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Molecular and Crystal Structure of an Azobenzene-Linked Amphiphile, $\mathrm{C_{33}H_{54}N_3O_3Br}$

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Azobenzene-linked, single-chain ammonium amphiphile ($\mathrm{C_{12}AzoC_5N^+Br^-}$), $\mathrm{C_{33}H_{54}N_3O_3Br}$, was crystallized from their solutions in water and ethanol. The crystals are triclinic with the space group $P\bar{1}$, $Z=2$, $a=3.4243(6)$, $b=0.8525(1)$, $c=0.6073(1)$nm, $\alpha=73.45(1)$, $\beta=88.03(1)$, and $\gamma=93.32(1)°$. The structure was solved by the direct method and refined anisotropically by the block-diagonal least-squares procedure: $R=0.06$ for 2917 observed reflections ($2\theta<105°$, Cu$K\alpha$, $\lambda=0.154184$ nm). The crystal structure consists of regularly stacking bimolecular layers in which $\mathrm{C_{12}AzoC_5N^+Br^-}$ molecules incline about 30° to the layer surface. As expected from spectroscopic analyses, the azobenzene chromophore shows a head-to-tail (J-like) aggregation between adjacent molecules. The pronounced planarity of the azobenzene chromophore found in this crystal structure enables parallel stacking of phenyl groups in the J-like aggregation.

Biological membranes are organized assemblies consisting mainly of proteins and lipids. Their gross organization has been proposed by Singer and Nicolson[1] in the form of a fluid mosaic model, in which lipid molecules are assembled in the bilayer form. For better understanding of structural details of biological membranes and their relation to specific functions, it is essential to accumulate structural information about various lipid bilayers on the atomic level. However, it has been difficult to obtain single crystals of amphipathic lipids suitable for X-ray diffraction, which could provide the most detailed information about their preferred molecular conformations and interactions. Such information has been obtained so far only for a few amphipathic lipids.[2]

In order to establish the physicochemical basis of the biological membrane and to develop membrane-related chemical processes, one of the authors (T. K) has studied the formation of the bilayer structure using a large variety of amphiphiles containing a single,[3] double,[4] and triple[5] alkyl chains as the hydrophobic tail part. It has been shown that many of these compounds form stable molecular (monolayer and bilayer) membranes spontaneously when dispersed in water. Furthermore, in the case of dialkyldimethylammonium bromides with alkyl chain lengths of $n=18$ and 16, single crystals suitable for X-ray diffraction could be provided[6] and detailed structure analyses are now in progress. In the case of single-chain ammonium amphiphiles containing azobenzene chromophore (abbreviated as $\mathrm{C}_n\mathrm{AzoC}_m\mathrm{N^+Br^-}$, hereafter, the general formula shown in Fig. 1), a

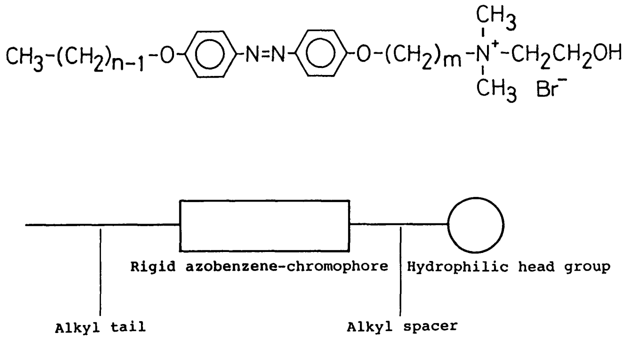


Fig. 1. Chemical formula and its schematic illustration for azobenezene-linked single chain amphiphiles (abbreviated as $\mathrm{C}_n\mathrm{AzoC}_m\mathrm{N^+Br^-}$). n and m represent a number of carbons in the alkyl tail and spacer parts, respectively.

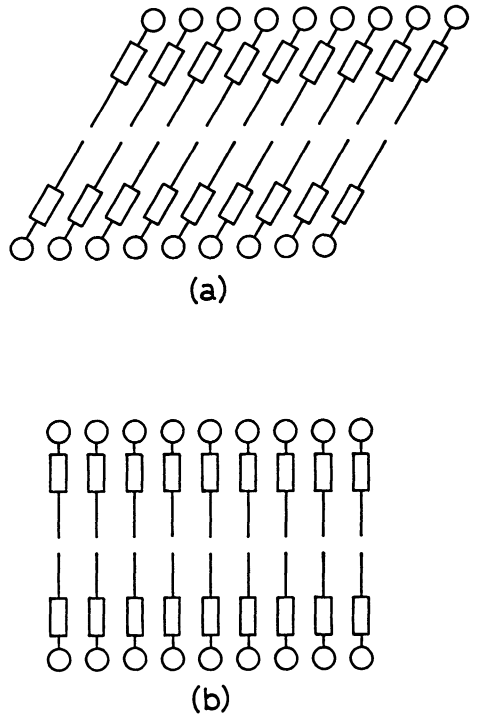


Fig. 2. Schematic illustration of two aggregation forms for $\mathrm{C}_n\mathrm{AzoC}_m\mathrm{N^+Br^-}$ amphiphiles in crystalline bilayer. (a) J-like aggregate showing head-to-tail chromophore orientation. (b) H-aggregate showing highly parallel chromophore orientation.

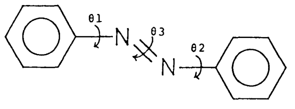
Table 1. Fractional Coordinates and Equivalent Isotropic Temperature Factors[26)] for Non-Hydrogen Atoms of $C_{12}AzoC_5N^+Br^-$ with Estimated Standard Deviations in Parentheses

Atom	x	y	z	$B_{eq}\times 10^2$/nm^2
Br	0.9335 (1)	0.3596 (1)	0.9798 (1)	501
O (1)	0.9976 (1)	0.1844 (6)	1.3215 (8)	473
O (2)	1.1856 (1)	−0.3977 (6)	1.4408 (8)	446
O (3)	1.2892 (1)	−1.4853 (6)	0.7964 (9)	501
N (1)	1.0639 (2)	0.2391 (6)	1.6589 (9)	359
N (2)	1.2245 (2)	−0.8960 (7)	1.0696 (10)	405
N (3)	1.2497 (2)	−0.9864 (7)	1.1669 (10)	426
C (1)	0.9935 (2)	0.2223 (8)	1.5329 (12)	405
C (2)	1.0276 (2)	0.3271 (8)	1.5728 (12)	401
C (3)	1.0949 (2)	0.3704 (10)	1.6658 (16)	602
C (4)	1.0550 (3)	0.1249 (10)	1.8946 (13)	561
C (5)	1.0784 (2)	0.1469 (8)	1.4971 (11)	342
C (6)	1.1140 (2)	0.0520 (9)	1.5642 (13)	422
C (7)	1.1223 (2)	−0.0506 (8)	1.4025 (12)	387
C (8)	1.1533 (2)	−0.1695 (8)	1.4867 (12)	389
C (9)	1.1604 (2)	−0.2743 (8)	1.3297 (12)	393
C (10)	1.1940 (2)	−0.5175 (8)	1.3424 (12)	357
C (11)	1.1772 (2)	−0.5383 (8)	1.1469 (12)	408
C (12)	1.1888 (2)	−0.6634 (9)	1.0641 (12)	421
C (13)	1.2213 (2)	−0.6222 (9)	1.4485 (12)	414
C (14)	1.2319 (2)	−0.7474 (8)	1.3646 (12)	392
C (15)	1.2154 (2)	−0.7695 (18)	1.1677 (11)	360
C (16)	1.2588 (2)	−1.1121 (8)	1.0688 (12)	384
C (17)	1.2419 (2)	−1.1387 (8)	0.8737 (11)	378
C (18)	1.2533 (2)	−1.2617 (9)	0.7899 (12)	426
C (19)	1.2861 (2)	−1.2167 (9)	1.1712 (12)	428
C (20)	1.2979 (2)	−1.3436 (9)	1.0883 (13)	450
C (21)	1.2805 (2)	−1.3669 (8)	0.8953 (12)	390
C (22)	1.3172 (2)	−1.6003 (9)	0.8913 (13)	484
C (23)	1.3201 (2)	−1.7104 (9)	0.7407 (13)	474
C (24)	1.3506 (2)	−1.8361 (9)	0.8027 (14)	521
C (25)	1.3520 (2)	−1.9418 (10)	0.6437 (15)	566
C (26)	1.3826 (3)	−2.0654 (11)	0.6831 (16)	670
C (27)	1.3819 (3)	−2.1693 (11)	0.5178 (17)	695
C (28)	1.4135 (3)	−2.2886 (11)	0.5411 (16)	674
C (29)	1.4124 (3)	−2.3877 (11)	0.3704 (18)	726
C (30)	1.4424 (3)	−2.5098 (12)	0.3962 (18)	752
C (31)	1.4419 (3)	−2.6113 (12)	0.2334 (19)	859
C (32)	1.4725 (4)	−2.7331 (13)	0.2605 (23)	1045
C (33)	1.4713 (4)	−2.8371 (16)	0.0966 (24)	1243

Table 2. Torsion Angles for Various Azobenzene-Linked Compounds

Compound	Torsion angles**		
	$\theta 1$/°	$\theta 2$/°	$\theta 3$/°
$C_{12}AzoC_5N^+Br^-$	−177.7	−179.0	−179.9
trans-Azobenzene[15)]*	−163.3	163.3	180.0
	−173.1	173.1	180.0
4,4′-Dimethylazobenzene[12)]*	−170.6	170.6	180.0
	−174.5	174.5	180.0
4-Amino-2′,3-dimethyl-azobenzene[13)]	−177.1	−173.9	177.7
1,4-Bis(phenylazo)benzene[14)]	178.4	178.9	179.0
2,2′,4,4′,6,6′-Hexamethyl-azobenzene[18)]*	161.2	−161.2	180.0
2,2′,4,4′,6,6′-Hexa-*t*-butyl-azobenzene[19)]	−132.9	−119.5	161.6
2,2′,4,4′,6,6′-Hexaisopropyl-azobenzene[20)]*	−155.5	155.5	180.0
2-Thiocyanatoazobenzene[21)]	−123.4	−118.7	−171.7

* Molecule has an inversion symmetry at the center of azobenzene moiety. ** Torsion angles are defined as follows:



series of compounds were synthesized to investigate the relationship between their aggregation behavior and the alkyl chain length in the tail(n) and spacer(m) portions.[7)] In terms of absorption spectroscopy, their molecular aggregation states were classified into four types. They are the monomeric chromophore in micelles and in molecularly dispersed solutions, the dimeric chromophore in the liquid crystalline bilayer, and the H-aggregate and the J-like aggregate in the crystalline bilayer (Table 5 in Ref. 7). The latter two aggregates are particularly interesting because of their chromophore orientations. The H-aggregate implies the highly parallel chromophore orientation and the J-like aggregate implies the head-to-tail orientation (Fig. 2). We already investigated the structure of the H-aggregate in a cast film of $C_8AzoC_{10}N^+Br^-$ by the X-ray diffraction technique.[8)]

In this paper, we discuss the detailed structure of the J-like aggregate in a single crystal of $C_{12}AzoC_5N^+$-Br^- on the basis of the structure obtained by X-ray crystal analysis. Since this is the first analysis of a totally synthetic amphiphile on the atomic level, the results will be useful for the better understanding of the biological membrane. Furthermore, it will also play an important part in the elucidation of the energy transfer mechanism, which occurs characteristically in the azobenzene-linked amphiphiles with J-like chromophore orientation.

Experimental and Structure Determination

$C_{12}AzoC_5N^+Br^-$ (25 mg) was dissolved in a boiling solution (30 ml) of water and ethanol (water/ethanol=1/1 by volume rotio). Yellow, plate-like crystals with thickness of about 0.02 mm were obtained by keeping the solution for a week or two at room temperature. However, these plate-like crystals were usually multiple crystals with the common axis normal to the plate surface. Needle-like crystals were also obtained by storing the solution in a refrigerator. In this case crystals were very thin (about 0.01 mm) and small

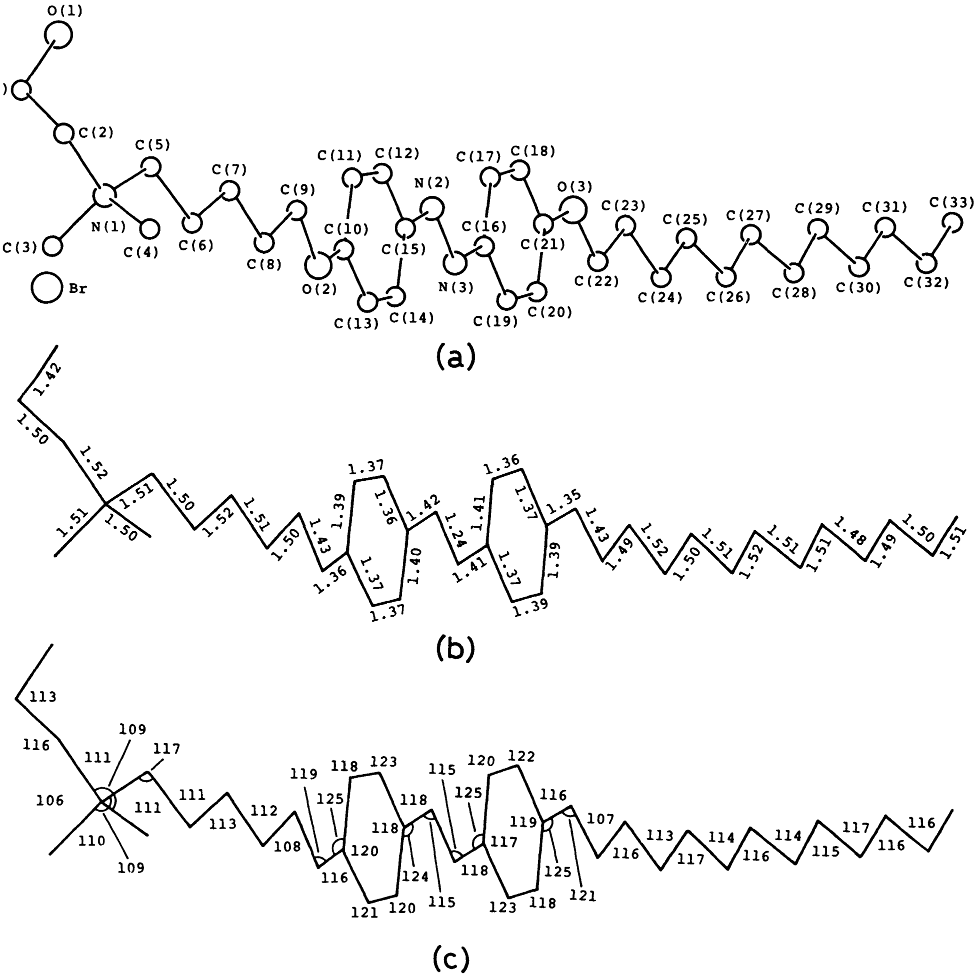


Fig. 3. Atomic labelling (a) and bond lengths (1×10/nm) (b) and angles (ϕ/°) in $C_{12}AzoC_5N^+Br^-$. Maximum estimated standard deviations for bond lengths and angles are 0.002 nm and 1°, respectively.

in width (about 0.1 mm) but long (2—3 mm). Diffraction experiments showed that these two types of crystals have identical lattice constants and intensity distribution. One of the latter type of crystals was used for the intensity measurements.

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 15—66°. By using the ω-scan mode at a scan rate of 6°/min, intensities of 4423 reflections were measured up to 105° in 2θ. The scan width was $\Delta\omega$=(2.8+0.15 tanθ)°. 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. A total of 2917 reflections greater than $3\sigma(F_o)$ were used in the following analysis, where $\sigma(F_o)$ is the standard deviation estimated from the counting statistics.

Crystal Data: $C_{33}H_{54}N_3O_3Br$, *F.W.*=620.7, triclinic, space group $P\bar{1}$, Z=2, a=3.4243(6), b=0.8525(1), c=0.6073(1) nm, α=73.45(1), β=88.03(1), γ=93.32(1)°, V=1.6941(4) nm^3, D_x=1.215, D_m=1.21 gcm^{-3}.

Determination and Refinement of the Structure

The direct method with the *MULTAN* 78 program[9] was applied for $P1$ and $P\bar{1}$ space groups. Only for the latter space group, a rigid azo moiety and two planar zigzag alkyl chains could be readily discerned. The vector between two bromide ions related by the centrosymmetric operation was clearly appeared in the three-dimensional Patterson map, which also supported the $P\bar{1}$ space group for the $C_{12}AzoC_5N^+Br^-$ crystal. After several cycles of block-diagonal least-squares refinement starting from the atomic coordinates obtained by the direct method, difference Fourier syntheses revealed the remaining two carbon atoms in the alkyl chain and a terminal oxygen atom in the hydrophilic head group. 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 $\sum w(|F_o|-|F_c|)^2$ with w=1.0 for all reflections. After several cycles some hydrogen atoms with unacceptable geometry were

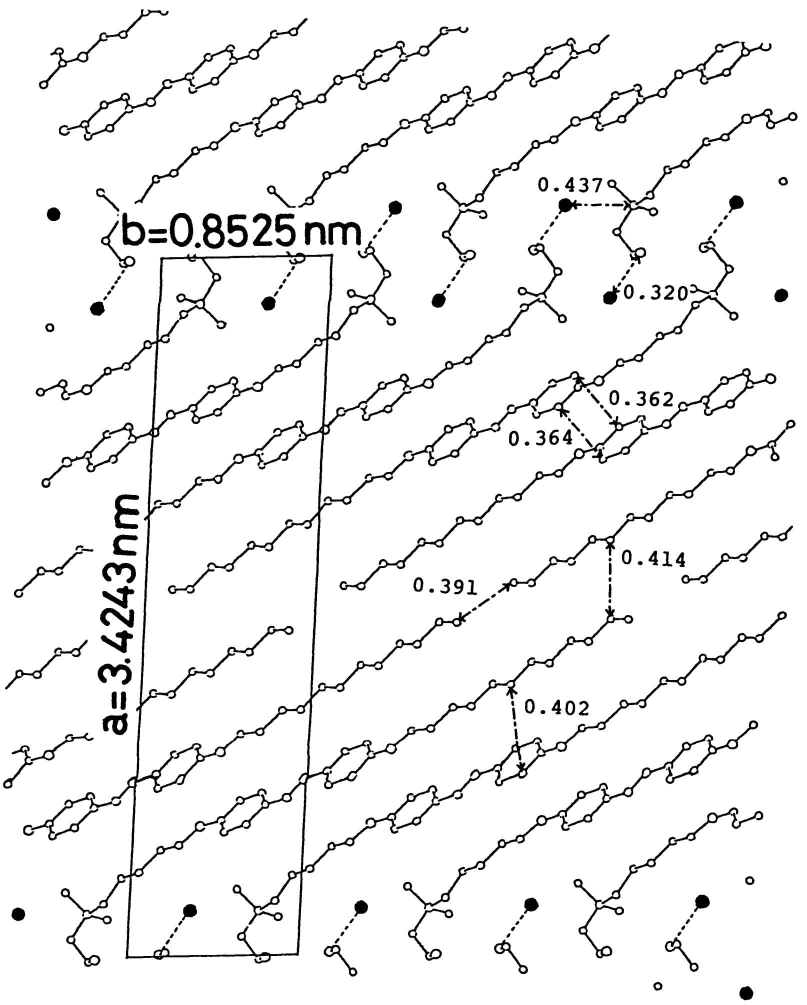


Fig. 4. Packing of $C_{12}AzoC_5N^+Br^-$ projected onto the *c*-plane. Hydrogen bonds between bromide anions and hydroxyl groups are indicated by broken lines (ORTEP[22] drawing).

excluded from further calculations. At the final stage of refinements the full-matrix least-squares calculation was carried out but no significant improvement was obtained. The final R value was 0.059 (R_w=0.060) for all nonhydrogen atoms and 51 hydrogen atoms. The final atomic parameters for non-hydrogen atoms are given in Table 1.[10]

The atomic scattering factors were taken from International Tables for X-Ray Crystallography, Vol. IV.[11] 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 Structure of $C_{12}AzoC_5N^+Br^-$. Bond distances and angles are shown in Fig. 3, together with its atomic labelling. The average bond length between carbon atoms in the benzene rings is 0.138 nm. Judging from the bond distances in Fig. 3, there are no evidence for the quinonoid character in both benzene rings, unlike in 4,4′-dimethylazobenzene[12] and 4-amino-2′,3-dimethylazobenzene.[13] The C(11)–C(10)–O(2), C(14)–C(15)–N(2), C(17)–C(16)–N(3) and C(20)–C(21)–O(3) bond angles deviate by 4—5° from the standard value (120°) for sp^2 carbon atoms, presumably in order to relieve the close approach between the C(9) and C(11), C(14) and N(3), C(17) and N(2), and C(20) and C(22) atoms, respectively. This type of deviation has been found in many other compounds,[12–15] in which substituent groups are located approximately in the benzene ring plane. The mean C–C bond distance and C–C–C bond angle in the alkyl spacer and tail parts are 0.150(1) nm and 115(1)°, respectively. They are in good agreement with values previously found in other amphiphilic compounds with long hydrocarbon chains.[16,17]

The most striking feature of the $C_{12}AzoC_5N^+Br^-$ molecule is the planarity of the azobenzene chromophore. Table 2 shows two torsion angles (θ_1 and θ_2) between the azo group and the benzene rings, and at the azo group itself (θ_3) for various compounds. In

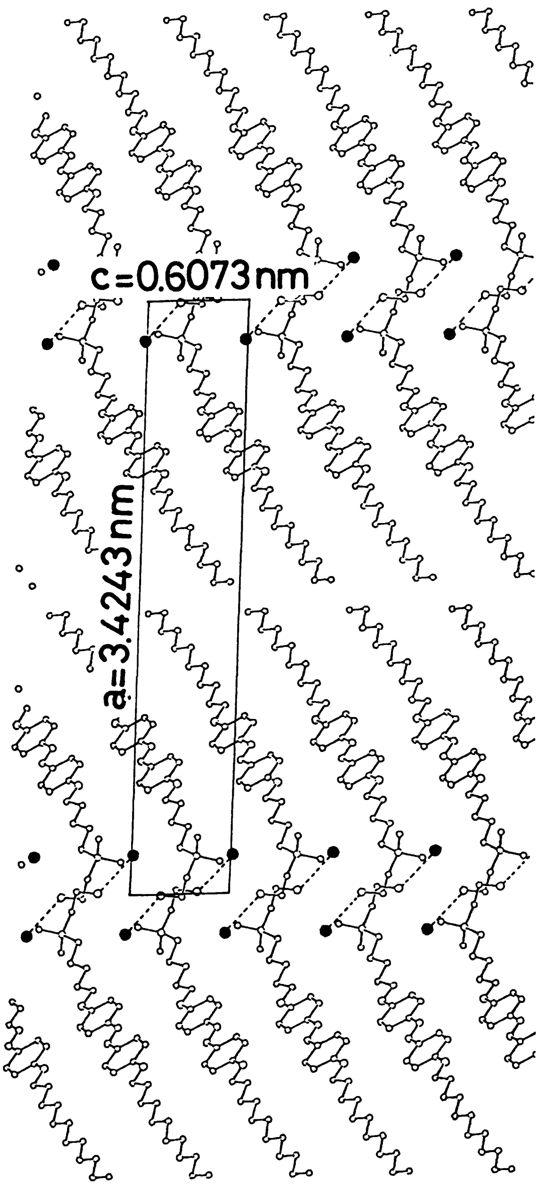


Fig. 5. Packing of $C_{12}AzoC_5N^+Br^-$ projected onto the *b*-plane (ORTEP[22]).

many cases, the deviation from the planar conformation is more than 6°, while in the $C_{12}AzoC_5N^+Br^-$ case the deviation is less than 2.3°. So far, this kind of planarity for an azobenzene chromophore has been found only in the case of 1,4-bis(phenylazo) benzene,[14] where adjacent molecules along the *b*-axis had very strong interactions and are separated by only 0.325 nm. Another example of the comparatively good planarity has been found in 4-amino-2′,3-dimethylazobenzene where there is delocalization of π-electrons between phenyl groups and an azo moiety.[13] The pronounced planarity of the azobenzene moiety in the $C_{12}AzoC_5N^+Br^-$ crystal enables parallel stacking of head and tail phenyl rings in the J-like aggregation (Fig. 4). Furthermore, the zigzag planes of the spacer alkyl and the tail alkyl parts of $C_{12}AzoC_5N^+Br^-$ are linked to the azobenzene plane with small torsion angles, 7.3° and 4.1°, respectively. Consequently, the whole molecule is like a long thin plate (Figs. 4 and 5).

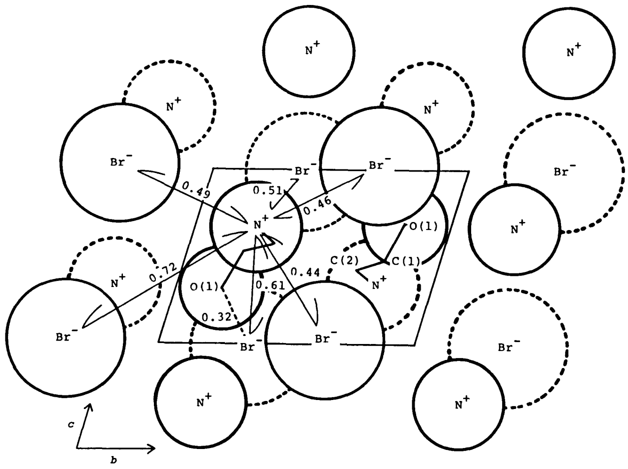


Fig. 6. Schematic illustration of hydrophillic layer of $C_{12}AzoC_5N^+Br^-$ projected onto the *a*-plane. Solid and broken circles represent atoms on the upper and lower side of the hydrophillic layer, respectively. Distances (l/nm) are indicated.

Crystal Structure of $C_{12}AzoC_5N^+Br^-$. The crystal structure of $C_{12}AzoC_5N^+Br^-$ can be considered as a structure regularly stacked by the bimolecular layers along the *a* direction (Figs. 4 and 5). Within the bimolecular layer, two molecules related by the inversion symmetry face each other in the tail-to-tail fashion with the contact distance of 0.391 nm and their molecular axes inclined by about 30° to the layer surface (Fig. 4). The structure also can be seen as an alternation of the hydrophobic and hydrophilic layers stacked along the *a* direction. The hydrophilic layer, consisted of ammonium cations, bromide anions and terminal hydroxyl groups from both sides of bimolecular layers, coincides in position with the (100) plane of the crystal (Figs. 4 and 5). In particular, ammonium cations and bromide anions make a plane parallel to the (100) plane within the deviation of 0.004 nm. Two such planes are contained in one hydrophilic layer. This type of planarity among charged atoms in an amphiphilic salt crystal have been found in many other cases.[23,24] Figure 6 shows one of the planes composed of charged atoms and 2-hydroxyethyl groups. 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). Terminal atoms, $N(1)^+$, C(2), C(1), and O(1) are nestled near the center of four equivalent bromides. A nitrogen cation locates at the point with long distances (0.61 and 0.72 nm) from bromide anions on the same side as O(1) atom. The distances from the other side of bromides are rather short (0.44—0.51 nm). The terminal hydroxyl group, which is the only one potential hydrogen bonder in the $C_{12}AzoC_5$-N^+Br^- molecule, takes part in the hydrogen bond formation with bromide anion. Hydrogen bond distance (0.32 nm) and angle ($\angle OHBr^- = 160.7°$) are those of commonly observed. In addition to this hydrogen bond, electrostatic interactions between ammonium cations and bromide anions stabilize the hydrophilic phase and link the neighboring bimolecular layers tightly, while hydrophobic alkyl chains and azobenzene moieties are stabilized only by the van der Waals interactions.

The cross-section of the benzene ring, S_B which is the largest part in the hydrophobic chain, could be estimated as 0.234 nm^2 by using van der Waals radii 0.18 and 0.12 nm for atoms C and H, respectively. While it is possible to calculate the molecular area, S_M, at the bilayer surface from the lattice constants: $S_M = 0.497$ nm^2($= bc \times \sin\alpha$). In order to balance the cross-sections of the hydrophilic and hydrophobic part, molecules have to incline by 62° ($= \cos^{-1}(S_B/S_M)$) from the layer normal. Thus, the azobenzene chromophores are obliged to form J-like aggregate (head-to-tail orientation). On the other hand, hydrophobic chains from both sides interdigitate each other in the bilayer of the cast film of $C_8AzoC_{10}N^+Br^-$.[8] This is another stable aggregation (H-aggregate), since the cross-section of the interdigitated hydrophobic chain, 2 S_B is comparable to the molecular area S_M.

Some short interatomic distances between the neighboring azobenzene chromophores are shown in Fig. 4 together with those between alkyl tail parts. Absorption spectra of azobenzene in J-like aggregates of $C_nAzoC_mN^+Br^-$ show a shift to longer wavelength (red shift) than those of the molecularly dispersed azobenzene chromophores. This shift has been attributed to the intermolecular interactions between azobenzene chromophores in J-like aggregates.[7] The distance about 0.36 nm between overlapped phenyl groups seems to be short enough for red shift by reference to theoretical calculations for dye molecules in J-aggregates.[25] In order to clarify the relationship between a molecular and/or packing structure and spectroscopic behavior, further analyses for several compounds with different n and m of $C_nAzoC_mN^+Br^-$ are now carring on.

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