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A Synthetic Hydroxy Acid That Shows Tubular-Shaped Structure in Solid-State and Ionophoric Activity in Phospholipid Bilayers

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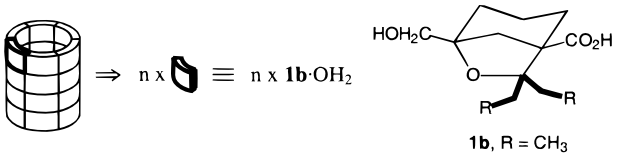
ABSTRACT



In this contribution, we describe the ability of compound (±)-1b and six molecules of water to form in solid-state hexameric aggregates, which self-assemble to give hollow tubular structures. Single-crystal X-ray analysis shows that these tubes are open-ended, with irregular shape and internal van der Waals pore diameter between 6 and 9 Å. In addition, transmembrane sodium transport activity was also assessed for (±)-1b using dynamic Na^+-NMR technique.

In this Letter, we describe the synthesis and characterization of a new class of organic tubular material,[1] based on self-assembling of aggregates joined by extensive hydrogen-bonding networks. Our synthetic strategy was to construct two-dimensional (2-D) H-bonded arrays by using molecular descriptors with an unbalanced H-bond donor/acceptor ratio[2] and then promote reversion to channels by increasing steric demands through precise variations of the molecular structure and by readdressing the d/a imbalance by inclusion of one or more molecules of water.[3] For a successful design, the H-bonded pattern must be persistent, maintaining both the chemical connectivity and the associated symmetry operator.

Solid-State Structure. We have shown[4] that compound (±)-**1a** with three oxygenated functions, under anhydrous conditions, crystallizes exhibiting 2D H-bonded arrays in which each molecule is connected through four H-bonds by exclusive participation of the carboxylic acid and hydroxyl functions. Crystallization of its higher homologue (±)-**1b**[5]

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(1) Considerable effort has been devoted toward the rational design of tubular structures because of their potential utility in molecular transport, inclusion chemistry, chemical catalysis, and manufacture of novel optical and electronic devices: (a) Ying, J. Y.; Mehnert, Ch. P.; Wong, M. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 56−77. (b) Kresge, C. T.; Leonowicz, M. E.; Roth, W. J.; Vartuli, J. C.; Beck, J. S. *Nature* **1992**, *359*, 710−712. (c) Schnur, J. M. *Science* **1993**, *262*, 1669−1676. (d) Fuhrhop, J.-H.; Spiroski, D.; Boettcher, C. *J. Am. Chem. Soc.* **1993**, *115*, 1600−1601. (e) Iijima, S. *Nature* **1991**, *354*, 56−58. (f) Harada, A.; Li, J.; Kamachi, K. *Nature* **1993**, *364*, 516−518.

(2) Desiraju, G. R. *J. Chem. Soc., Chem. Commun.* **1990**, 426−428.

(3) Similar effects may also be in control of the spherical hexameric assembly of resorcinarenes through 60 H-bonds: MacGillivray, L. R.; Atwood, J. L. *Nature* **1997**, *389*, 469−472.

(4) Ruiz-Pérez, C.; Rodríguez, M. L.; Martín, J. D.; Pérez, C.; Morales, P.; Ravelo, J. L. *Acta Crystallogr. Sect. C* **1990**, *46*, 1507−1509.

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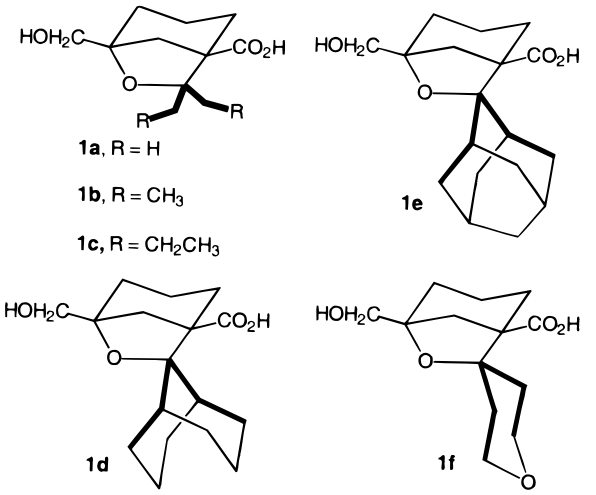

from damp carbon tetrachloride/*n*-hexane provides thin plates (0.67 × 0.17 × 0.07 mm) for the X-ray analysis. In the crystalline state,[6] **1b** presents a hydrate structure. The structure is dominated by a hexameric ring of alternating (+)- and (−)-**1b** molecules held together by O2−H2···O4 hydrogen bonds (Figure 1a). This ring system, which is the striking feature of this molecular assembly, joins by *c* translation to another one via hydrogen bonds involving six water molecules, each one using one of its hydrogen atoms for that link to O2. Moreover, they act as donors of other hydrogen bond to O1 and accept a strong hydrogen bond from O3 which reinforces the ring system. The rings stack in this way to give columns defining the *c* axis (Figure 1b), all through the crystal, creating a channel which holds disordered water molecules (one O6 per ring with a population of 0.2 each). The key structural requirement for

(5) Compound **1b** was synthesized by condensing the dilithium salt of 3-methylenecyclohexanecarboxylic acid with 3-pentanone, following the synthetic sequence reported for compound **1a** (ref 4).

(6) Crystal structure analysis: Intensity data were collected on a Philips PW 1100 four circle with graphite oriented monocromator Cu Kα radiation (λ = 1.5418 Å) $\omega/2\theta$ scans, temperature 170 K (Oxford Cryostream device),[6a] cell determination with 21 reflections. Lorentz and polarization corrections, direct methods,[6b] refinement against F_o and H atoms from difference Fourier synthesis. Secondary extinction correction.[6c] A disordered water molecule (O6) was located on a 3-fold inversion axis (Figure 1a) and was allowed to refine up to a site-occupancy factor of 0.20(6) and isotropic thermal vibration. The maximum positive residual peak in the final difference map (0.91e Å^{-3}) occurs in positions close to the O6 molecule (1.67 Å) that could be considered as possible alternative locations for the O6 water molecule although it was impossible to refine. Calculations were mainly performed with XTAL[6d] set of programs, and the scattering factors were taken from the *International Tables for X-ray Crystallography*.[6e] Crystal data ($C_{13}H_{22}O_4 \cdot 1.033H_2O$, M_r = 260.69), rhombohedral, space group *R*-3, a = 34.9204(25), c = 6.5875(12) Å, V = 6956.8(15) Å^3, Z = 18, ρ_{calcd} = 1.12 g cm^{-3}; final *R* indices: R = 0.087 and R_w = 0.083 (for 1810 observed reflections (θ_{max} = 65° and $I > 2\sigma(I)$ criterion) for 262 variables. Crystallographic data (excluding the structure factor tables) for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 119462. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: Int. +-(1223) 336 033; e-mail: deposit@ccdc.cam.ac.uk. (a) Cosier, J.; Glazer, J. A. M. *J. Appl. Crystallogr.* **1986**, *19*, 105. (b) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Burla, M. C.; Polidori, G.; Camalli, M.; Spagna, R. SIR97 a package for crystal structure solution by direct methods and refinement. University of Bari (Italy), 1997. (c) Zachariasen, W. H. *Acta Crystallogr.* **1967**, *23*, 558. (d) Hall, S. R.; Flack, H. D.; Stewart, J. M. XTAL3.2 Eds.; Univs. of Western Australia: Perth Australia, 1992. (e) International Tables for X-ray Crystallography, Kynoch Press: Birmingham, England, 1974; Vol. IV.

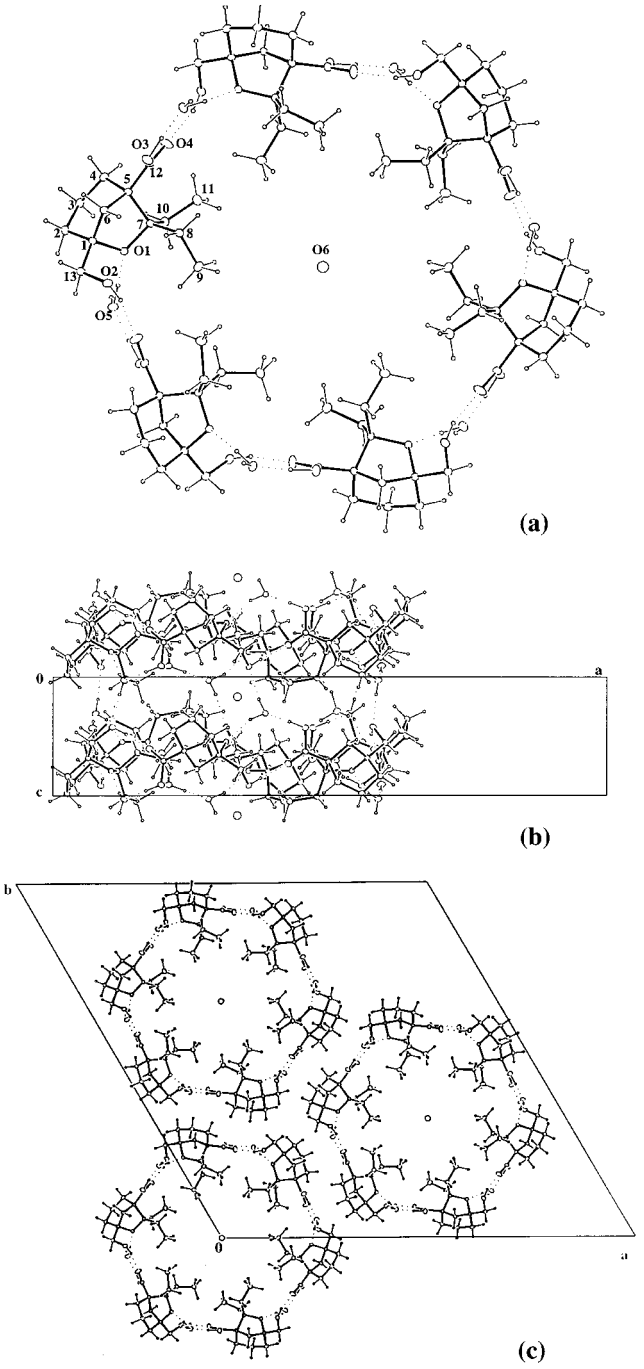

Figure 1. (a) Structure of the hexameric association (one of the three in the unit cell) displaying the numbering system and the hydrogen network (dotted lines). (b) Packing of the hexameric units along the *b* showing the location of the water molecules. (c) Crystal packing along the *c* axis. Hydrogen bonds (Å, deg): O5···O1 = 2.712(4), H5bw···O1 = 1.83(7), O5−H5bw···O1 = 159(6); O5···O2 (x, y, z − 1) = 2.753(5), H5aw···O2 = 1.89(7), O5−H5aw···O2 = 172(6); O2···O4 (1/3+x−y,-1/3+x,2/3-z) = 2.711(6), H2O···O4 = 1.81(7), O2−H2O···O4 = 166(5); 66(5); O3···O5 (1/3 + y, 2/3 − x + y, 1/3z) = 2.563(5), H3O···O5 = 1.52 (7), O3 H3O···O5 = 177 (10); C3···O2 (2/3 − y, 1/3 + x − y, −2/3 + z) = 3.650 (8), H3b···O2 = 2.76(7), C3−H3b···O2 = 145(3); C2···O5 (2/3 − y, 1/3 + x − y, 1/3 + z) = 3.476(10), H2b···O5 = 2.62(5), C2−H2b···O5 = 144(3).

producing a multiply ring-stacked tubular structure was the spatial disposition of hydrogen-bond donor and acceptor sites on both faces of the hexameric ring structure. The molecules forming the hexameric unit are related by 3-fold inversion center, and the ethyl groups are oriented on the inner part of the ring, forming an irregular cylindrical hydrophobic surface of minimum and maximum diameter of 5.7 and 9.4 Å, respectively (Figure 2). Further, these tubular assemblies are

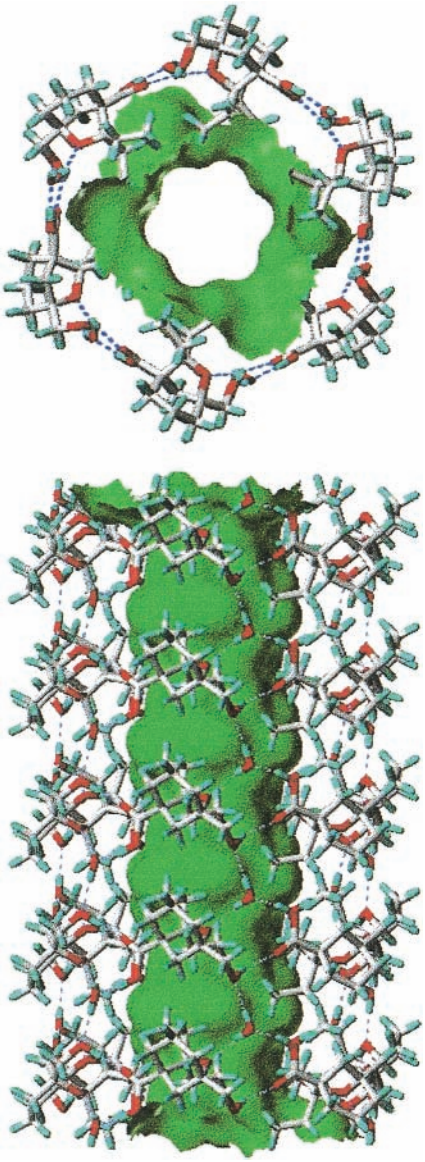

Figure 2. Two pictures of the tubular structure (along and across its axis) showing the Connoly surface and the channel inside it.

held together by weak CH···O interactions in which the O2 hydroxyl group and ordered (O5) water molecule are involved. Structural comparison is made with the previously reported structure with methyl substituents at C-7(**1a**). There are no major discrepancies in the molecular structure; however, the hydrogen network is quite different, showing the important role played by the ordered water molecule (O5) in the formation of the tubular structure.[7] In that structure,[4] O−H···O bonds, only between hydroxyl groups, form an extended 2D-sheet leaving the carbonyl group free of strong interaction. Examination of the **1a** structure showed that there were not solvent-accessible voids in the crystal lattice and that **1a** forms a closer-packed lattice than that present in compound **1b** as reflected by the density (1.29 vs 1.12 g cm^{-3}) and by the total packing coefficient (0.689 vs 0.614), respectively. The observed layer to channel reversion[8] is probably due to the steric factor hindrance cause by the change of methyl substituent to ethyl. Such a change may prevent layer arrangements to force the hydroxyl and carboxyl functions to form H-bonds, resulting in the ring-shaped structure with more pore volume. The ethyl groups pointing into the channel sterically interfere which each other to protect the structure against collapse (Figure 1a). This molecular determinant provides a guideline for synthetic targeting of potential members of this family by varying the nature of CH_2R groups. Thus, the homologues (±)-**1c**−**f**, were synthesized.[9] Single crystals of the expected pore-forming derivatives (±)-**1c**−**e** proved as difficult to obtain as those of (±)-**1b**. After numerous attempts of crystallization from damp CCl_4/*n*-hexane mixtures, it was found that hydrated single crystals could be obtained for these compounds; however, they were too small for structural determination using a typical laboratory X-ray source.[10] As expected, the solid-state aggregation of (±)-**1f**[11] does not occur to form cyclic hexamers, but rather as anhydrous 2D H-bonded arrays such as (±)-**1a**. This can be probably due to the effect of tying the CH_2 end groups into a syn conformation.

Transport Experiments. The ability of compounds **(±)-1a**−**f** to transport Na^+ through a phosphatidylcholine bilayer can be assessed directly by using the ^{23}Na-NMR technique developed originally by Riddell.[12] Large unilamellar vesicles (LUV) were prepared from fresh egg yolk phosphatidylcholine (PC) by the dialytic detergent removal technique introduced by Reynolds[13] (20 mM PC, 20% volume entrapment, $[Na^+]$ = 200 mM). Dysprosium (external solution, 5mM, tripolyphosphate) was added to create a 10−15 ppm shift difference of ^{23}Na inside from ^{23}Na outside. Incorporation of **(±)-1a**−**f** was accomplished by microliter injection of the appropriate stock solution (2 mM) at 25 °C. Final concentrations were typically 20−140 mM. As can be seen in Table 1, compounds (±)-**1b**−**e** were found to facilitate the transmembrane transport of sodium cations across the

(7) Attempts to obtain a hydrate structure of compound **1a** failed.

(8) Meléndez, R. E.; Hamilton, A. D. *Top. Curr. Chem.* **1998**, *198*, 97−129.

(9) Compounds **1c**−**f** were synthesized by condensing the dilithium salt of 3-methylenecyclohexanecarboxylic, respectively, with: 4-heptanone, bicyclo[3.3.1]nonan-9-one, 2-adamantanone, and tetrahydro-4*H*-pyran-4-one, following the synthetic sequence reported for compound **1a** (ref 4).

(10) Though perfect and single, these crystals measured only a few tens of microns along one edge.

(11) To be published.

(12) (a) Riddell, F. G.; Hayer, M. K. *Biochim. Biophys. Acta* **1985**, *817*, 313−317. (b) Riddell, F. G.; Arumugam, S. *Biochim. Biophys. Acta* **1989**, *984*, 6−10. (c) Riddell, F. G.; Tompsett, S. J. *Biochim. Biophys. Acta* **1990**, *1024*, 193−197.

(13) Mimms, L. T.; Zanpighi, G.; Nozaki, Y.; Tanford, C.; Reynolds, J. A. *Biochemistry* **1981**, *20*, 833−840.

Table 1. Intravesicular ^{23}Na Line Widths as a Function of Ionophore Concentration

	$\Delta\nu_{1/2}$ (Hz)					
concn (μM)	(±)-**1a**	(±)-**1b**	(±)-**1c**	(±)-**1d**	(±)-**1e**	(±)-**1f**
0	9.00	9.00	9.00	9.00	9.00	9.00
20	9.01	9.34	9.30	9.25	9.50	9.00
40	9.02	9.80	9.71	9.50	9.94	9.00
60	9.03	10.12	10.00	9.80	10.30	9.01
80	9.04	10.52	10.34	10.10	10.70	9.02
100	9.06	10.96	10.66	10.45	11.10	9.03
120	9.06	11.25	10.95	10.73	11.45	9.03
140	9.06	11.66	11.22	11.00	11.90	9.03

lipid bilayer of the LUVs. It is also the case that structural variations of the CH_2R groups lead to differences in flux rate[14] that are well outside experimental error (Figure 3).

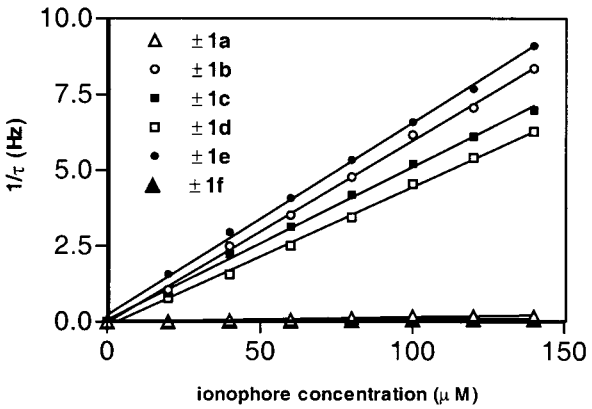

Figure 3. Mean lifetime ($1/\tau_{in}$) of the $^{23}Na^+$ "inside" vs ionophore concentration.

Most importantly, compounds **(±)-1a** and **(±)-1f** are inactive at any concentration, indicating that indiscriminate damage to bilayers does not occur. Although the significant transport dependence with the conformation of the CH_2R groups disfavors a carrier mechanism, and the induction of membrane defects in a detergent-like manner is unlikely, the mechanism of transport induced by compounds (±)-**1b**−**e** remains to be elucidated. Obviously, if the observed solid-state structure for (±)-**1b** is formed in a bilayer, the predominantly hydrophobic water-filled pore should contribute significantly to the stabilization of ions. Because its inside is lined by hydrophobic ethyl chains, it is unlikely that a permeant ion will shed its hydration shell. This consideration should favor hydrated ions throughput by simple electrostatic principles, unless the physical opening appears to be small enough to prevent entry of hydrated cations into the pore. It would be interesting to know if such simple molecules could overcome the dielectric barrier presented by the membrane, providing a water-filled pathway that allows passive flux of hydrated cations through a central pore.[15] Work toward these ends is in progress.

Acknowledgment. This work was supported by grants (PB97-1123, PB96-0001-C03) from the Ministry of Education and Culture and the Ramón Areces Foundation. H.C. thanks the AECI, Spain, for a Predoctoral Fellowship. This article is dedicated to Professor José Elguero on the occasion of his 65th birthday.

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(14) The rate for the efflux of $^{23}Na^+$ from the vesicles is determined by the equation $k = 1/\tau = \pi(\Delta\nu - \Delta\nu_0)$, where $\Delta\nu$ is the line width at half-height of the observed resonance line in the presence of ionophore and $\Delta\nu_0$ is the corresponding value in its absence: Sandstrom, *J. Dynamic NMR Spectroscopy*; Academic Press: London, 1982; Chapter 6.

(15) For recent general reviews related with synthetic channels and pores, see: (a) Gokel, G. W.; Murillo, O. *Acc. Chem. Res.* **1996**, *29*, 425−432. (b) Fyles, T. M.; van Straaten-Nijenhuis, W. F. in *Comprehensive Supramolecular Chemistry*, Reinhoudt, D. M. Ed.; Elsevier Science Ltd.; Oxford, 1996; Vol. 10, pp 53−57. (c) Voyer, N. *Top. Curr. Chem.* **1996**, *184*, 1−37. (d) Meillon J.-C.; Voyer, N. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 967−969. (e) Hartgerink, J. D.; Clark, T. D.; Ghadiri, M. R. *Chem. Eur.* **1998**, *4*, 1367−1372. (f) Gokel, G. W. *Chem. Commun.* **2000**, 1−9. (g) Bayley, H. *Curr. Opin. Biotechnol.* **1999**, *10*, 94−103.