molecule and coincides with the *bc*-plain of the unit cell. The dimethylammonium moiety has a chemically very simple structure compared with the hydrophilic parts of the natural amphiphilic compounds, such as phosphatidylethanolamine and phosphatidylcholine. The cross-sectional area of hydrophilic part of $2C_{18}N^+2C_1Br^-$, however, is comparatively larger than those of the above phospholipids, because of the conformationally fixed bulky dimethylammonium moiety and a bromide anion. Since the cross section of one hydrocarbon chain is about 0.20 nm², two hydrocarbon chains of $2C_{18}N^+2C_1Br^-$ molecule are tilted by 45° (=$\cos^{-1}(0.40/0.567)$) in order to accomodate to the large area of 0.567 nm² of the hydrophilic part (Fig. 2). Because of this tilting, two methyl carbon atoms at the tail end of the long alkyl chains are aligned parallel to the bilayer surface.

During the refinement procedure, statistically four water molecules with half occupancies were found in a unit cell. This number of water molecule is in good agreement with the results from chemical analysis and density measurement. Because of a short contact between OW1 and OW2 (0.18 nm), it is not possible to accomodate these four water molecules within the same unit cell. Figure 3 shows four types of their occurence and hydrogen bonding networks with bromide anions. Each unit cell in Fig. 3 contains two water molecules with full occupancy. Types A and B have no centrosymmetric relationships between water oxygens. In both cases, the water oxygen OW1 links to bromide anions by hydrogen bonds with lengths of 0.327 and 0.337 nm. Another water oxygen OW2 links to a bromide anion and a water oxygen OW1 in the

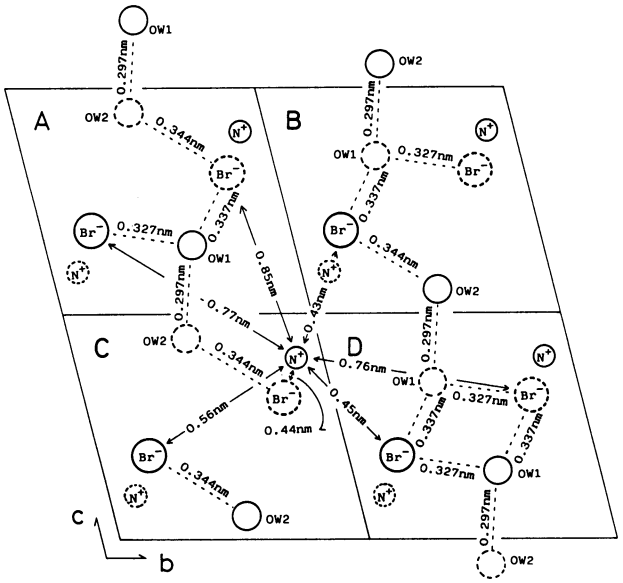

Fig. 3. Schematic illustration of hydrophillic layer of $2C_{18}N^+2C_1Br^-\cdot H_2O$ projected onto the *bc*-plane. Two water oxygen atoms are accomodated in a unit cell. Each unit cell, A, B, C, and D, has a different pair of water oxygens and hydrogen bonding scheme. Hydrogen bonds between bromide anions and water oxygen atoms are indicated by broken lines together with their bond lengths. Solid and broken circles represent atoms on the upper and lower side of the hydrophillic layer, respectively.

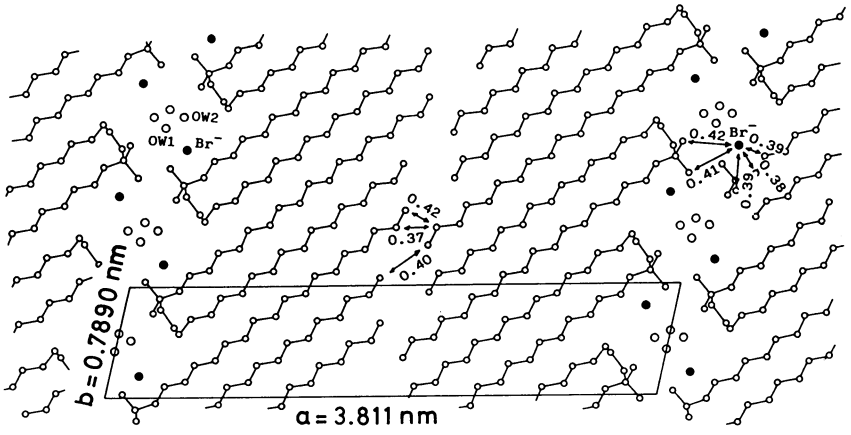

Fig. 2. Packing of $2C_{18}N^+2C_1Br^-\cdot H_2O$ projected onto the *ab*-plane (ORTEP[15] drawing). Four water oxygen atoms, OW1 and OW2 and their centrosymmetrical mates, are located on their positions in the unit cell. Distances (*l*/nm) between arrowed atoms are indicated short contacts in the structure.

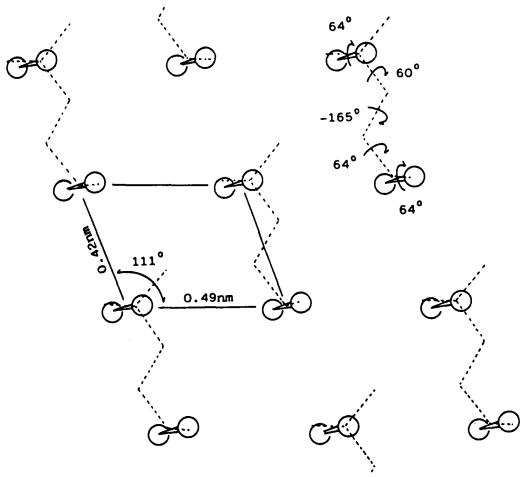

Fig. 4. The triclinic packing (*T//*) of the hydrocarbon chains in $2C_{18}N^{+}2C_1Br^{-}$. The chain axis C_s is a normal to the figure. The dotted lines denote the folding part and dimethyl groups in the molecule.

neighbouring unit cell with bond lengths 0.344 and 0.297 nm, respectively. Types C and D have a pair of oxygen atoms OW2 and OW1, respectively. In these cases, oxygen atoms are related by a centrosymmetry at the center position of each unit cell. The combination of the unit cells of AA, BB, BD, and DA along the *c*-axis makes a continuous hydrogen bond network along this direction within a hydrophilic layer. While the combination of BA, CA, BC, and CC is not possible because of a short OW2···OW2 contact (0.23 nm). The rest of the combination gives a discontinuous hydrogen bond network. Although there are many ways to accomodate water molecules in a unit cell, as a statistical point of view, one asymmetric unit contains two water oxygen atoms OW1 and OW2 with half occupancies as a whole.

The short intermolecular contacts between carbon atoms at the tail portions are 0.37 (C(417)···C(417)), 0.40 (C(318)···C(418)), 0.42 (C(417)···C(418)), 0.42 (C(317)···C(417)), and 0.43 nm (C(418)···C(418)) (Fig. 2). Rest of them are more than 0.48 nm. The short distances between carbon atoms in the adjacent alkyl chains are in the range of 0.39—0.43 nm including those between chains in the same molecule. On the other hand, there are several strong interactions in the hydrophilic region. Bromide anions link to water molecules by a hydrogen bonding with a distance of about 0.34 nm as mentioned above. Other short contacts of the bromide anion are with methyl carbons of C(1)(0.41 nm), C(2)(0.42 nm) and alkyl carbons of C(31)(0.39 nm), C(32)(0.40 nm), C(33)(0.40 nm), C(41) (0.38 nm), and C(42)(0.39 nm). Thus, the bromide anion is very neatly nestled among the head portions of the amphiphiles and water molecules.

Ammonium cations and bromide anions make a plane parallel to the bilayer surface within the deviation of 0.013 nm (Table 1). Two such planes are contained in one hydrophilic layer (Fig. 2). This kind of planarity among charged atoms have been reported in many other amphiphilic salt crystals.[10-13] A nitrogen cation is surrounded by six bromide anions (four on the same plane and two on the other plane in the same hydrophilic layer) (Fig. 3). Three of them are located at the place with somewhat long distances (0.56—0.77 nm) from a nitrogen cation because of the hydrogen-bonded water molecules with these bromide anions. The distances from the rest of bromide anions are rather short (0.43—0.45 nm). Therefore, the plus charge on the ammonium cation seems to be canceled exclusively by these three bromide anions.

The hydrocarbon chains pack latterally according to the triclinic (*T//*) packing mode.[14] The subcell dimensions are: a_s=0.42, b_s=0.49, c_s=0.26 nm, α_s=88, β_s=104 and γ_s=111° (Fig. 4). The packing cross section per chain perpendicular to its long chain axis is 0.193 nm^2, giving a well packed chain matrix. It is remarkable that all the hydrocarbon chains pack identically as it were no folding part in the amphiphile molecule.

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