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Tracking the Structural Changes in a Series of Cholesterol Solvates

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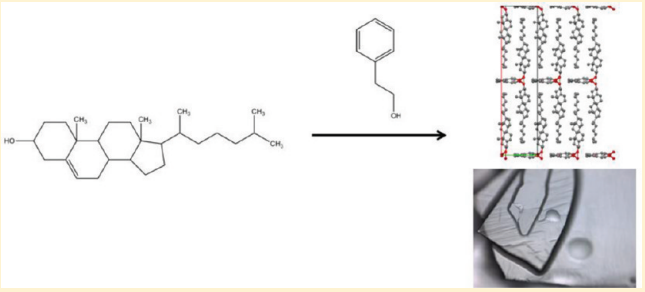
Supporting Information

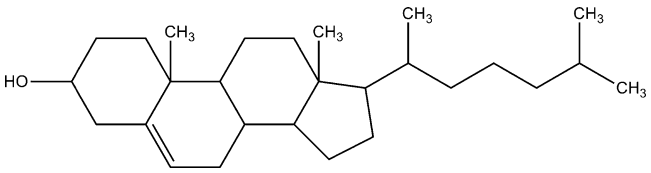
ABSTRACT: This article describes the isolation of seven new solvates of cholesterol using solvents of varying carbon chain length and overall size from propanol through to phenylethanol. The structural similarities that exist between these novel solid forms and also those cocrystals and solvates already observed are discussed.



INTRODUCTION

Cholesterol is one of the major steroids within mammalian cells and forms an integral part of the cell membranes. The molecular structure of cholesterol is ideally suited to these surroundings, as it consists of a hydroxyl group, that interacts with the polar headgroup of the phospholipid, and a tetracyclic steroid body bonded to an eight carbon alkyl chain that interacts with the hydrophobic fatty acid chains of the phospholipid bilayer of the cell membrane (Scheme 1).

Scheme 1. Molecular Structure of Cholesterol



Once orientated within the membrane, one of the roles of cholesterol is to alter the physicochemical properties of the cell membrane by subtly altering the interactions of the surrounding fatty acid chains. It has been shown through DSC and X-ray diffraction measurements that the addition of cholesterol to the membrane structure causes changes in the physical behavior of the phospholipid layers with respect to temperature. Pure lipid layers possess a gel/liquid-crystal transition at elevated temperatures whereby the chains "melt" and become less ordered. The addition of >40 mol % cholesterol causes the disappearance of this phase transition, resulting in an "intermediary" structure that possesses a bilayer thickness between that of the gel and liquid-crystal states.[1,2] Furthermore, a study by Lund-Katz et al. demonstrated, using surface pressure measurements, that the addition of cholesterol to a membrane causes it to become more condensed and hence harder which has an effect on the functioning of transmembrane proteins.[3]

Despite the functional use of cholesterol in the body, it has received notoriety in the press for being a contributing factor in causing heart disease if present in high levels in the bloodstream. Cholesterol is also a major component of gallstones as the anhydrous form as well as in the form of the monohydrate, and so there have been a number of studies relating to the growth of this phase onto calcium carbonate, the other main contributor to gallstones.[4−7] Further theoretical studies of cholesterol have been made investigating formation of nanoparticles and also the applicability of the GROMOS force field, designed primarily for biological systems, to model the crystalline structures of smaller membrane sterols.[8,9] At the heart of all these studies, is the investigation of how cholesterol molecules interact with one another and surrounding materials. Further investigation of the solid-state forms of cholesterol and how the molecules interact with one another in these systems can contribute to our understanding of cholesterol in these other environments.

In the solid-state, cholesterol crystallizes in a number of different forms in addition to the two anhydrous phases.[10−14] In the Cambridge Structural Database (CSD),[15,16] there is a monohydrate (CSD refcode: CHOLES20),[17] a hemimethanol solvate (CHOLME02),[18] two hemiethanol solvates (CHOLEU01, CHOLEU10),[19] an isobutylphosphocholine/*tert*-butanol cocrystal (MEQKAU),[20] and finally a cocrystal with 4-iodophenol (WOMHAI).[21] The structures of all these compounds can be broadly categorized into two distinct molecular architectures depending on the size of the additional solvent or coformer. The majority of these compounds crystallize in a bilayer structure with alternating hydrophobic and hydrophilic regions. The molecules of cholesterol are positioned end-to-end such that the isopropyl groups at the end of the alkyl chains interact with one another. The second architecture is more compact,

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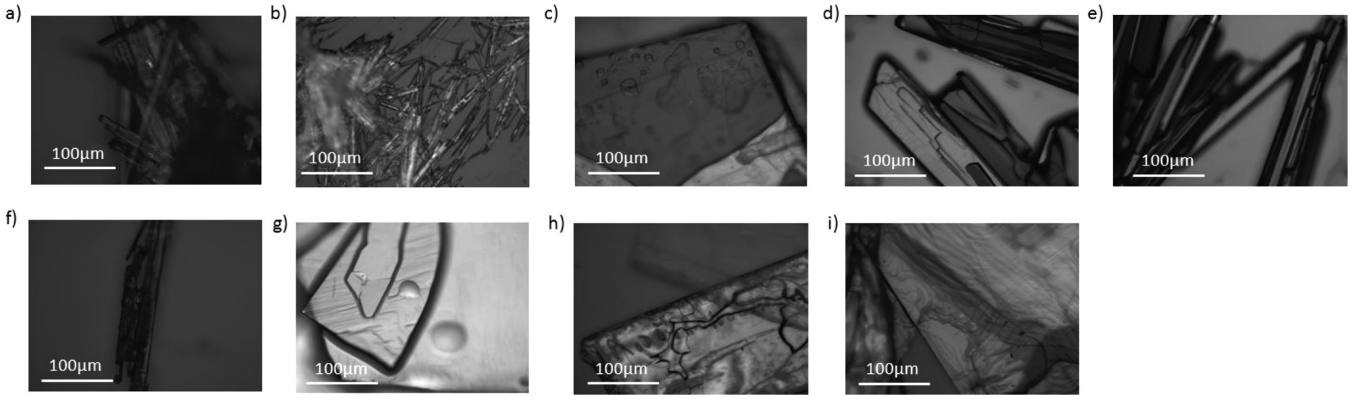

Figure 1. Crystal morphologies of the seven new cocrystals compared to the morphology of the methanol and ethanol solvates. Solvates of cholesterol with (a) propanol, (b) butanol, (c) pentanol, (d) hexanol, (e) phenol, (f) benzyl alcohol·water, (g) phenylethanol, (h) methanol, or (i) ethanol.

where the cholesterol molecules from neighboring chains are intercalated with one another in a head-to-tail arrangement. In this study we have attempted to systematically investigate the effect of the size and rigidity of the solvent on the final crystal structure so that a comparison can be made with those structures already found in the CSD.

EXPERIMENTAL SECTION

Sample Preparation. Cholesterol and solvents were purchased from Sigma Aldrich and used as received.

Formation of Compounds 1−7. These compounds were obtained from a 1:1 mixture of diethylether and the target solvent (propanol, butanol, *etc.*). Cholesterol (103 mg, 0.266 mmol for **1**; 100 mg, 0.259 mmol for **2**; 98 mg for **3**; 106 mg, 0.275 mmol for **4**; 101 mg, 0.261 mmol for **5**; 151 mg, 0.391 mmol for **6**; and 256 mg, 0.663 mmol for **7**) was dissolved in 3 cm^3 of the 1:1 mixture of diethyl ether and target solvent. The solvent was left to evaporate, allowing crystals to form. In the case of propanol, after a few days, some small crystals appeared on the side of the vial which were pushed into the mother liquor in order to grow a suitable crystal for single crystal X-ray diffraction. For compound **5**, an excess of phenol was added (44 mg, 0.468 mmol) to 3 cm^3 of diethyl ether. The cocrystals from pentanol and hexanol needed further addition of cholesterol in order to form the precipitate on evaporation of the diethyl ether.

Crystal Morphology. Figure 1 shows the morphology of the crystals from each of the crystallizations. The crystals from propanol, butanol, and benzyl alcohol show a needle-like morphology whereas the crystals from the other solvents possessed a lathe or plate morphology—these are compared with the crystals of the methanol and ethanol solvates.

Differential Scanning Calorimetry. DSC plots were obtained using dynamic DSC (DSC 822e, Mettler Toledo, U.K.). Samples were prepared by carefully weighing between 2.31 and 6.03 mg of each sample into a 40 μL aluminum pan, which was then hermetically sealed with a pinhole in the lid. An empty pin-holed 40 μL aluminum pan was used as a reference. Both pans were subjected to a nitrogen atmosphere. The pans were then heated at a rate of 10 °C/min from 293 to 463 K (well above the melting point of cholesterol). The temperature and heat flow of the DSC instrument were calibrated with indium and zinc. The results were analyzed using Mettler STAR software. Figure ES1 of the Supporting Information shows the DSC and TGA traces for each of the samples.

Thermal Gravimetric Analysis. TGA measurements were performed on a Mettler Toledo TGA 751e. Each sample (7.86−22.48 mg) was placed in a ceramic pan. An empty ceramic pan was used as a reference, and both pans were subjected to a nitrogen atmosphere. The pans were then heated from 303 to 463 K at 10°/min. The results were analyzed using Mettler STAR software.

Phase Identification—X-ray Powder Diffraction. A small quantity (1−50 mg) of each recrystallized sample was analyzed using transmission foil XRPD data collected on a Bruker AXS D8-Advance transmission diffractometer equipped with a θ/θ geometry, primary monochromated radiation (Cu Kα_1, λ = 1.54056 Å), a Bruker Vantec 1D position sensitive detector (PSD), and an automated multiposition $x-y$ sample stage.[22] Samples were mounted on a 28 position sample plate supported on a polyimide (Kapton, 7.5 μm thickness) film. Data were collected from each sample in the range 4−35° 2θ with a 0.015° 2θ step size and a 1 s·step^{-1} count time. Figure ES2 of the Supporting Information contains the X-ray powder diffraction patterns of all the bulk samples and confirms the identity of the cocrystals. Slight differences in the diffraction patterns can be attributed to the data collection temperature (293 K cf. 123 K for the single crystal experiments) and preferred orientation.

Crystal Structure Determination. X-ray diffraction intensities were collected with Mo Kα radiation on a Bruker KAPPA Apex II CCD diffractometer equipped with an Oxford Cryosystems Cryostream-*Plus* variable-temperature device operating at 123 K.[23] Absorption corrections were carried out using the multiscan procedure *SADABS* (Sheldrick, 2004, based on the procedure described by Blessing, 1995).[24,25] The structures were solved by direct methods and refined by full-matrix least-squares against F^2 using all data (SHELX).[26] Due to the wavelength of the X-ray source (0.71073 Å), the absolute configuration of the cholesterol molecule was not determined but the absolute structure was chosen to reflect the chirality observed for the vast majority of structures in the literature in order to make a direct comparison of the crystal structures. All hydrogen atoms attached to carbon atoms were geometrically placed, and those participating in hydrogen bonding, i.e. hydroxyl hydrogens, were found in the difference map. All non-H atoms were modeled with anisotropic displacement parameters. Where disorder was present, the bond lengths and angles were restrained to values found in the CSD.

Additional programs used included Materials Mercury 2.4,[27] PLATON as incorporated in WINGX.[28,29] Mercury, ChemBioDraw 12.0, and GIMP 2.6[30] were used in the production of the figures.

RESULTS AND DISCUSSION

Cholesterol was crystallized with seven alcoholic solvents of increasing size: propanol, butanol, pentanol, hexanol, phenol, benzyl alcohol, and phenylethanol. The descriptions of the crystal structures follow, and the crystallographic parameters for the compounds under study and also those of previous studies can be found in Tables 1 and 2.

Thermal Analysis. Figure ES1 of the Supporting Information shows the thermal analysis plots for each of the cocrystals. It can be observed that each of the cocrystals **1**−**4** shows a desolvation event followed by the melting of the cholesterol at ∼423 K. The desolvations of the propanol and butanol solvates

Table 1. Crystal Structure Refinement Details for Compounds 1−7

	1	2	3	4	5	6	7
chemical formula	$C_{57}H_{100}O_3$	$C_{58}H_{102}O_3$	$C_{59}H_{104}O_3$	$C_{60}H_{106}O_3$	$C_{33}H_{52}O_2$	$C_{61}H_{102}O_4$	$C_{62}H_{102}O_3$
M_r	833.37	847.40	861.62	875.45	480.75	899.43	895.44
crystal system, space group	monoclinic, $P2_1$	monoclinic, $P2_1$	monoclinic, $C2$	monoclinic, $C2$	monoclinic, $P2_1$	monoclinic, $P2_1$	monoclinic, $C2$
a, b, c (Å)	15.0249(12)	15.0634(9)	42.9792(15)	42.982(2)	11.5935(9)	15.699(2)	42.676(4)
	6.1107(5)	6.0931(4)	10.3021 (4)	10.3695(7)	6.1697(5)	7.5133(9)	10.2044(11)
	28.663(2)	29.1765(18)	6.2807 (2)	6.2684(4)	21.1002(16)	23.492(3)	6.3396(6)
β (deg)	97.386(4)	96.583(3)	96.225(2)	96.166(3)	105.446(4)	94.643(9)	97.501(6)
V (Å^3)	2609.8(4)	2660.2(3)	2764.55(17)	2777.7(3)	1454.8(2)	2761.8(6)	2737.1(5)
Z	2	2	2	2	2	2	2
D_x (mg m^{-3})	1.061	1.058	1.108	1.047	1.098	1.082	1.086
temp (K)	123(2)	123(2)	123(2)	123(2)	123(2)	123(2)	123(2)
no. of reflns for cell	7248	9921	9902	6988	5735	4652	6021
$2\theta_{max}$ (deg)	48.90	53.06	50.52	49.84	52.64	48.70	52.64
μ (mm^{-1})	0.062	0.062	0.065	0.061	0.066	0.065	0.064
reflns collected	20182	26928	30565	19989	12342	15071	11188
unique [R_{int}]	8364[0.0230]	10339 [0.0248]	5162[0.0364]	4924[0.0600]	5789[0.0214]	8204 [0.0319]	5354[0.0254]
no. $I > 2u(I)$	6666	7994	4468	3915	5222	6354	4755
T_{min}, T_{max}	0.69, 0.75	0.64, 0.75	0.66, 0.75	0.63, 0.75	0.65, 0.75	0.65, 0.75	0.53, 0.75
params	603	583	310	351	329	616	327
R1 [$F > 4u(F)$]	0.0428	0.0642	0.0676	0.0774	0.0466	0.0391	0.0475
wR2 (F2, all data)	0.039	0.1829	0.2146	0.2357	0.1290	0.0886	0.1335
S	0.998	1.044	1.061	1.092	1.027	1.016	1.045
ρ_{max} (e Å^{-3})	0.189	0.672	0.833	0.623	0.585	0.146	0.281
ρ_{min} (e Å^{-3})	−0.292	−0.752	−0.278	−0.286	−0.230	−0.153	−0.205

Table 2. Unit Cell Parameters for the Crystal Structures of Cholesterol in the Cambridge Structural Database[15,16]

	cholesterol form I	cholesterol form II	cholesterol monohydrate form III	cholesterol hemimethanol solvate	cholesterol hemiethanol solvate form I	cholesterol hemiethanol solvate form II	cholesterol isobutyl-phosphocholine isobutanol solvate	cholesterol 4-iodophenol cocrystal
CSD Refcode	CHOEST20	CHOEST21	CHOLES20	CHOLME02	CHOLEU01	CHOLEU10	MEQKAU	WOMHAI
crystal system, space group	triclinic, $P1$	triclinic, $P1$	triclinic, $P1$	triclinic, $P1$	triclinic, $P1$	monoclinic, $P2_1$	monoclinic, $C2$	monoclinic, $P2_1$
a, b, c (Å)	14.172(7)	27.565(10)	12.390(30)	12.2735(17)	12.787(2)	12.775(2)	16.994(10)	6.302(<1)
	34.209(18)	38.624(16)	12.410(30)	34.237(7)	35.310(11)	68.668(15)	11.314(7)	10.295(<1)
	10.481(5)	10.748(4)	34.360(60)	6.2739(8)	12.225(1)	12.213(2)	28.164(15)	41.964(3)
α, β, γ (deg)	94.64(4)	93.49(3)	91.90(10)	90.224(14)	97.80(2)			
	90.67(4)	90.90(3)	98.10(10)	93.705(10)	100.40(2)	100.43(1)	104.07(3)	91.03(<1)
	96.32(4)	117.15(3)	100.80(10)	91.576(14)	99.06(2)			
V (Å^3)	5033(4)	10151(7)	5128	2629.8(7)	5284(2)	105367(3)	5252	2722.3(3)
Z	8	16	8	4	8	16	2	2

are quite clean events; the desolvations of the pentanol and hexanol show other events surrounding the desolvation which may be due to an increase in disorder of the solvent molecule in the crystal lattice before it leaves the lattice. The TGA plots show that compounds **1**, **2**, and **4** (Figures ES1a, ES1b, and ES1d) all lose a mass that is equivalent to the stoichiometry that is found in the crystal structure analysis (7, 12, and 9%). The hexanol solvate shows a change in the gradient of the TGA which signifies a change from the loss of residual solvent to the loss of solvent from the crystalline lattice. The second loss is equivalent to the quantity of solvent in the crystal structure.

The proportion of solvent that is lost from compound **3** (Figures ES1c) is a little lower than would be expected (7% cf. 10%), but refining the crystal structure with a lower occupancy of solvent resulted in extra electron density surrounding the solvent molecules; that is, the stoichiometry is correct at 2:1 cholesterol/pentanol. A possible explanation could be that during the prepartion of the sample and subsequent equilibration of the pan inside the TGA instrument, some solvent was lost from the crytal structure; for crystal structure analysis, the crystals were placed under oil before putting them onto the instrument under a nitrogen cold stream which would preserve the solvent in the crystal.

Compounds **5** and **7** (Figures ES1e and ES1g) show an endothermic event at ∼373 K with a constant loss of mass throughout the experiment. There was no separate endothermic event that could be assigned to the melting of cholesterol. Intriguingly, hot-stage microscopy showed that the samples remained beyond 373 K and melted at the melting point of cholesterol with indications that solvent was being lost on heating. Figures ES1e and ES1g show that, at the melting point of cholesterol, compound **5** has lost 18% of its mass and compound **7** has lost 14% of its mass, which are consistent with the weights of solvent in the crystal structures identified by

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single crystal diffraction (20% and 14%, respectively). Beyond the melting point, the mass reduced in each of these solids indicated the sublimation of cholesterol.

Compound **6** (Figure ES1f) showed two endothermic peaks at ~330 K which could be attributed to the loss of benzyl alcohol and water. Increasing the end point of the experiment to 230 K showed no further events that could be attributed to the melting of another compound. The mass lost at the melting point of cholesterol was 21%, which is greater than the mass of benzyl alcohol and water, which indicates that during heating it is likely that some of the cholesterol was lost through sublimation.

Crystal Structure Analysis. *2:1 Cholesterol/Propanol Solvate (1).* In 2008, Uskoković investigated the effects of varying the crystallization conditions on the morphology of crystals of cholesterol.[12] As part of this study, Uskoković crystallized cholesterol from a mixture of propanol and water and analyzed these crystals using both DSC and X-ray powder diffraction. Despite the use of propanol in the reaction mixture, the author did not observe any changes in the diffraction pattern or the thermal analysis trace that would suggest the presence of another phase. Nevertheless, the formation of a potential propanol solvate was investigated due to the previous successful isolation of methanol and ethanol solvates by various groups.[18,19]

A new compound was observed and found to crystallize in space group $P2_1$ with two molecules of cholesterol and one propanol molecule in the asymmetric unit (Figure 2). All three

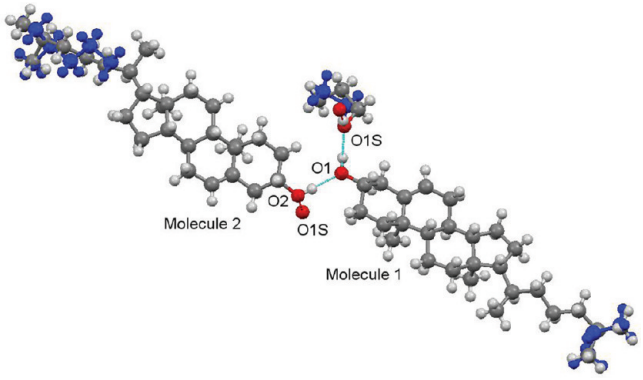

Figure 2. Asymmetric unit of compound **1**. The main component of the disorder is colored by atom and the minor component colored blue. The color coding for this and subsequent figures is as follows: carbon, gray; oxygen, red; hydrogen, white.

molecules show some degree of disorder, with molecule **2** showing the greatest proportion. Molecule **1** has small differences in orientation of the isopropyl end group, whereas molecule **2** shows disorder along the whole length of the alkyl chain. The propanol molecule is disordered over two positions with the oxygen and primary carbon residing in the same position. These latter two places of disorder reside close to one another in the crystal structure, and so it is probable that the disorder in each region is related to one another. However, assigning the same occupancies to the cholesterol and propanol disorder, one observed a slight increase in the *R*-factor indicating a poorer fit to the data. The disorder was better modeled as 65:35 and 54:46 for the cholesterol and propanol, respectively.

The three molecules interact through hydrogen bonding between the alcohol moieties on each of the molecules to form chains along the *b*-direction (O1···O1S, 2.650(3) Å; O2···O1, 2.676(2) Å; O1S···O2, 2.683(3) Å). Neighboring chains are packed through the application of the 2_1-screw axis so that the cholesterol molecules interact in a head-to-tail manner. Due to the paucity of hydrogen-bonding groups in the alkyl chain, the cholesterol molecules only interact through van der Waals contacts, and so one observes different molecular confomations of the two independent molecules.

This structure is not extensively layered into hydrogen bonded and hydrophobic regions, in contrast to the cases of the methanol and ethanol solvates. Instead, the propanol molecules are in discrete locations in the crystal structure (Figure 3).

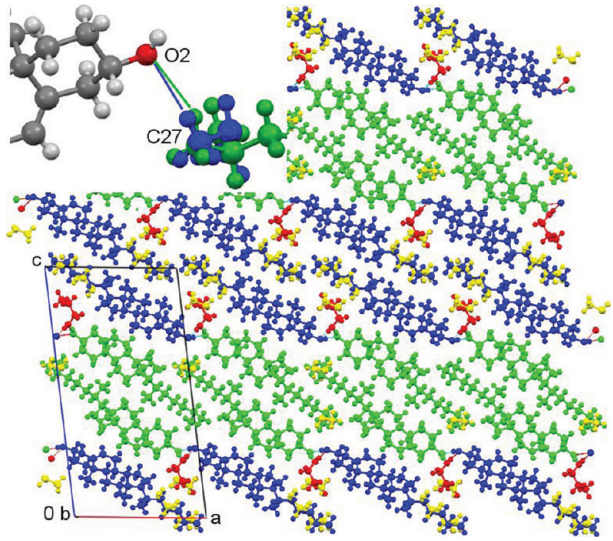

Figure 3. Packing diagram of compound **1** viewed down the *b*-direction. The molecules are colored by symmetry equivalent. Molecule **1** is green; molecule **2** is blue; propanol is red; and the disordered components are yellow. The inset shows the close interaction between C27 of molecule **1** and O2, where the position of C27 in both components does not alter significantly.

While molecule **2** and the solvent show a great deal of disorder, molecule **1** shows relatively little disorder, which could be attributed to a weak CH···O interaction between the hydrogen attached to C27 and the hydroxyl group (O2) of a neighboring molecule (Figure 3, inset).

2:1 Cholesterol/Butanol Solvate (2). Compound **2** is isostructural with compound **1** (see Table 1), but only one cholesterol molecule (molecule **2**) shows any disorder in the alkyl chain while the solvent remains disordered. As one might expect, the hydrogen bonding interactions are a similar length to those observed in compound **1** (O1···O1S, 2.670(4) Å; O2···O1, 2.704(3) Å; O1S···O2, 2.703(4) Å). The weak hydrogen bond that was observed in compound **1** is retained in this structure at a slightly shorter distance (H27A···O2S, 2.63 Å; cf. 2.73 Å).

2:1 Cholesterol/Pentanol Solvate (3). Extending the alkyl chain length of the solvent molecule further to pentanol produces a new crystalline structure that crystallizes in *C*2 (compound **3**). There is one molecule of cholesterol and half a molecule of pentanol that lies perpendicular to the cholesterol molecule in the asymmetric unit; the solvent has been modeled

as disordered over two sites with occupancy 0.35:0.15, but in reality the solvent is likely to be disordered over many other sites with occupancies too low for the refinement of the atomic positions. The cholesterol hydrogen bonds to a symmetry equivalent molecule as well as the disordered solvent (O1···O1 2.623(4) Å; O1···O1S 2.644(9) and 2.712(9) Å; O1···O1SA 2.717(6) Å). There are two values for the distance between O1 and O1S due to the two positions the oxygen atom of the solvent atom adopts.

As far as the packing is concerned, the cholesterol molecules are arranged in a head-to-tail manner but there is a clear distinction in the packing of the molecules in this structure compared to the packing in compounds **1** and **2** (Figure 4, cf. Figure 3).

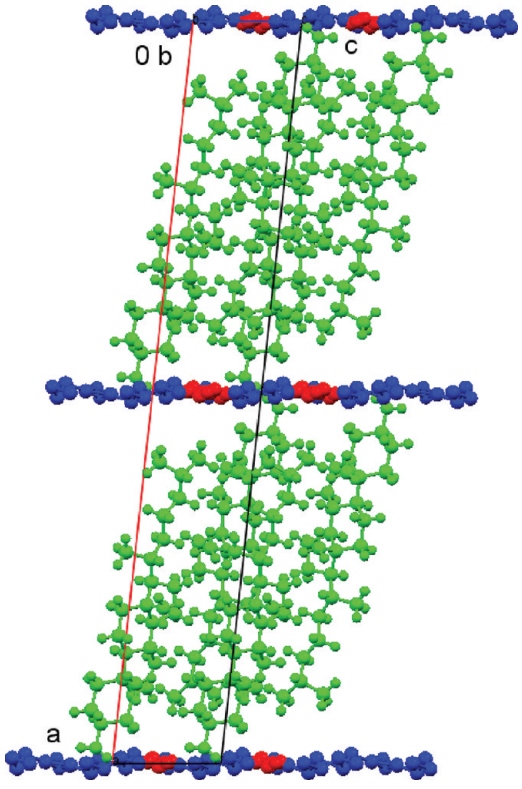

Figure 4. Packing diagram of compound **3** viewed down the *b*-direction. The disordered solvent molecules are colored blue and red, depicting the major and minor components, respectively.

The solvent molecules in **1** and **2** were observed to be in discrete locations with some interaction between the alkyl chain of the solvent and the steroid moeity of cholesterol. This occurs due to the pseudoparallel orientation of the solvent with respect to the cholesterol molecule. In compound **3**, the perpendicular orientation of the solvent allows it to form a layer at $a = 0, {}^{1}/_{2}$, *etc.* with the cholesterol molecules sandwiched between these. This change to a more layered structure is most likely due to the increase in disorder of the solvent compared to the propanol and butanol solvates. The solvent molecules are disordered such that the oxygen atoms are in a position to interact with the hydroxyl groups of cholesterol molecules that are equivalent by translation along the *b*-direction (Figure 5).

*2:1 Cholesterol/Hexanol Solvate (**4**).* The solvate with hexanol, compound **4**, is isostructural with compound **3**. The cholesterol molecule in this structure shows slight disorder around the isopropyl group, which equates to a 70:30 ratio of components. This solvate shows the same layered structure as

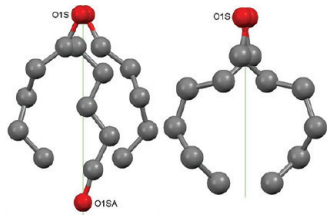

Figure 5. Disordered solvent molecules in compound **3** (left) and compound **4** (right). The solvent is disordered over the *bc*-plane, as depicted in Figure 4. The solvent molecules are disordered over the 2-fold rotation (green line). The second molecule of pentanol (O1SA) will have another site of occupation that can be generated *via* the 2-fold rotation but has been omitted for clarity.

compound **3**, with the solvent molecules arranged perpendicular to the direction of the cholesterol molecules. Table 1 shows that the unit cell parameters for compound **4** are very similar in the *a*-direction, as the contributions to this direction from the solvent molecules in both structures are disordered in the *bc*-plane. The *b*- and *c*-directions show some differences in lengths that can be attributed to the change in solvent length and orientation. The cholesterol molecule hydrogen bonds to a symmetry equivalent molecule and also to the two components of the disordered solvent molecule (O1···O1 2.608(5) Å; O1···O1S 2.70(3) Å; O1···O1S 2.63(3) Å) (Figure 5).

*1:1 Cholesterol/Phenol Cocrystal (**5**).* The attempted crystallization of phenol with cholesterol was to investigate whether the iodine substituent on iodophenol was an important factor in the crystallization of the WOMHAI in the intercalated structure rather than the bilayer structure observed for the other solvates.

Compound **5** crystallizes with one molecule of cholesterol and one molecule of phenol in space group $P2_1$ and with cell parameters that are different from those of the previous compounds (Table 1). However, if one looks closely at the unit cell parameters, one can actually convert the *P*-centered cell into a distorted *C*-centered cell through the application of the following matrix transformation to the *P*-centered cell (−1 0 −2 1 0 0 0 −1 0). The resulting unit cell lengths from this transformation are similar to those found for compounds **3** and **4**, with the angles necessarily different from 90° (a = 43.979 Å, b = 6.305 Å, c = 10.305 Å, α = 89.853°, β = 103.371°, γ = 92.248°). Therefore, there is a similarity between the structures despite the large differences in the cell parameters.

The molecules interact through hydrogen bonds between the hydroxyl groups to form chains along the *b*-axis (O1···O1S 2.754(2) Å; O1S···O1 2.6193(19) Å). The neighboring chains are intercalated so that the cholesterol molecules are arranged head-to-tail with only short contacts between hydrogen atoms on each of the molecules on different chains (Figure 6). The depth of intercalation is not as great as that found in compound **3**; therefore, there is not a definitive layer of solvent molecules (cf. Figure 4).

*2:1:1 Cholesterol/Benzyl Alcohol/Water Solvate (**6**).* While the products of the previous crystallizations could be isolated over the course of a day, the product from the crystallization of cholesterol from benzyl alcohol took over a week to appear. X-ray analysis of the crystals showed that water had been incorporated into the structure of cholesterol along with a targeted benzyl alcohol molecule. Compound **6** crystallizes with two molecules of cholesterol, one molecule of benzyl alcohol, and one of water in space group $P2_1$. The *a*- and *b*-parameters for this compound

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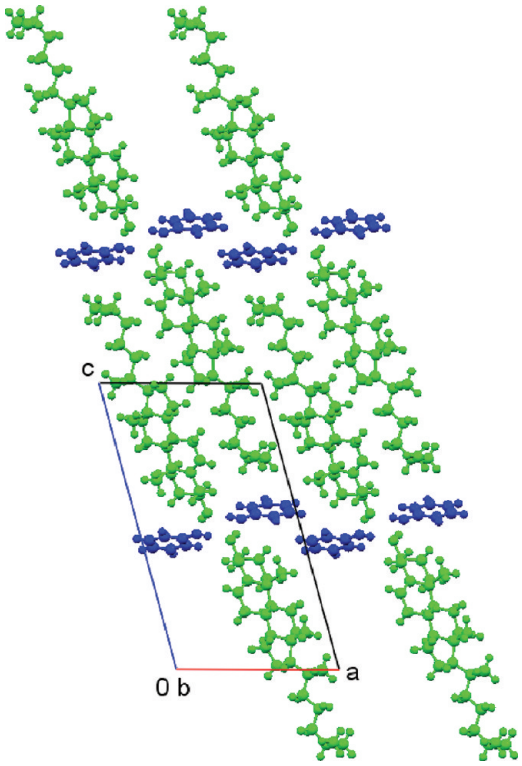

Figure 6. Packing diagram of compound **5** viewed along the *b*-direction. The phenol molecules are colored blue. The layers are not as well differentiated as those found in compounds **3** and **4**.

are similar to those of the first two compounds; however, the *c*-parameter is significantly shorter. Despite the similarity of the cell parameters, the packing of the molecules is significantly different, which is due to the incorporation of the water molecule. The water allows for further hydrogen bonding, which enables the dimerization of the formally isolated hydrogen bonded chains. The cholesterol molecules in the chain interact favorably so that the flat sides of the ring system (α-side) are adjacent to one another, as opposed to the case of compound **1**, where the ring systems of neighboring molecules are perpendicular to one another (Figure 7).[8] Chains of molecules are formed along the *b*-direction using all three components (O1S···O1 2.663(2) Å; O1···O2 2.771(2) Å; O2···O2S 2.670(2) Å; O2S···O1S 2.770(3) Å) with an additional hydrogen bond between the water molecule and a benzyl alcohol of another chain (O2S···O2 2.760(2) Å) (Figure 8).

*2:1 Cholesterol/Phenylethanol Solvate (**7**).* The last solvate studied in this series was that of cholesterol with phenylethanol. These molecules crystallize together in a 2:1 ratio in monoclinic space group *C*2. The cholesterol molecule is fully ordered apart from the hydroxyl hydrogen atom, which is disordered over two sites due to the disorder in the solvent. The phenylethanol resides on the 2-fold axis and is found to be disordered over this site (Figure 9). The hydrogen bonding in this crystal is very simple, with chains of molecules running along the *c*-direction. Due to the disorder of the solvent oxygen atom, there are two distances quoted for the interaction between cholesterol and the solvent: one which is slightly below average for this type of

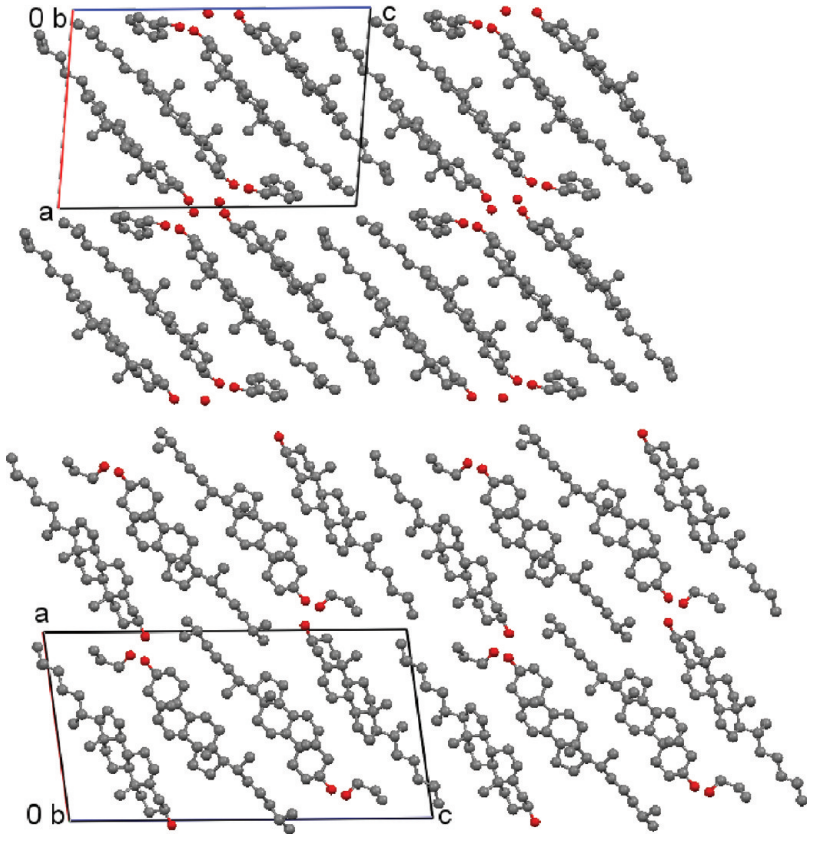

Figure 7. Comparison of the packing diagrams of compounds **1** (lower) and **6** (upper) viewed along the *b*-direction. The cholesterol molecules in compound **6** interact via their α-sides, which has previously been calculated as being a favorable motif.[8]

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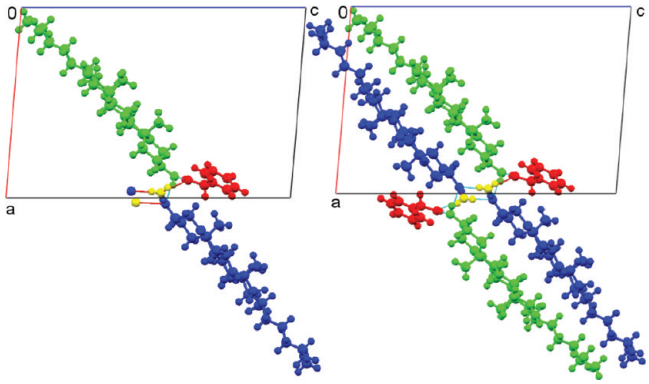

Figure 8. Single hydrogen bonded chain (left) equivalent to other compounds. The water molecules allow further hydrogen bonding to neighboring chains to form "dimers" (right).

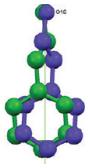

Figure 9. Disordered phenylethanol molecule. The different components that are related by the 2-fold rotation (green) are highlighted in blue and green.

interaction and one which is slightly above (O1···O1S 2.494(4) Å; O1···O1S 2.824(3) Å; O1···O1 2.604(3) Å).

The crystal structure is isostructural to both compounds **3** and **4**. The cholesterol molecules are intercalated with those from neighboring chains so that a layered structure is formed. The solvent molecule is orientated so that the phenyl group lies in the *bc*-plane at $a = 0, {}^{1}/_{2}$, *etc.* The solvent molecules in this solvate are all orientated in the same way, mimicking the behavior in compound **4**, as opposed to compound **3**, where the disordered components are orientated in opposite directions.

Comparison with Known Structures. There are eight structures present in the database for which the structure has been fully characterized, and their cell parameters can be found in Table 2. While there are subtle differences in the structures leading to differences in cell parameters, the overall molecular architectures show two distinct variations. The bilayer architecture is exhibited by both of the anhydrous forms, the monohydrate, and the methanol and ethanol solvates. A good example of this is the monohydrate (Figure 10, left). All of these structures except for the high temperature polymorph of the hemiethanol solvate crystallize in *P*1 with multiple molecules in the unit cell, where, in many of the cases, different molecules are related by pseudosymmetry. In the monohydrate, there are four different molecular conformations (despite being $Z = 8$) (Figure 10, right), which indicates the pseudosymmetry in the crystal structure as discussed by Hsu et al. in their paper.[14]

The second type of architecture is exhibited by the two cocrystals MEQKAU and WOMHAI. In these structures, the cholesterol molecules interact in a head-to-tail manner with other chains that are related by a 2_1-screw axis (Figure 11). This type of architecture seems to reduce the disorder and thermal motion exhibited by the cholesterol molecules as the tail groups are in close proximity to the strong hydrogen bonds. The

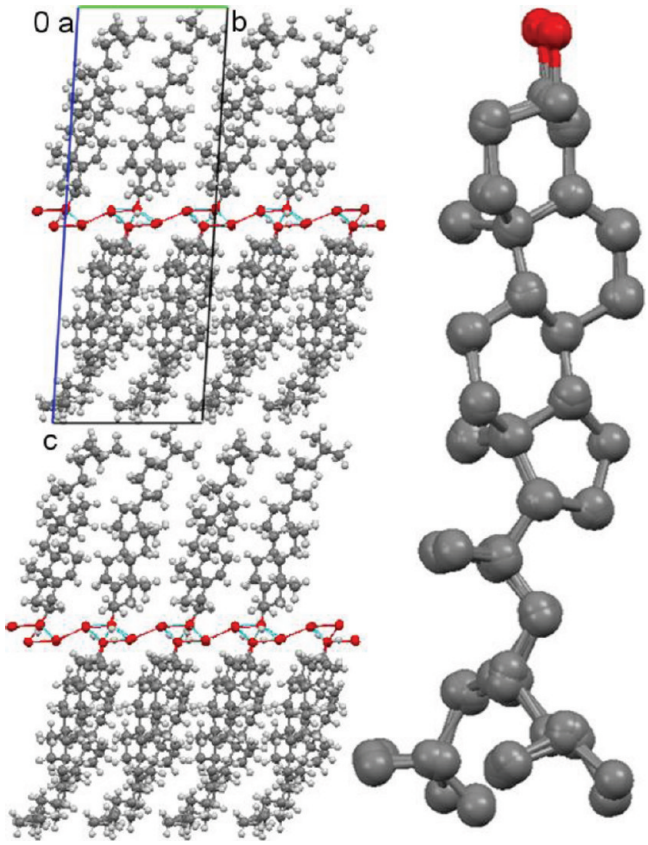

Figure 10. Packing diagram of cholesterol monohydrate showing the bilayer structure (left). A molecular overlay of the independent molecules and their conformational variability (right).

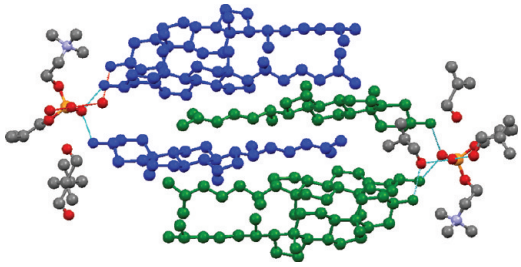

Figure 11. Head-to-tail packing in MEQKAU. Colors depict the different orientations of cholesterol molecules. Atom colors: carbon, gray; oxygen, red; nitrogen, blue; phophorus, orange.

question that was posed at the outset of this investigation was how big does the coformer have to be before a change from the bilayer to the intercalated structure was invoked?

From the outset, a novel molecular packing was observed for the simplest alcohols studied: propanol and butanol. In these structures the packing of the cholesterol molecules is in the head-to-tail arrangement, which is the distinctive feature of the second type of molecular architecture, but instead of observing the layers of alternating hydrophilic and hydrophobic regions, one observes that the solvent molecules are located in discrete locations in the crystal structure. The crystal structures of compounds **5** and **6** show no isostructurality with any of the five other solvates isolated as part of this study, or between themselves, but the two structures show a stepwise change from one architecture (compounds **1** and **2**) to the other (**3**, **4**, and **7**). If one considers compound **6**, the dimer unit in this

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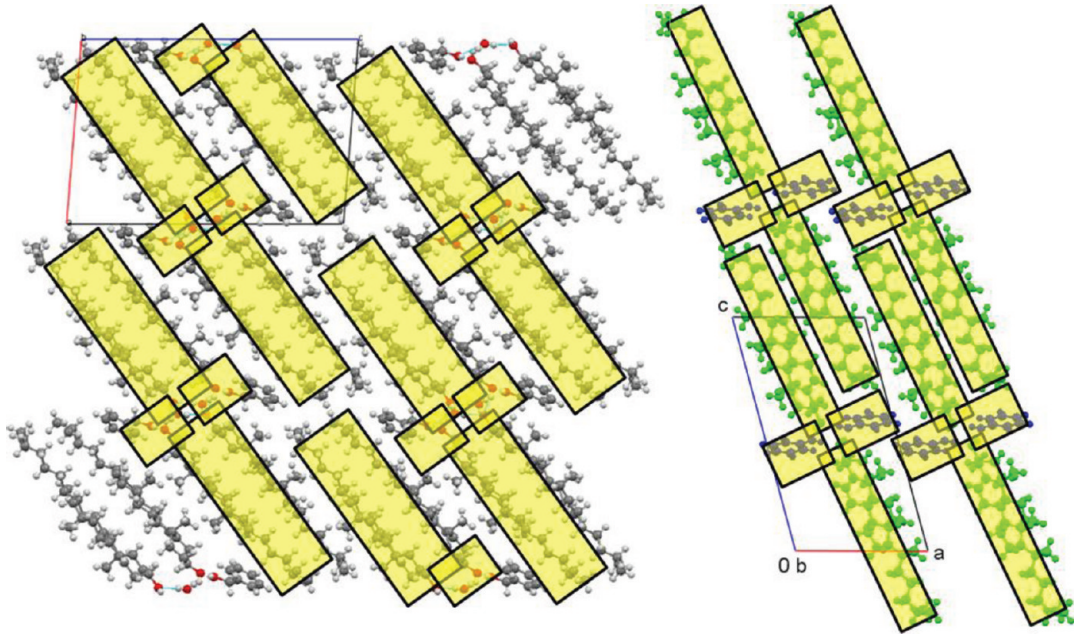

Figure 12. Crystal structures of compound **6** (left) and compound **5** (right) with an overlay depicting the similarity of the dimer chain of compound **6** and the single hydrogen bonded chain in compound **5**.

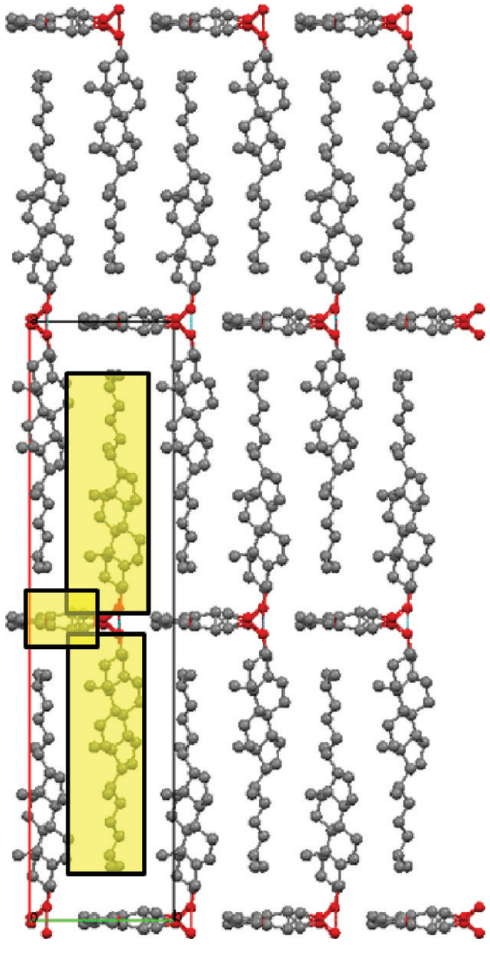

Figure 13. Crystal structure of compound **7**. Note that the phenylethanol participating in the hydrogen bonded chain lies only on one side of the cholesterol chain (cf. compound **5**).

structure resembles the single hydrogen bonded chains found in the phenol solvate (compound **5**), where the phenol molecules reside at both sides of the cholesterol chain when viewed along the *b*-axis (Figure 12 (right)). Figure 12 shows a block representation of the hydrogen bonded chains in order to show the similarity in the packing of these chains more clearly. When depicted like this, it is clear that the chains are "tilted" to a different extent in each of the structures. The lower the degree of "tilt", the closer the structure is to the layered structure observed in the iodophenol cocrystal (WOMHAI). Figure 13 shows a similar overlay of the block representation over the structure of compound **7**. There is a change in the hydrogen bonded chain to only include the solvent molecule on one side of the cholesterol molecules that allows neighboring chains to pack closely so that the layered hydrophilic and hydrophobic structures can be formed. The disorder of the pentanol and hexanol molecules mimics the role of the bulkier additives rather than following the packing arrangement observed in the smaller alcohols: propanol and butanol.

■ CONCLUSIONS

In this paper we have described the formation of seven new solvates of cholesterol. All of these novel forms adopt crystal structures that are removed from the bilayer structure observed for the simple solvates presently in the CSD. In each of these structures, the cholesterol molecules interact in a head-to-tail manner as opposed to tail-to-tail structure of the bilayer. The solvates of the simplest two alcohols and benzyl alcohol form a new molecular architecture where the solvent molecules are found in discrete regions in the crystal structure. Phenol forms an intermediary structure with more extended hydrophobic and hydrophilic regions than the previous solvates. The solvates with pentanol, hexanol, and phenylethanol are all isostructural with each other and are essentially isostructural with an

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iodophenol cocrystal in the CSD but for the change in space group symmetry.

■ ASSOCIATED CONTENT

Supporting Information

DSC and TGA analysis of the solvates along with the powder X-ray diffraction results of the solvates formed. This material is available free of charge via the Internet at http://pubs.acs.org.

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