Cholic acid as host for long linear molecules: a series of co-crystals with *n*-alkylammonia†

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Co-crystals of cholic acid (CA) with *n*-alkylammonia (n = 10, 12, 14, 16) represent the first examples of CA's co-crystals with molecules of comparable size. In one of the compounds a completely new type of bilayer arrangement was found. The host–guest ratio in crystals was found to be 1 : 1 in three cases and 2 : 1 in one case. In 1 : 1 complexes CA molecules are assembled in bilayers and *n*-alkylammonia guests are included in the hydrophobic zones between those layers in a kind of *sandwich-type* structure. In the 2 : 1 complex the CA molecules include guest molecules in a different way: bilayers are not parallel but instead they cross and form one-dimensional hydrophobic channels into which guest molecules are included. In the case of 1 : 1 co-crystals the bilayers of CA are separated much more than in previously reported structures in order to accommodate large *n*-alkylammonia molecules. In spite of the different bilayer arrangements the characteristic spacing in the plane of CA bilayers is remarkably similar to the majority of other CA crystal structures known to date.

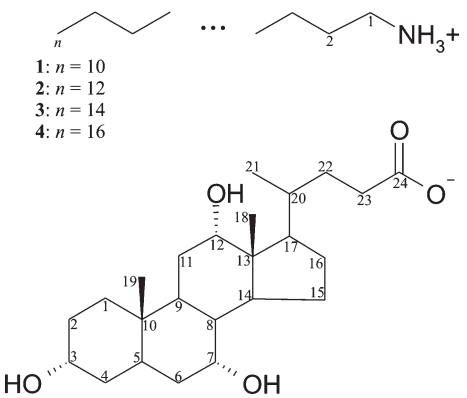
Introduction

Cholic acid (CA) and related compounds have attracted considerable attention in the context of host–guest chemistry and have proved capable of including a wide variety of organic substances.[1–4] In supramolecular and biomimetic chemistry these compounds can be used as an architectural component of supramolecular hosts suitable for molecular recognition by size, shape, polarity, chirality and chemical environment.[1,3,5–8] The attractiveness of this class of compounds lies in their facial amphiphilicity which leads to their assembly in the form of bilayers with hydrophilic and hydrophobic surfaces. The molecules of CA are connected by hydrogen bonds on their hydrophilic faces. This is the most dominant mode of assembly of the CA molecules in the crystalline state.[1] Further organization of the crystal structure can be represented as stacking of these bilayers in various directions. Such bilayers can shift relative to each other to form one-dimensional channels of variable cross-sections between the bilayers, which leads to their versatile inclusion capability. Molecules of usually smaller sizes can be placed into these cavities.[1] It was shown that the size of these cavities can be controlled and expanded by a suitable modification of host molecules.[4] To some degree, the size and shape of the host framework are influenced by the size of the guest molecule itself in a sort of guest-dependent polymorphism.[1] In a work reported previously, the authors managed to obtain fine control over the size of the cavities by varying the length of the alkyl chain in the series of alkylammonium deoxycholates.[3] This prompted us to investigate the behaviour of the CA host frameworks in co-crystals with alkylammonia of longer chain lengths (Scheme 1).

The investigation of the assembling modes of catanionic moieties in crystalline form is of interest because aqueous mixtures of oppositely charged surfactants, named catanionic surfactants,[9–11] provide the possibility to design various supramolecular structures. Following the great interest in the design and study of novel surfactants, here we focused our attention on structural characterization of the novel catanionic amphiphiles comprised of oppositely charged surfactants, an anionic cholate and cationic *n*-alkylammonium part of varying alkyl chain length (n = 10, 12, 14, 16; compounds **1**–**4** in Scheme 1).

Results and discussion

The series of the *n*-alkylammonium cholates were characterized by X-ray diffraction in order to compare their



Scheme 1 Chemical structures of the *n*-alkylammonium cholates with standard numbering of CA.

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Table 1 Crystallographic data for compounds **1**–**4**

Compound number	**1**	**2**	**3**	**4**
Chain length	10	12	14	16
Chemical formula	$C_{34}H_{65}NO_6$	$C_{60}H_{111}NO_{12}$	$C_{38}H_{73}NO_6$	$C_{40}H_{77}NO_6$
Formula moieties	$C_{10}H_{21}NH_3^+$, $C_{24}H_{39}O_5^-$, H_2O	$C_{12}H_{25}NH_3^+$, $C_{24}H_{39}O_5^-$, $C_{24}H_{40}O_5$, $2H_2O$	$C_{14}H_{29}NH_3^+$ $C_{24}H_{39}O_5^-$, H_2O	$C_{16}H_{33}NH_3^+$ $C_{24}H_{39}O_5^-$, H_2O
M_r	583.9	1038.50	639.97	668.03
Host : guest ratio	1 : 1	2 : 1	1 : 1	1 : 1
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P\,2_1$	$P\,2_1$	$P\,2_1$	$P\,2_1$
a/Å	14.5255(5)	18.291(7)	14.6044(7)	14.593(4)
b/Å	7.9210(3)	7.973(3)	7.9746(4)	7.990(2)
c/Å	14.9241(5)	20.566(7)	17.2816(7)	18.702(8)
β/°	98.607(3)	95.049(3)	102.785(3)	109.52(3)
V/Å^3	1697.78(10)	2987.5(19)	1962.79(16)	2055.3(12)
Z	2	2	2	2
Calc. density/g cm^{-3}	1.14	1.154	1.083	1.055
θ range/°	3.0–76.4	2.16–76.42	2.6–76.3	2.5–76.8
μ/mm^{-1}	0.60	0.622	0.556	0.520
Total data collected	3975	6901	4591	4764
Unique data	3831	6715	4417	4624
Observed data [$I > 2\sigma(I)$]	3317	3461	1771	2175
R_{int}	0.012	0.059	0.058	0.044
Final R [$I > 2\sigma(I)$]]	$R1$ = 0.0508	$R1$ = 0.0849	$R1$ = 0.0725	$R1$ = 0.0724
	$wR2$ = 0.1433	$wR2$ = 0.2197	$wR2$ = 0.1369	$wR2$ = 0.1746
R indices (all data)	$R1$ = 0.0649	$R1$ = 0.1941	$R1$ = 0.2437	$R1$ = 0.1898
	$wR2$ = 0.1548	$wR2$ = 0.2747	$wR2$ = 0.1885	$wR2$ = 0.2316
GOF	1.091	1.035	1.013	1.040
Data/restraints/parameters	3831/6/390	6715/15/657	4417/6/426	4624/6/444

crystalline structures. In compounds **1**, **3**, and **4** two oppositely charged surfactants in equimolar aqueous solution assemble to form catanionic co-crystals; the 1 : 1 ratio of oppositely charged moieties insures neutrality. At neutral pH no single crystals of compound **2** were obtained. For crystals to be formed an acidic solution (pH $\sim$ 3) was required. In the crystals of **2**, where the anion–cation ratio is 2 : 1, one out of two anion (CA) molecules must be in its neutral form in order to keep the charge balance. By crystallographic analysis alone it is difficult to determine the protonation state of individual CA molecules, especially if the crystal structure is of relatively poor quality due to the partial disorder (Table 1).

Crystal structures

The crystal packings of compounds **1**–**4** are shown on Fig. 1. All compounds crystallize in the space group $P\,2_1$. The host–guest ratio is 1 : 1 in the case of **1**, **3** and **4** and 2 : 1 in the case of **2**. All crystal structures include one molecule of water except **2** which includes two. In crystal structures **1**, **3** and **4**, the molecules of CA orient themselves to form bilayers with hydrophilic interior and hydrophobic external surfaces. These bilayers are stacked parallel to each other in a *sandwich-type* structure (Fig. 1).[12,13] In-between the bilayers long hydrophobic alkyl chains of *n*-alkylammonia molecules are inserted. The NH_3^+ polar heads of *n*-alkylammonia molecules are immersed into the hydrophilic interior of the CA bilayers to form hydrogen bonds with the OH groups of CA and with the water molecules. In **1** the alkyl chain is straight whereas in **3** and **4** it is bent around the fourth and fifth C atom. The effect of increasing the chain length in these three structures is the elongation of the crystallographic *c* axis caused by widening of the space between bilayers. The α and β classification[4] of stacking modes of CA bilayers is not applicable because the hydrophobic sides of CA molecules are not in direct contact due to the large separation by long alkyl chains of the guest molecules. Characteristic torsion angles of CA of the side-chain of the CA molecules in different compounds are given in Table 2. Torsion angles ψ_1 are very similar and are around $-60°$ in all of the structures. Angles ψ_2 are consistent with two most common values in other CA crystals representing the *trans* and *gauche* conformations of the carboxyl group.[1]

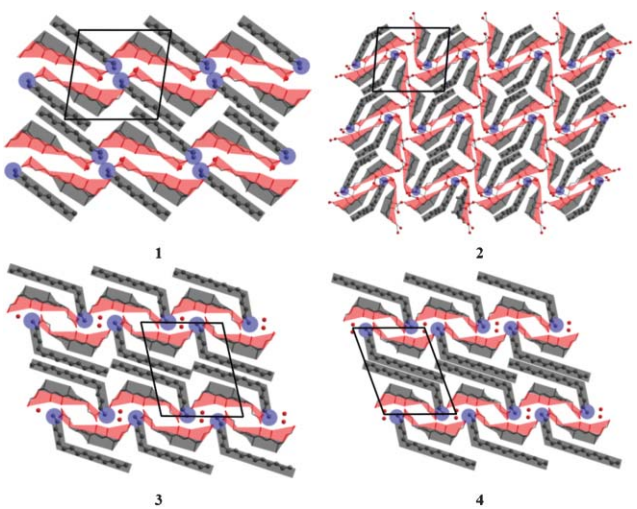

Fig. 1 Comparison of bilayer arrangements of CA molecules in the crystal structures **1**–**4**. The hydrophilic sides of CA molecules are shown in red, the NH_3^+ groups of alkylammonia in blue and hydrophobic regions in grey. The host–guest ratio in structures **1**, **3** and **4** is 1 : 1 and they form a *sandwich-type* structure typical of CA; the host–guest ratio is 2 : 1 in structure **2** and the bilayers are not parallel.

Table 2 Characteristic torsion angles[a] of the CA molecules in compounds **1**–**4**

	ψ_1	ψ_2	ψ_3
1	−61.8(4)	−164.2(3) *trans*	−179.1(4)
2	−61.3(12)	−173.2(8) *trans*	167.0(9)
2′	−60.0(9)	−158.1(6) *trans*	74.7(9)
3	−64.2(9)	64.0(10) *gauche*	142.4(8)
4	−63.6(9)	62.4(10) *gauche*	143.3(8)

[a] ψ_1 = C13–C17–C20–C21, ψ_2 = C17–C20–C22–C23, ψ_3 = C20–C22–C23–C24.

The crystal structure of **2** is different from the structures **1**, **3** and **4** as well as from all of the CA host frameworks known to date.[1] The difference is illustrated in Fig. 1. Two independent molecules in the asymmetric unit (denoted **2** and **2′** in Table 2) give rise to two distinct bilayers. Unlike in the *sandwich-type* structures of **1**, **3** and **4** the bilayers are not parallel but instead intersect at an angle, thus forming a *crossing-bilayers* structure. In this way one-dimensional hydrophobic channels are formed in which guest molecules of $n = 12$ alkylammonia are enclosed. The bilayers are not independent—there are hydrogen bonds between molecules belonging to different bilayers. This is not surprising because the hydrophilic regions must intersect as well. Hydrogen bonding is made possible by an unusual torsion angle ψ_3 for molecule **2′** (Table 2) which differs by more than 90° from its analogue in molecule **2**. A similar torsion angle is found in only one other structure in the CSD (refcode ZUPKIE).[14] Both CA molecules in **2** are of the *trans* type. The NH_3^+ heads of guest molecules are located in this bilayer intersection region, while their alkyl chains occupy the hydrophobic channels.

CSD analysis

In order to compare the CA host frameworks in the title structures with analogous frameworks previously known, the general search was made in the most recent version of the CCDC database (version 5.28 + 2 updates).[15] The search revealed 113 crystal structures of CA (multiple structure determinations are represented with best structure) of which 93 crystallize in space group $P\,2_1$, 13 in $P\,2_12_12_1$, 6 in $P\,1$ and 1 in $P\,6_522$ (Fig. 2).

Until now the largest guest molecule included by CA was mono-substituted benzene with the longest dimension of 9.36 Å (CCDC refcode HURPEP).[16] In the title structures the shortest of the guest molecules is decylammonium in **1** with a length of 11.77 Å, whereas the longest distance of 18.3 Å is found in hexadecylammonium in the crystal structure **4**. This demonstrates the ability of CA to include molecules of almost double the size than previously reported. Interestingly, in spite of a different type of bilayer arrangements in the title structures, in all of them the bilayers are parallel to the crystallographic *b* axis (two-fold screw axis). Moreover, the lengths of the *b* axes are remarkably similar (in the range 7.92–7.97 Å, Table 1). This characteristic dimension is found in the majority of CA crystal structures (Fig. 2).

With respect to the hydrogen bond connectivity that leads to the formation of bilayers, all known CA structures can be classified into 5 types (denoted T1–5) with only a handful of exceptions. By far, the most dominant mode is a side-to-side connection of CA molecules *via* cyclic O–H···O hydrogen bonds of the OH groups attached to C atoms in the sequence C3···C7···C24···C12···C3 (T1 on Fig. 2). This mode uses a 2_1 screw axis for the generation of the bilayer and gives rise to an anti-parallel orientation of the molecules within the bilayer.[17] There can be two or even four molecules in the asymmetric unit. In one case of the T1 type (QOQFAD)[17] the combination of 2_1 axis in the plane of bilayers with another 2_1 perpendicular to it produces a unique case of $P\,2_12_12_1$ space group. This structure possesses special host framework.[17] The structures of type T2 also belong to the $P\,2_1$ space group but CA bilayers are formed parallel to the *a* axis without making use of 2_1 screw axis. This leads to the parallel orientation of CA molecules within the bilayer and all of the structures have two independent molecules to achieve this. In the T2 type the crystallographic 2_1 axis is instead used for the stacking of the bilayers in the crystal packing. The structures of T2 type have the shortest characteristic distance of about 7.5 Å. Six of the T3 structures crystallize in the $P\,1$ space group and one (ZEJFAV)[18] crystallizes in $P\,2_1$. It is interesting to note that all T3 structures utilize a pseudo-2_1 screw axis to generate the bilayers, while the ZEJFAV structure uses the combination of a pseudo-2_1 and a proper crystallographic 2_1 screw axes. In T3 type the CA molecules are connected by rings (similar to the rings in T1) and with open chains of hydrogen bonds. This kind of connectivity stretches the characteristic dimension slightly above 8 Å. In the type T4 structures the guest molecules participate in the hydrogen bonding between the CA molecules. This results in further increase of the characteristic dimension to around 8.5 Å. Finally the T5 type structures all belong to the $P\,2_12_12_1$ space group and do not form bilayers; their structure is previously described as a *crossing type*.[4] Curiously, the only structure of this type falling close to the 8 Å peak is the structure of the guest-free CA (JEYDEW).[19] The other structures are clustered close to 12 Å. This reflects the much stronger dependence of characteristic dimension on the guest size in the case of the *crossing-type* than in the case of the *bilayer-type* structures.

Structures **1**–**4** can not be classified into any of the aforementioned types. This is partially due to the size of the guest molecules, their interference with the CA hydrogen bonds and presence of crystal water. On the other hand, their characteristic distance in the plane of bilayers agrees very well with the majority of the other CA structures. The CSD analysis together with the novel arrangements in the four title compounds suggest that CA molecules are able to preserve this characteristic spacing in a variety of hydrogen bonding schemes. This reveals a striking versatility of CA as a host molecule: the characteristic dimension of about 8 Å seems to be an intrinsic property of CA side-to-side packing in bilayers independent of the hydrogen bond pattern and for the most part independent of the guest molecule. Moreover, the similar length of the *b*-axis in compound **2** suggests that the CA molecular packing is conserved not only with respect to the changes in size of the guest molecules, but that it may even be preserved in completely different bilayer arrangement, namely in the *crossing-bilayers* structure.

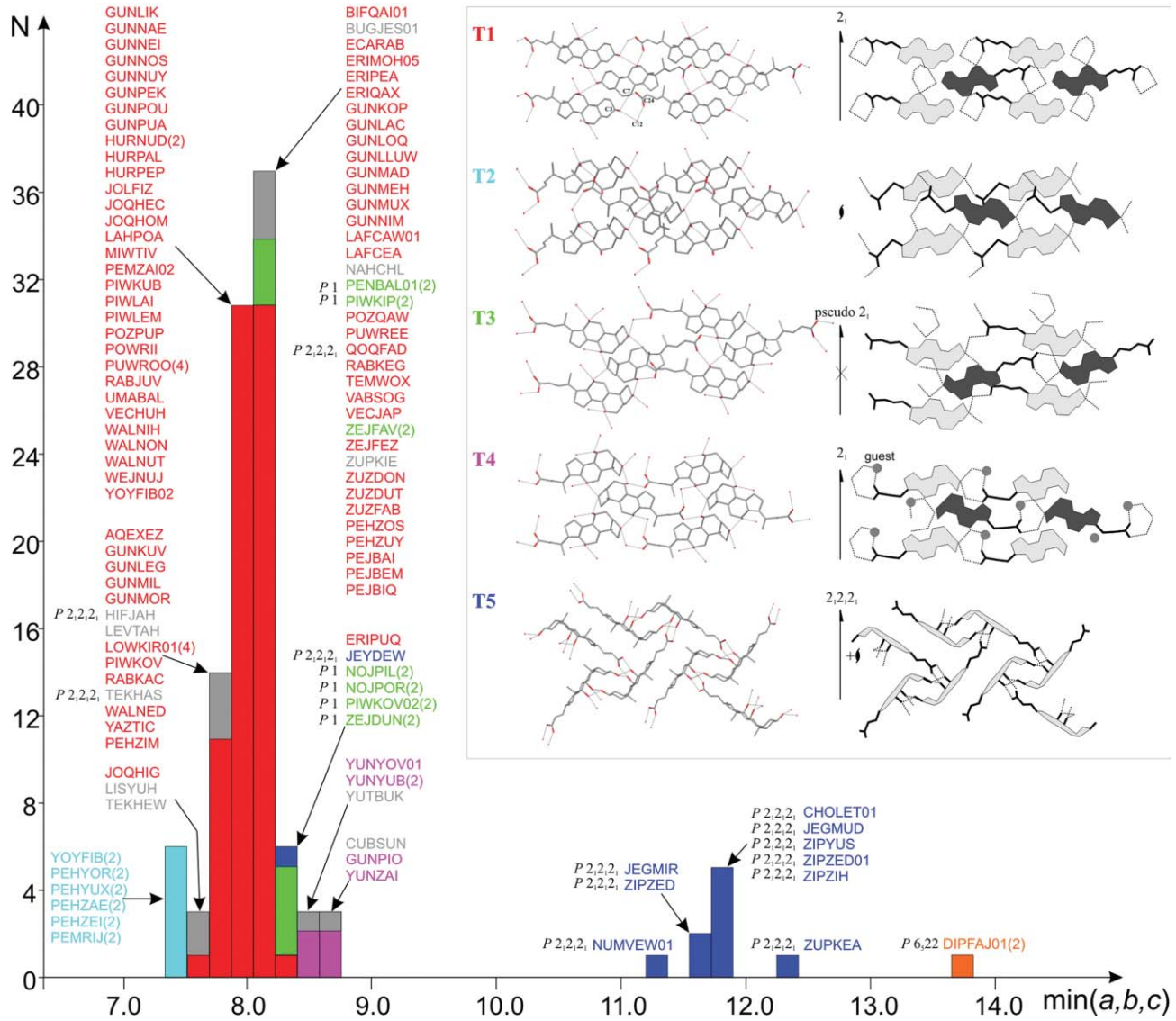

Fig. 2 The distribution of shortest cell axes in the crystal structures of CA in the CSD. The length of the shortest cell axis in all structures corresponds to the characteristic (side-to-side) distance between the CA molecules in the plane of the bilayers. According to the type of hydrogen bonds between the CA molecules within the bilayer, the structures can be classified into five types (T1–T5) with only a few exceptions (shown in grey). Clear clustering of structures around 8 Å and a overwhelming majority of T1 (red) structures is noticeable. The numbers in parentheses after the CCDC refcode are the number of CA molecules in the asymmetric unit. The space group is shown only if different than $P\ 2_1$.

Conclusions

To date the majority of crystal structures of CA are host–guest inclusion compounds with guest molecules smaller in size than CA. In this work it has been shown that CA as a host molecule is able to include guest molecules of considerably larger dimensions than any previously reported. Indeed, the shortest guest molecule, the 10-alkylammonium in compound **1**, is still longer than the longest molecule crystallized with CA so far. In structures **1**, **3** and **4**, the CA host frameworks show a large degree of similarity to the previously described *bilayer-like* structures, the only difference being larger separation of the CA bilayers. On the contrary, the organization of bilayers in the crystal structure of **2** is dissimilar to any previously reported. On the other hand, structures **1**–**4** have a characteristic dimension in the plane of bilayers in common with the majority of the CA host frameworks. This shows the ability of CA as a host molecule not only to include much larger guest molecules but also to keep certain organizational features intact. Demonstrated flexibility and versatility of the CA molecules is of potential interest in designing new host frameworks in the host–guest chemistry of this compound.

Experimental procedures

Materials

The preparation and purification of *n*-alkylammonium chlorides, general formula R-NH_3Cl (with R = C_nH_{2n+1} and n = 10, 12, 14 and 16), *i.e.* decylammonium, dodecylammonium, tetradecylammonium, and hexadecylammonium chloride were described earlier.[20] The purity of surfactants was checked by

elemental analysis and surface tension measurements. Sodium cholate hydrate (3α, 7α, 12α-trihydroxy-5β-cholanic-acid sodium salt; $C_{24}H_{39}O_5Na$), SigmaUltra, min. 99% (CAS 73163-53-8) was used without further purification. To minimize the effect of micro-organisms on the solutions, all solutions were freshly prepared before the experiment. The aqueous solutions of the relevant *n*-alkylammonium chlorides were prepared at concentrations higher than the respective critical micellar concentration, and heated at approximately 70 °C, above the highest Krafft point of *n*-alkylammonium chlorides used. The equimolar and equivolume heated aqueous solutions of the *n*-alkylammonium chlorides and sodium cholate were mixed, and stirred for 15 min. Afterwards, this first set of the samples was stored at room temperature, with pH close to the neutral value (pH ∼ 6). The second set of the compounds was prepared likewise along with variation in preparing the solutions of the *n*-alkylammonium chlorides while acidifying them with HCl, so according to this modification, after mixing the samples were in the acid region (pH ∼ 3) at room temperature. The microcrystalline products of white fibre-like structures precipitated immediately after cooling the system to room temperature, and were collected after 24 h by filtration. Catanionic salts were washed with cold water to remove co-precipitated NaCl salt and dried in vacuum at room temperature.

The identification of investigated compounds was performed by elemental analysis and expressed as weight percent (wt%) for different *n*-alkylammonium cholates.‡ The single colourless crystals were prepared by dissolving the appropriate *n*-alkylammonium cholates in water by heating followed by slow cooling to ∼4 °C, allowing slow crystallization for some weeks. Attempts to prepare single crystals with $n = 10$, 14 and 16 from acidic medium or single crystals with $n = 12$ from neutral medium using different solvents (water, methanol, ethanol, acetone, or acetonitrile) remained unsuccessful.

Crystal structure determination

Crystals of *n*-alkylammonium cholates **1**–**4** suitable for single-crystal X-ray analysis were grown from water solutions by slow evaporation at room temperature. The results of the X-ray structure determinations are summarized in Table 1. All measurements were performed on an Enraf Nonius CAD4 X-ray diffractometer with graphite-monochromated Cu Kα radiation ($\lambda = 1.5412$ Å) at room temperature. Data reduction was performed using XCAD[21] and WINGX[22] program package. Structures were solved by direct methods using the SHELXS package and refined by the package SHELXL97.[23] Molecular geometry calculations were done by PLATON98.[24] Plots of the molecules and crystal packings were prepared by MERCURY.[25] Atomic scattering factors were those included in SHELXL97. The hydrogen atom coordinates were calculated geometrically and refined using the SHELX97 riding model. Structure **2** contains partial disorder in the central part of the alkyl chain. The attempt to refine the structure with partial occupancy in the disordered part has not improved the model.

‡ Elemental analysis was performed on a Perkin-Elmer Analyser PE 2400 Series 2. Elemental analysis % (calcd): compound number **1** C 69.91 (69.94); H 11.20 (11.22); N 2.39 (2.40). Compound **2**: C 69.36 (69.39); H 10.73 (10.77); N 1.34 (1.35). Compound **3**: C 71.36 (71.32); H 11.45 (11.49); N 2.20 (2.19). Compound **4**: C 71.88 (71.91); H 11.60 (11.62); N 2.09 (2.10).

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