Acta Crystallographica Section B
Structural Science
ISSN 0108-7681

Structure of the inclusion complex of β-cyclodextrin with lipoic acid from laboratory powder diffraction data

Csaba-Pal Rácz,[a] Gheorghe Borodi,[b]* Mihaela Maria Pop,[b] Irina Kacso,[b] Szabolcs Sánta[a] and Maria Tomoaia-Cotisel[a]

[a]Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University of Cluj-Napoca, 11 Arany J. Str., Cluj-Napoca 400028, Romania, and [b]National Institute for R&D of Isotopic and Molecular Technologies, PO Box 700, Cluj-Napoca R-400293, Romania

Correspondence e-mail: borodi@itim-cj.ro

Received 30 August 2011
Accepted 1 February 2012

The crystal structure of the inclusion complex of β-cyclodextrin with lipoic acid was determined from laboratory powder diffraction data. Thermogravimetric data was used to estimate the number of water molecules in the crystal structure. Lipoic acid is included in β-cyclodextrin through its primary face with the five-membered ring reaching the center plane of the cyclodextrin cavity and its fatty acid chain adopting a bent conformation. Lipoic acid and β-cyclodextrin form a channel-like packing which is stabilized by guest–host hydrogen bonding and close contacts, host–host intermolecular interactions and hydrogen bonding involving the water molecules.

1. Introduction

α-Lipoic acid is a medium-chain (C8) fatty acid with sulfur atoms at C1 and C3 (Fig. 1). As the C3 atom is chiral, lipoic acid exists as two enantiomers or stereoisomers: *R*-(+)-lipoic acid and *S*-(−)-lipoic acid.

α-Lipoic acid (LA) is a potent antioxidant (Jones *et al.*, 2002). The racemic mixture of α-lipoic acid, *RS*-(+/−)-α-lipoic acid (*rac*-LA), has been utilized clinically and in therapeutic applications such as diabetic neuropathy (Tang *et al.*, 2007), metabolic syndrome (Sola *et al.*, 2005), burning mouth syndrome (Femiano *et al.*, 2002; Femiano & Scully, 2002) and peripheral artery disease (Vincent *et al.*, 2007). *Rac*-LA and the natural form *R*-lipoic acid (*R*-LA) are widely available as nutritional supplements marketed as dietary antioxidants (Packer *et al.*, 1995, 2001). Based on positive outcomes in various models, *R*-LA has been recommended for the prevention and treatment of both Alzheimer (Holmquist *et al.*, 2007) and Parkinsons' (Bharat *et al.*, 2002) diseases.

Owing to its tendency to polymerize, LA is relatively unstable to light and heat and suffers poor aqueous solubility, leading to poor absorption and low bioavailability (Packer *et al.*, 2001). Also, it has a sulfide smell and an irritating taste. Owing to these drawbacks, the development of a product containing LA with higher solubility and stability is of high

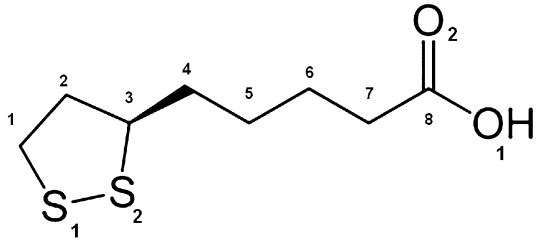

Figure 1
Lipoic acid (LA) molecule and the atom-numbering system.

Table 1
Experimental details.

Crystal data	
Chemical formula	$C_{42}O_{35}\cdot C_8O_2S_2\cdot O_9$
M_r	1400.62
Crystal system, space group	Monoclinic, *C*2
Temperature (K)	295
a, *b*, *c* (Å)	18.892 (9), 24.463 (4), 15.737 (6)
β (°)	110.519 (5)
V (Å^3)	6812.18 (3)
Z	4
Radiation type	Cu $K\alpha$, λ = 1.5405 Å
Specimen shape, size (mm)	Powder, disc of 20 mm diameter
Data collection	
Diffractometer	Bruker D8 Advanced
Specimen mounting	Plate holder
Data collection mode	θ–2θ
Scan method	Step
2θ values (°)	$2\theta_{min}$ = 2.999, $2\theta_{max}$ = 49.979, $2\theta_{step}$ = 0.01
Refinement	
R factors and goodness of fit	R_p = 0.092, R_{wp} = 0.128, R_{exp} = 0.033, $R(F^2)$ = 0.12487, χ^2 = 30.581
No. of data points	4699
No. of parameters	167
No. of refined parameters:	
Lattice	4
Positional	98
Thermal	4
Texture	0
Profile	17
Background	18
Zero-shift	1
No. of restraints:	
Rigid bodies	8
Bond restraints	72
Bond-angle restraints	65
Phase restraints	1

interest. Complexation with cyclodextrins (CDs) is a known approach to favorably enhance the physicochemical properties of compounds.

CDs are macrocyclic oligosaccharides that consist of glucose units that are α-1,4-glycosidically linked in a cyclic manner (Fig. 2). CDs are characterized by a 'hollow' truncated conical shape; the primary face (with smaller radius) of the truncated cone comprises the primary hydroxyl groups of the glucose units and the secondary face comprises (twice as many) secondary hydroxyl groups. CDs consist of a hydrophobic cavity relative to the hydrophilic periphery of the molecule and they represent important host molecules in supramolecular chemistry owing to the fact that they are capable of accommodating various kinds of guest molecules into their cavities (Szejtli, 1998; He *et al.*, 2008; Wenz *et al.*, 2006; Hapiot *et al.*, 2006; Rodriguez & Elola, 2009). A successful method used to increase not only the aqueous solubility but also the stability of the biologically active molecules is their complexation with CDs. Numerous scientific articles describe the advantages of drugs complexed with cyclodextrins, such as: increased solubility, enhanced bioavailability, improved stability, the masking of bad taste or odor, reduced volatility, transformation of liquid or gas into the solid form, reduced side effects and the possibility of a drug release system (Uekama & Hirayama, 1996; Erden & Celebi, 1988; Ribeiro *et al.*, 2007).

The effect of β-CD on the solubility and stability of LA were recently investigated (Maeda *et al.*, 2010; Takahashi *et al.*, 2011), but no crystal structure of the complex has been determined so far.

In the current work the β-CD complex with LA was obtained by evaporative crystallization from a water:methanol (60:40) solvent mixture, leading to a powder-like substance.

X-ray powder diffraction (XRPD) is routinely used for the qualitative identification of the CD complex formation and only a few cases of crystal structure determination are reported (Guo *et al.*, 2011; Marques *et al.*, 2008; Borodi *et al.*, 2008; Pop *et al.*, 2002). Synchrotron radiation is used for two of the reported structures (Borodi *et al.*, 2008; Pop *et al.*, 2002) to substantially improve the XRPD pattern resolution and therefore the number of observed reflections to be used in the crystal structure determination process. Marques *et al.* (2008) proposed hypothetical structure models of inclusion compounds based on laboratory XRPD data and Monte Carlo structural optimization, but the structures could not be refined due to the low crystallinity of the samples. The recent work of Guo *et al.* (2011) compares the structural results from the structure determination performed by X-ray diffraction from laboratory powder diffraction data and the results of a single-crystal experiment. This comparison shows that the crystal structure determination of cyclodextrin inclusion complexes of such complexity is accessible from powder diffraction data to a certain extent.

In this paper we report the crystal structure of the β-CD/LA inclusion complex, as solved from laboratory XRPD. To our knowledge this is one of the first crystal-structure determinations of a β-inclusion complex from laboratory XRPD data. We confirm that this type of data obtained with a laboratory X-ray source combined with structure determination and refinement techniques can lead to a detailed characterization of the structural characteristics of the inclusion compounds.

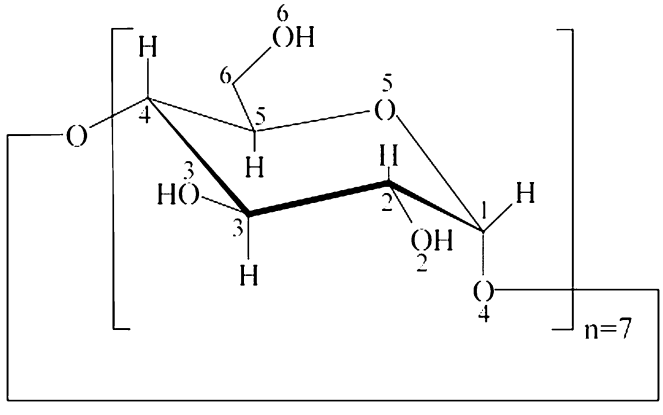

Figure 2
Cyclodextrin molecule and the atom-numbering system.

2. Crystal structure determination of the β-CD–LA inclusion complex

2.1. Indexing and model building

The powder-diffraction pattern was indexed using the programs *XCell* (Neumann, 2003) and *Ito* (Visser, 1969) implemented in the program *MS Reflex Plus* of Accelerys Material Studio suite (Accelerys Software Inc., 2010). The indexing procedure resulted in a monoclinic cell (Table 1) with *C*2 (Z = 4) as the most likely space group. With *X-Cell* we obtained a relative figure of merit, Rel.FOM = 2.27, and a figure of merit, FOM = 1014. With the *ITO* method we obtained a De Wolff figure of merit, M_{20} = 23.2. With both methods applied all the reflections were indexed.

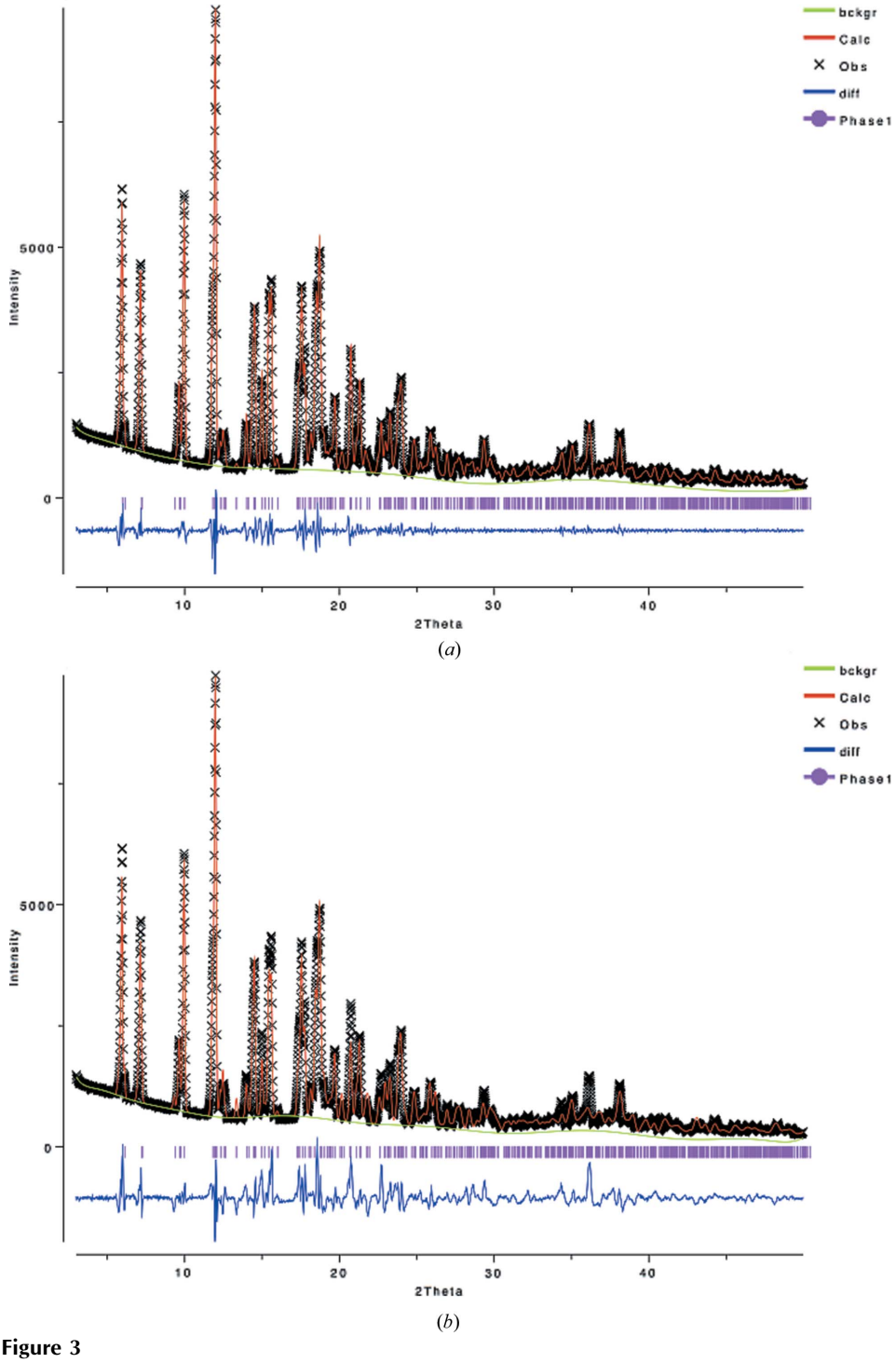

Figure 3
Powder-diffraction patterns of the β-CD–LA (1:1) inclusion complex after (*a*) LeBail and (*b*) Rietveld refinements; experimental pattern (crosses), the final calculated pattern after refinement (line) and the difference (experimental − calculated) pattern (line, bottom).

The initial model of the β-CD inclusion complex with LA was constructed from single-crystal structures found in the Cambridge Structural Database (Allen, 2002); the β-CD model was taken from the CSD entry with the reference code BCDEXD03 (Steiner & Koellner, 1994) and the lipoic acid model from THOCAR01 (Stroud & Carlisle, 1972). Owing to the complexity of other known crystal structures of inclusion compounds (Guo *et al.*, 2011; Borodi *et al.*, 2008; Pop *et al.*, 2002) and the associated difficulties encountered even in the determination of crystal structures from single-crystal data (Tsorteki *et al.*, 2005), molecular-mechanics simulation (*HyperChem*™; Hypercube Inc., 2002) was carried out on the guest molecule prior to the crystal structure determination process. It was assumed that positioning a more probable geometry of the guest molecule (by geometry optimization in vacuum) would lead to a better crystal structure model for the refinement stage.

The next step was to determine the configuration and position of LA and the position of β-CD in the unit cell. By using the Parallel Tempering option in the program *Dash* (David *et al.*, 2006) the β-CD and LA molecules were first treated as rigid bodies and at a later stage the torsion angles of both β-CD and LA molecules were allowed to vary. Under these conditions a structural model was obtained in which LA was included in the β-CD and had substantial changes of the torsion angles around the C3—C4 bond compared with the starting model (Fig. 1). The structure determination procedure led to a possible solution with a minimum of *ca wR*

Table 2
The characteristics of the β-CD molecule.

D = distances between atoms O4(G_n)$\cdots$O4(G_{n+1}); φ = angles between atoms O4(G_{n-1})$\cdots$O4(G_n)$\cdots$O4(G_{n+1}); d = deviations from the least-squares plane through the seven O4(G_n) atoms; α = dihedral angle between the O4(G_n) plane and the least-square plane through C2(G_n), C3(G_n), C5(G_n) and O5(G_n); D3 = intramolecular distances between atoms O3(G_n)$\cdots$O2(G_{n+1}). Torsion angle τ_a = O5(G_n)—C5(G_n)—C6(G_n)—O6(G_n) and τ_b = C4(G_n)—C5(G_n)—C6(G_n)—O6(G_n).

Residue	D (Å)	φ (°)	d (Å)	α (°)	$D3$ (Å)	τ_a (°)	τ_b (°)
G1	4.933 (17)	135.2 (4)	0.273 (7)	65.1 (5)	2.97 (3)	−87.6 (18)	36 (2)
G2	5.516 (11)	122.7 (3)	−0.437 (15)	87.1 (4)	2.639 (14)	−137 (3)	−15 (3)
G3	5.455 (17)	105.2 (2)	0.115 (9)	88.1 (5)	2.919 (16)	−44.5 (12)	73.1 (12)
G4	4.911 (17)	160.7 (4)	0.29 (2)	83.1 (6)	2.93 (2)	−95 (2)	32 (3)
G5	5.464 (14)	101.7 (3)	−0.243 (13)	74.3 (7)	2.47 (2)	−103 (2)	28 (2)
G6	4.010 (15)	135.6 (3)	−0.079 (12)	84.5 (8)	2.83 (2)	−154.2 (10)	−23.1 (12)
G7	4.776 (13)	134.4 (4)	0.081 (13)	78.5 (8)	3.416 (15)	−37 (3)	89 (3)

Table 3
Intramolecular close contact distances related to the primary —OH groups of β-CD.

The first index is related to the usual notation for the glycoside unit as given in Fig. 1. The second index corresponds to the glycoside unit number in the β-CD macrocycle.

Atoms	Length (Å)
Primary —OH groups of β-CD	
O61$\cdots$O42	2.49 (4)
O61$\cdots$O52	2.55 (3)
O62$\cdots$O43	2.495 (14)
O62$\cdots$O53	2.401 (19)
O63$\cdots$O53	2.48 (2)
O64$\cdots$O45	2.44 (2)
O64$\cdots$O55	2.49 (3)
O66$\cdots$O47	2.78 (2)
O66$\cdots$O57	2.473 (15)
O67$\cdots$O57	2.89 (5)

= 0.35 (*wR* defined as in David *et al.*, 2006).

2.2. Rietveld refinement of the crystal structure model

Prior to the Rietveld refinement of the crystal structure model, a Le Bail fitting (Le Bail *et al.*, 1988) was carried out using the procedure included in the *GSAS* package (Larson & Von Dreele, 2000; Toby, 2001). The background and profile parameters from the Le Bail fit (R_p = 0.0369, R_{wp} = 0.0526) were used as starting points in the Rietveld refinement. In order to retain a chemically realistic model several types of restraints were implemented during the Rietveld refinement procedure. Each glucose unit together with its secondary O atoms was defined as a separate rigid body, and rotation and translation were allowed relative to the glycosidic plane. As the primary units of the β-CD host molecule are more flexible they were not included in the rigid-body definition. In the case of the LA molecule the five-membered ring was also defined and maintained as a rigid body during the whole refinement process. In addition, planar-group restraints (Nowell *et al.*, 2002) were applied to the seven O4(G_n) atoms with s.u.s being 0.5 Å. Bond-distance and bond-angle restraints were applied to the glycosidic O4(G_n) atoms and to the LA atoms of the fatty acid chain. As ideal restraint values for bond distances and angles, the averages of the corresponding quantities were used, calculated from the Cambridge Structural Database (Allen & Kennard, 1993). Initially, strong restraints (weighting factors $f_d = f_a = f_p = 1000$, defined in Larson & Von Dreele, 2000) were applied but in subsequent cycles they could be reduced gradually to f_d = 5, f_a = 5 and f_p = 0.5). Based on the TGA results, nine O atoms of the water molecules were included in the crystal structure and refined as follows: the positions of two O atoms were determined from the Fourier map and the other seven O atoms were positioned in solvent-accessible voids calculated with *PLATON* (Spek, 1990). The number of water molecules and information about the stability of the complex were determined from TGA and DSC analyses. Since the amount of crystallization water in CDs can be affected by ambient conditions (Bilal *et al.*, 1995), the TGA trace was recorded immediately after crystallization and showed approximately 11% weight loss between 323 and 393 K, corresponding to about nine water molecules.

The DSC trace showed a melting temperature of the β-CD-LA complex that is $\sim$10 K higher compared with the LA, indicating an increased thermal stability of LA by complexation with β-CD. This is in agreement with an improvement of the degradation profiles of the inclusion complexes with LA reported in the literature (Tong *et al.*, 1995; Takahashi *et al.*, 2011).

The H atoms of both β-CD and LA were not considered owing to the fact that for such large molecules they could not be refined.

Details of the structure refinement are given in Table 1; observed, calculated and difference patterns after the LeBail and Rietveld refinements are shown in Figs. 3(*a*) and (*b*).

3. Results and discussion

3.1. Molecular packing and hydrogen bonding of the β-CD-lipoic acid (1:1) inclusion complex

The overall conformation of the β-CD moiety is well characterized by two structural parameters: the macrocyclic plane through the O4(G_n) atoms and the tilt angles ($|90 - \alpha|$) of the glucose units with respect to this plane, which are derived from the dihedral angles (α) of the macrocyclic plane with the least-squares plane through the C2,C3, C5 and O5 atoms of each glucose unit (Table 2).

The seven glycosidic O4(G_n) atoms in β-CD–LA (1:1) form a distorted heptagon given the large deviations of the interatomic distances (4.00–5.5 Å) and interatomic angles (102–160°; Table 2) from the values of 4.38 Å and 128.6° in an ideal non-distorted heptagon. The tilt angles $|90 - \alpha|$ of the glucose units towards the pseudo-sevenfold axis of O4(G_n) are between 1.9 and 11.5° for G_2, G_3, G_4, G_6 and G_7 glucose units, which are similar to the average value (of 6°) encountered in

Table 4
Intermolecular O···O and S···O close contact distances.

The first index is related to the usual notation for the glycoside unit as given in Fig. 1. The second index corresponds to the glycoside unit number in the β-CD macrocycle.

Primary —OH groups of β-CD		Secondary –OH groups of β-CD		Lipoic acid (LA) with β-CD and water (O90–O98)		Water (O90–O98) with β-CD	
Atoms	Length (Å)	Atoms	Length (Å)	Atoms	Length (Å)	Atoms	Length (Å)
				LA–β-CD			
O63···O63^{i}	2.45 (2)	O27···O37^{ii}	2.449 (16)	O1···O52^{iii}	2.43 (2)	O90···O26^{iv}	2.49 (2)
O64···O67^{v}	2.62 (5)	O37···O37^{ii}	2.802 (16)	O1···O42^{iii}	2.55 (3)	O90···O35^{iv}	2.47 (2)
				O2···O42^{iii}	2.44 (3)	O90···O22^{iii}	2.81 (2)
				LA–water		O92···O24^{vi}	2.47 (3)
				S2···O91	2.21 (4)	O92···O44^{vi}	2.81 (3)
				O2···O92	2.82 (4)	O92···O33^{vi}	2.93 (3)
						O93···O25^{vii}	2.85 (6)
						O94···O21^{viii}	2.48 (3)
						O94···O37^{viii}	2.70 (2)
						O94···O27^{ix}	2.72 (2)
						O94···O25^{iv}	2.81 (2)
						O94···O23^{iii}	2.47 (2)
						O96···O32^{iii}	2.82 (2)
						O97···O22^{viii}	2.49 (3)
						O97···O32^{viii}	2.45 (3)
						O97···O26^{ix}	2.86 (3)
						O97···O35^{ix}	2.45 (2)
						O98···O$_{67}$^{x}	2.62 (4)
						O98···O55^{xi}	2.47 (3)
						O98···O64^{xi}	2.45 (3)

Symmetry codes: (i) $2-x, y, 1-z$; (ii) $2-x, y, 2-z$; (iii) $\frac{3}{2}-x, -\frac{1}{2}+y, 1-z$; (iv) $-\frac{1}{2}+x, -\frac{1}{2}+y, -1+z$; (v) $\frac{3}{2}-x, \frac{1}{2}+y, 1-z$; (vi) $1-x, -1+y, 1-z$; (vii) $x, y, -1+z$; (viii) $-1+x, y, -1+z$; (ix) $1-x, y, 1-z$; (x) $-1+x, y, z$; (xi) $\frac{1}{2}-x, -\frac{1}{2}+y, 1-z$.

β-CD complexes (Makedonopoulou *et al.*, 1999). The larger tilt angles of the G_1 (25°) and G_5 (16°) glucose units compared with the average observed in CD complexes indicate a narrowing of the CD primary face at the G_1 and G_5 side of the CD host. The average intramolecular distances O2(G_n)—O3(G_n) (2.83 Å) within each glucose unit are at suitable hydrogen-bond distances. In the case of the intramolecular distances O3(G_n)—O2(G_{n+1}) of consecutive glucose units, the somewhat larger distances related to G_1 (2.97 Å), G_3 (2.92 Å), G_4 (2.93 Å) and G_7 (3.42 Å) glucose units (Table 2) indicate a slight enlargement of the β-CD secondary face. The seven glucose units have slightly distorted chair conformations with puckering theta angles between 0 and 7°, originating from the β-CD model used to construct the β-CD–LA inclusion compound. In the crystal structure determination and refinement of the β-CD–LA complex, the glucose units were treated as rigid bodies and therefore no further distortions of the glucose conformations were allowed.

The torsion angles τ_a at the exocyclic C5—C6 of the C5—C6—O6 primary groups correspond to syn-clinal (*sc*) for G_1, G_3 and G_7, anti-clinal (*ac*) for G_2, G_4 and G_5, and anti-periplanar (*ap*) for G_6.

The positioning of the O6 atoms with respect to the β-CD cavity was assessed in relation to the least-squares plane through C2–C3–C5–O5 of the corresponding glucose unit. The O6 atoms of G_2, G_3, G_4, G_5, G_6 and G_7 are outside the cyclodextrin cavity and only the O6 of G_1 points into the macrocycle cavity. The orientations of most O6 atoms outside the CD cavity accommodate the LA inclusion from the primary face of the β-CD cavity (see §3.2). In general, hydrogen bonds with O6(G_n) of neighbouring cyclodextrin molecules are formed if the O6(G_n) are in the same position as the C2–C3–C5–O5 plane. The fact that no O6 atom in the β-CD–LA inclusion complex satisfies this criterion explains the presence of a few possible hydrogen bonds between the O6 atoms of neighbouring cyclodextrins (Table 4) and their extensive involvement in the intramolecular hydrogen bonding (Table 3).

The cyclodextrins are packed in the channel-type head-to-head arrangement which was encountered in other complexes that crystallize in the *C*2 space group (Borodi *et al.*, 2008). The cyclodextrins form tubular dimers (Fig. 4*a*) in which the CDs' primary rims are linked together by two O6···O6 and two water (O98)···O6 intermolecular close contacts (Table 4). The two secondary rims of each dimer are also involved in two close contact interactions with secondary rims of adjacent dimers and with the water molecules (Table 4). The crystal structure contains nine water molecules out of which seven are positioned outside the CDs' cavities and two (O91, O95) are situated at the secondary faces of the β-CD dimers. In addition, O91 is in close contact with a sulfur (S2) atom of the LA molecule. All the water molecules located outside the CDs' cavities are involved in the CDs' hydrogen bonding: O90, O92, O93, O94, O96, O97 contribute to the hydrogen bonding between the secondary faces of the β-CD dimers and only one water molecule (O98) to the hydrogen bonding of the dimers' corresponding primary faces (Table 4). The other two water molecules (O91, O95) are in close contact with each other and with O97, most likely forming hydrogen bonds. Besides the nine water molecules present in the crystal structure there are

still three voids in the unit cell with volumes in the range 75–113 Å^3. These voids are located in the neighbourhood of the water molecules (O91, O92, O95) and therefore are likely to accommodate the disorder of these water molecules in the structure. Disorder is quite common in the cyclodextrin inclusion complexes since around 70% of the structures reported in the Cambridge Structural Database (CSD) are affected by disorder.

The channel packing of the CD molecules with the water molecules present in between the CD layers is shown in Figs. 5(*a*) and (*b*).

3.2. Mode of inclusion and guest conformation

Crystallization of the β-CD inclusion complex with racemic LA in the C2 chiral space group indicates that the complex is not a racemic compound and that a racemic conglomerate is likely to be formed.

The LA molecule is included into the cyclodextrin cavity through the primary rim (narrow face) with the five-membered ring close to the macrocycle O4(G_n) plane. The S1 atom of the guest LA molecule is located at a distance of d = 0.191 (17) Å from this plane. Both carboxyl O atoms, O1 and O2, of LA are in close contact with the G_2 glucose of a neighboring cyclodextrin molecule (Table 4). The two water molecules (O91, O95) located at the secondary face of the β-CDs are in close contact and one of them (O91) forms a S···O close contact with the S2 of the LA molecule. S···O close contacts between 2.0 and 3.25 Å are common especially in conjugated ring systems (Burling & Goldstein, 1993). When viewed down the *b* axis, two symmetry-related LA molecules have their fatty acid chains outside the hydrophobic CD cavity and they are filling the space between the slightly shifted CDs of a dimer (Fig. 4*b*). As a result, the fatty acid chain of LA

Table 5
Torsion angles (°) of the guest LA molecule after energy minimization and after Rietveld refinement.

Torsion angle	LA after geometry optimization (in vacuum)	LA after the Rietveld refinement
S1—S2—C3—C4	149	136
C1—C2—C3—C4	−171	−159
S2—C3—C4—C5	−61	98
C2—C3—C4—C5	60	−146
C3—C4—C5—C6	179	99
C4—C5—C6—C7	−178	−111
C5—C6—C7—C8	−179	157
C6—C7—C8—O2	178	63
C6—C7—C8—O1	−1	−141

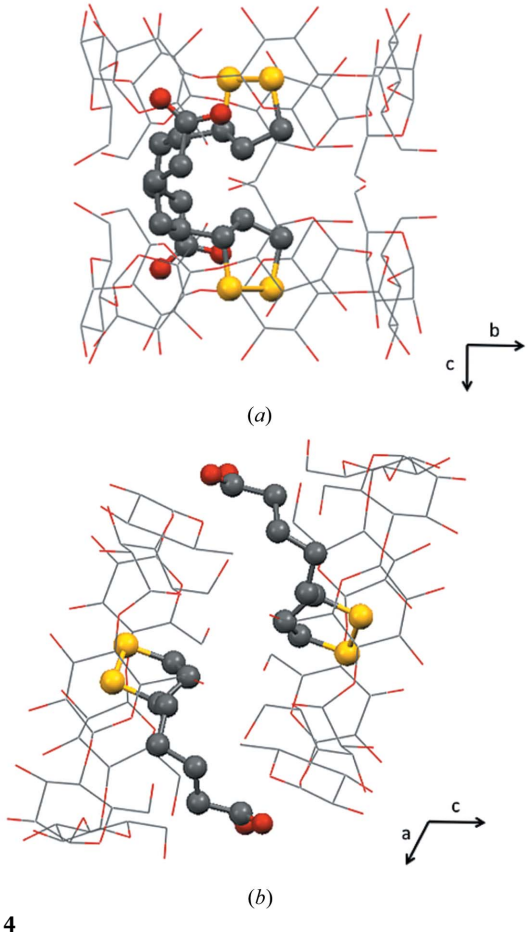

Figure 4
Molecular packing of the β-CD dimers with included lipoic acid (LA), viewed along (*a*) the *a* axis and (*b*) the *b* axis. The water O atoms are omitted for clarity.

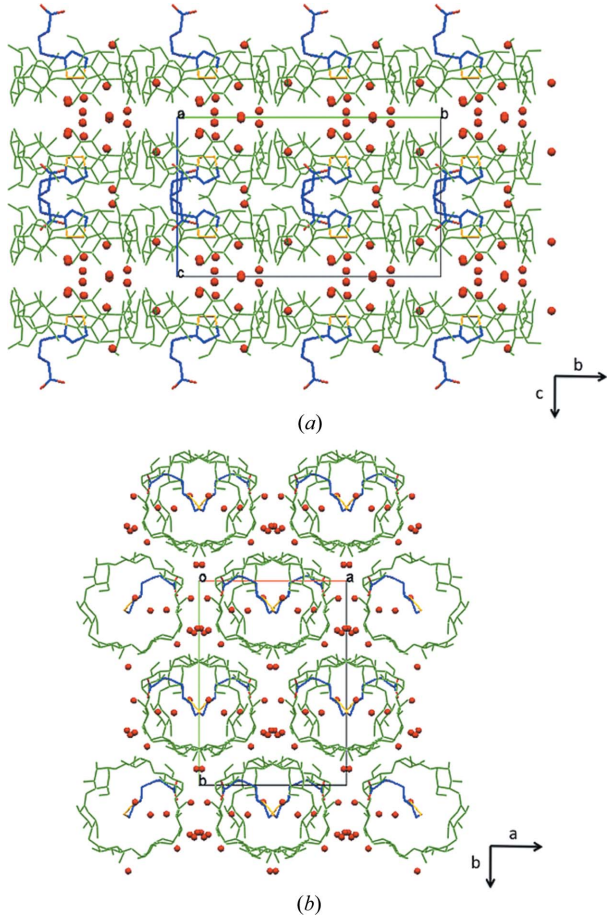

Figure 5
Molecular packing of the β-CD–LA inclusion complex, viewed along (*a*) the *a* axis and (*b*) the *c* axis.

adopts a bent conformation compared with the LA model after geometry optimization in a vacuum (Table 5). Unusual U-shaped conformations of the alkyl chains of saturated fatty acids were also found in their complexes with cucurbit[n]uril, a family of host molecules with cavities similar to cyclodextrins (Young *et al.*, 2008).

The channel-like packing of the LA inclusion complex in β-CD is stabilized by guest–host hydrogen bonding and close contacts, the β-CD–β-CD intermolecular interactions and hydrogen bonding involving the water molecules.

4. Conclusions

Model building by Parallel Tempering algorithm and Rietveld refinement based on rigid bodies and stereochemical restraints enabled the crystal structure determination of a large molecular system with 98 non-H atoms even from laboratory X-ray powder diffraction data.

The results showed that the water molecules positioned by Fourier synthesis and by voids calculation contribute to the intermolecular hydrogen bonding and consequently to the stabilization of the overall structure.

This work was supported by ANCS, project POSCCE ID536.

References

Accelrys Software Inc. (2010). *Materials Studio*, Release 5.5. Accelrys Software Inc., San Diego, CA, USA, http://accelrys.com/products/materials-studio/.

Allen, F. H. (2002). *Acta Cryst.* B**58**, 380–388.

Bharat, S., Cochran, B. C., Hsu, M., Liu, J., Ames, B. N. & Andersen, J. K. (2002). *Neurotoxicology*, **23**, 479–486.

Bilal, M., Brauer, C. D., Claudy, P., Germain, P. & Létoffé, J. M. (1995). *Thermochim. Acta*, **249**, 63–73.

Borodi, G., Bratu, I., Dragan, F., Peschar, R., Helmholdt, R. B. & Hernanz, A. (2008). *Spectrochim. Acta Part A*, **70**, 1041–1048.

Burling, F. T. & Goldstein, B. M. (1993). *Acta Cryst.* B**49**, 738–744.

David, W. I. F., Shankland, K., van de Streek, J., Pidcock, E., Motherwell, W. D. S. & Cole, J. C. (2006). *J. Appl. Cryst.* **39**, 910–915.

Erden, N. & Celebi, N. (1988). *Int. J. Pharm.* **48**, 83–89.

Femiano, F. & Scully, C. (2002). *J. Oral Pathol. Med.* **31**, 267–269.

Femiano, F., Scully, C. & Gombos, F. (2002). *Int. J. Oral Maxillofac. Surg.* **31**, 625–628.

Guo, P., Su, Y., Cheng, Q., Pan, Q. & Li, H. (2011). *Carbohydr. Res.* **346**, 986–990.

Hapiot, F., Tilloy, S. & Monflier, E. (2006). *Chem. Rev.* **106**, 767–781.

He, Y., Fu, P., Shen, X. & Gao, H. (2008). *Micron*, **39**, 495–516.

Holmquist, L., Stuchbury, G., Berbaum, K., Muscat, S., Young, S., Hager, K., Engel, J. & Münch, G. (2007). *Pharmacol. Ther.* **113**, 154–164.

Hypercube Inc. (2002). *HyperChem*, Version 7.52. Hypercube Inc., Florida, USA.

Jones, W., Li, X., Qu, Z. C., Perriott, L., Whitesell, R. R. & May, J. M. (2002). *Free Radic. Biol. Med.* **33**, 83–93.

Larson, A. C. & Von Dreele, R. B. (2000). *GSAS*. Report LAUR 86–748. Los Alamos National Laboratory, New Mexico, USA.

Le Bail, A., Duroy, H. & Fourquet, J. L. (1988). *Mater. Res. Bull.* **23**, 447–452.

Maeda, H., Onodera, T. & Nakayama, H. (2010). *J. Incl. Phenom. Macrocycl. Chem.* **68**, 201–206.

Makedonopoulou, S., Tulinski, A. & Mavridis, I. M. (1999). *Supramol. Chem.* **11**, 73–81.

Marques, J., Anjo, L., Maria, P. M., Marques, M. P. M., Santos, T. M., Almeida Paz, F. A. & Braga, S. S. (2008). *J. Organomet. Chem.* **693–18**, 3021–3038.

Neumann, M. A. (2003). *J. Appl. Cryst.* **36**, 356–365.

Nowell, H., Attfield, J. P. & Cole, J. C. (2002). *Acta Cryst.* B**58**, 835–840.

Packer, L., Kraemer, K. & Rimbach, G. (2001). *Nutrition*, **17**, 888–895.

Packer, L., Witt, E. H. & Tritschler, H. J. (1995). *Free Radic. Biol. Med.* **19**, 227–250.

Pop, M. M., Goubitz, K., Borodi, G., Bogdan, M., De Ridder, D. J. A., Peschar, R. & Schenk, H. (2002). *Acta Cryst.* B**58**, 1036–1043.

Ribeiro, L. S., Falcão, A. C., Patrício, J. A., Ferreira, D. C. & Veiga, F. J. (2007). *J. Pharm. Sci.* **96**, 2018–2028.

Rodriguez, J. & Elola, M. D. (2009). *J. Phys. Chem. B*, **113**, 1423–1428.

Sola, S., Mir, M. Q., Cheema, F. A., Khan-Merchant, N., Menon, R. G., Parthasarathy, S. & Khan, B. V. (2005). *Circulation*, **111**, 343–348.

Spek, A. L. (1990). *Acta Cryst.* A**46**, C34.

Steiner, T. & Koellner, G. (1994). *J. Am. Chem. Soc.* **114**, 5122–5128.

Stroud, R. M. & Carlisle, C. H. (1972). *Acta Cryst.* B**28**, 304–307.

Szejtli, J. (1998). *Chem. Rev.* **98**, 1743–1754.

Takahashi, H., Bungo, Y. & Mikuni, K. (2011). *Biosci. Biotechnol. Biochem.* **75**, 633–637.

Tang, J., Wingerchuk, D. M., Crum, B. A., Rubin, D. I. & Demaerschalk, B. M. (2007). *Neurologist*, **13**, 164–167.

Toby, B. H. (2001). *J. Appl. Cryst.* **34**, 210–213.

Tong, L. H., Pang, Z. Z. & Yi, Y. (1995). *J. Incl. Phenom. Mol. Recognit. Chem.* **23**, 119–126.

Tsorteki, F., Bethanis, K., Pinotsis, N., Giastas, P. & Mentzafos, D. (2005). *Acta Cryst.* B**61**, 207–217.

Uekama, K. & Hirayama, F. (1996). *The Practice of Medicinal Chemistry*, pp. 793–825. London: Academic Press.

Vincent, H. K., Bourguignon, C. M., Vincent, K. R. & Taylor, A. G. (2007). *J. Altern. Complement. Med.* **13**, 577–584.

Visser, J. W. (1969). *J. Appl. Cryst.* **2**, 89–95.

Wenz, G., Han, B. H. & Müller, A. (2006). *Chem. Rev.* **106**, 782–817.

Young, H. K., Hyunuk, K., Youngkook, K. & Kimoon, K. (2008). *Angew. Chem. Int. Ed.* **47**, 4106–4109.