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The effect of 1-deoxy-D-lactose upon the crystallization of D-lactose.

*Kevin P. Guiry[1], Simon J. Coles[2], Humphrey A. Moynihan[1], Simon E. Lawrence[1]**

[1] Department of Chemistry / Analytical and Biological Chemistry Research Facility, University College Cork, Cork, Ireland; [2]EPSRC National Crystallography Service, University of Southampton, Southampton, SO17 1BJ, UK

s.lawrence@ucc.ie

RECEIVED DATE:

* Phone: +353 21 490 3143, FAX: +353 21 427 4097

ABSTRACT

The synthesis and crystal structure of 1-deoxy-D-lactose is described, as is its use as an additive in the crystallization of D-lactose. This has resulted in the formation of αβ-D-lactose for the first time at room temperature. Single crystal analysis shows differences between this analysis and the structure previously obtained from powder data, particularly in the hydrogen-bonding patterns.

KEYWORDS: D-lactose, crystal polymorphism, 1-deoxy-D-lactose, αβ-D-lactose, tailor-made additives

INTRODUCTION

D-Lactose (**1**)**,** a disaccharide, is a by-product of the milk industry. Its main applications are as a pharmaceutical excipient[1] and as a food additive.[2] Quality is of high importance in these applications; hence crystallization control during processing and an understanding of how and why crystals form is crucial.[3] The size and shape of the crystals is determined by internal (structure and defects) and external factors (impurities, additives, temperature, solvents, supersaturation, agitation, etc).[4] In food and pharmaceutical formulations, surface interactions between particles are influential in determining the efficiency of processes such as compaction, dissolution etc.[5] These interactions help to define the bulk and surface properties of the material. Controlling the morphology of crystals during the crystallization

process is of great importance as undesired changes in this can have major implications in the manufacturing process.

D-Lactose exists in two anomeric forms, α and β-D-lactose,[6] shown in Figure 1. The most stable crystalline form of D-lactose is α-D-lactose monohydrate which is usually obtained through crystallization from aqueous solutions. It crystallizes in the chiral space group $P2_1$, with a = 7.982(2) Å, b = 21.562(3) Å, c = 4.824(1) Å, and β = 109.57(3)º.[7,8] The water molecule is hydrogen-bonded to the α-D-lactose molecules via, amongst others, the anomeric oxygen. Crystals of α-D-lactose monohydrate exhibit asymmetric shape and a polar morphology.[9]

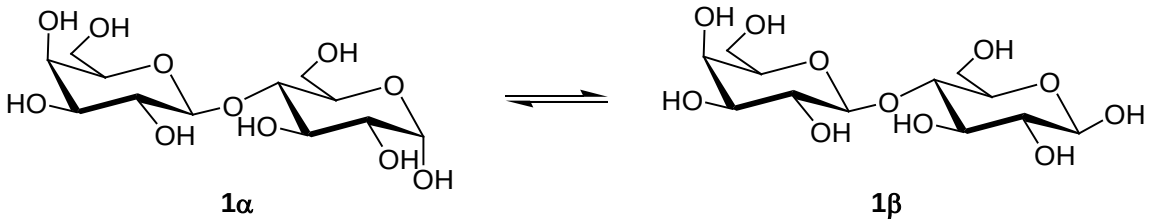
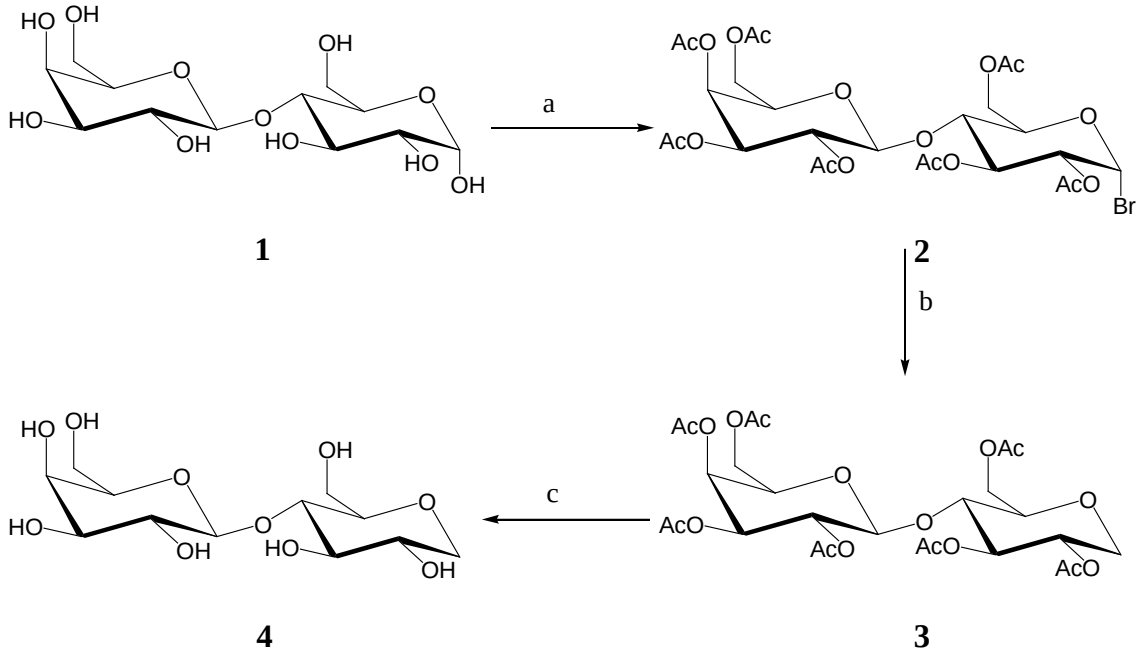


Figure 1. The molecular structures of the α and β anomers of D-lactose.

A consequence of aqueous crystallization is that, due to mutarotation, both anomers of D-lactose are simultaneously present in the solution with an equilibrium α/β ratio, at room temperature, of 37:63.[10] These conditions are believed to determine the characteristic morphology of α-D-lactose monohydrate crystals. This morphology is referred to as 'tomahawk-shaped'.[11] Such crystals only show growth in the [010] direction.[11]

For the purpose of crystallization of α-D-lactose monohydrate the β anomer can be considered an impurity and as such may act as a habit modifier.[12] This has been debated in the literature.[10,12-13] Reducing the concentration of β-D-lactose present leads to a more prismatic habit of α-D-lactose monohydrate crystals.[12] It was proposed that the behavior of β-D-lactose as a habit modifier was due to the similarity in the molecular structure of the two anomers which both contain the same galactosyl moiety linked to different anomers of glucose. Hence, the $(0\ \bar{1}\ 0)$ face of the crystal with tomahawk morphology can easily absorb β-D-lactose molecules which fit perfectly onto the glucose-moiety of the crystal. Once the β-D-lactose molecules have been adsorbed, they inhibit further growth. Adsorption on the (010) face is unlikely since at this face the lactose molecules are attached by their glucose groups which differ in the α and β-D-lactose forms. This theory explains the observed growth in one polar direction, [010], with inhibition of growth observed in the $[0\ \bar{1}\ 0]$ direction.

Crystallization of lactose in the presence of impurities such as mono- and disaccharides has resulted in significant habit modification.[14] However, the exact nature of the effects of crystallization in the presence of these additives are not clear. For example, the use of other disaccharides means that the exact relationship between these impurities and the crystallizing lactose moiety is complicated by the ability of the impurity to undergo mutarotation in some cases. For other crystal systems, e.g. glutamic acid, a tailor-made additive approach has been successful for both habit modification and for influencing polymorph outcomes.[15-24] This involves the addition of an impurity which has been designed to interact in some manner with the crystallizing material. Use of an additive where the mutarotation phenomenon can be avoided but which remains a close structural analogue of D-lactose will allow examination of the carbohydrate additive effect without the complication of mutarotation. Herein we report the preparation, crystal structure and effect on D-lactose crystallization of such an additive; 1-deoxy-D-lactose, **4**, Scheme 1. The issue of existence of different anomers does not arise for this compound.



Scheme 1. Preparation of 1-deoxy-D-lactose: (a)[25] AcBr/AcOH, room temperature, 1hr, 100%; (b) Bu_3SnH, AIBN, MeOH, 67°C, 1hr, 67%; (c) KOMe/MeOH, 1hr, 54%.

EXPERIMENTAL

General Procedures

Analytical grade toluene and acetonitrile were used as supplied by Sigma-Aldrich. All commercial reagents were used without further purification.

The ^{1}H NMR spectra was recorded at 300 MHz on a Bruker advance 300 spectrometer with tetramethylsilane (TMS) as the reference absorption peak. Chemical shifts (δ_H) are given in parts per million (ppm).

Infrared spectra were recorded as KBr discs (solids) on a Perkin Elmer Paragon 1000 FT-IR spectrometer. Melting points were measured on an Electrothermal 9100-Melting Point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck Silica Gel 60, F_{254}). Wet flash column chromatography was carried out using Merck silica gel 60, typically with a 30:1 ratio of silica to sample.

Powder X-ray diffraction was performed using a Philips PANalytical X'Pert Pro diffractometer with a PW 3830 generator, a PW 3710 MPD diffractometer and an X'Celerator detector (40 mA / 45 kV, CuKα radiation, 2θ from 5 ^{o} to 75 ^{o} continuous scan mode, step size of 0.0167 ^{o}, step time of 10.16 s) for the samples of 1-deoxy-D-lactose, αβ-D-lactose and α-D-lactose monohydrate, shown later in Figure 4. A Siemens D5000 diffractometer (40 mA / 40 kV, CuKα radiation, 2θ from 5 ^{o} to 45 ^{o} continuous scan mode, step size of 0.02 ^{o}, step time of 1.8 s) was used for αβ-D-lactose and anhydrous α-D-lactose, shown in the Electronic Supporting Information**.**

Mass spectra were recorded on a Kratos Profile HV-4 double focusing high-resolution mass spectrometer (EI) in electron impact (E.I.) mode with an ionization voltage of 70 eV.

Elemental analyses were performed by the Microanalysis Laboratory, University College Cork, using a Perkin Elmer 240 and an Exeter Analytical CE440 elemental analyzer.

1-Bromo-1-deoxy- 2,2',3,3',4',6,6'-hepta-*O*- acetyl-α-D-lactose (2) [25]

To a solution of α-D-lactose monohydrate (**1**) (3.0 g, 8.3 mmol) in acetic acid (20 mL) was added acetyl bromide (10.8 mL, 133.2 mmol) and the mixture was stirred at room temperature until a homogenous solution was obtained. The mixture was concentrated under reduced pressure at 70 ^{o}C and co-evaporated three times with dry toluene (20 mL) to give a foam. This was used without further purification. (5.66 g, 100%). (m.p. 68-70 ^{o}C), ν_{max}/ cm^{-1} (KBr): 2970 (s), 1752 (s), 1372 (m), 1218 (s), 1050 (m). δ_H

($CDCl_3$): 1.96-2.18 (21H, m), 3.87-3.89 (2H, m), 4.09-4.19 (4H, m), 4.49-4.52 (2H, m), 4.85 (1H, dd, *J* 8, 3Hz), 4.97 (1H, dd, *J* 4, 4Hz), 5.13 (1H, dd, *J* 4, 6Hz), 5.36 (1H, d, *J* 3Hz), 5.56 (1H, m), 6.52 (1H, d, *J* 4Hz).

1-Deoxy-2,2',3,3',4',6,6'-hepta-*O*- acetyl-D-lactose (3)

To a solution of AIBN (0.162 g, 0.98 mmol) in dry toluene (8 mL) was added drop-wise tributyltin hydride (2.6 mL, 9.7 mmol). The resultant solution was added to the crude **2** (5.8 g, 8.3 mmol) in dry toluene (20 mL) under nitrogen at 67 ^{o}C over 1 h while stirring. Once the addition was complete, the reaction was kept at 67 ^{o}C for 30 min. The solution was concentrated under reduced pressure and the resulting residue dissolved in acetonitrile (50 mL). The acetonitrile layer was then extracted with hexane (3 x 40 mL) and subsequently concentrated under reduced pressure. The crude compound was purified using column chromatography over silica gel (diethyl ether). The product was obtained as a white foam. (3.37 g, 67 %), (m.p. 73-75 ^{o}C), ν_{max}/ cm^{-1} (KBr): 2964 (s), 1751 (s), 1371 (m), 1233 (s), 1052 (m).

δ_H/ppm ($CDCl_3$): 2.02-2.17 (21H, m), 3.29 (1H, m), 3.47- 3.54 (1H, m), 3.71 (1H, m), 3.89 (1H, m), 4.04-4.15 (4H, m), 4.47-4.50 (2H, m), 4.94- 4.98 (2H, m) 5.09- 5.22 (2H, m), 5.36 (1H, m). MS-ESI: Found *m/z*, 643.1861, Calculated for $C_{26}H_{36}O_{17}Na$ $[M+Na]^+$, 643.1850. Elemental analysis calculated ($C_{26}H_{36}O_{17}$): C, 50.32; H, 5.85. Found C, 49.97; H, 5.73.

1-Deoxy-D-lactose (4)

To a solution of **3** (1.75 g, 2.82 mmol) dissolved in dry methanol (17.5 mL) was added potassium methoxide (0.1145 g, 1.633 mmol) while slowly stirring. The resulting solution was stirred at room temperature overnight. The product was isolated by filtration and washed with cold methanol (20 mL x 2). Re-crystallization from boiling methanol produced needle-like crystals. (0.49 g, 54 %), (m.p. 207-209 ^{o}C) ν_{max}/ cm^{-1} (KBr): 3402 (w,br), 2899 (s), 1458 (m), 1098 (s), 1016 (m). δ_H/ppm (D_2O): 3.13-3.23 (2H, m), 3.34- 3.69 (7H, m), 3.80- 3.88 (4H, m), 4.31- 4.33 (2H, m). MS-ESI: Found *m/z*, 325.1148, Calculated for $C_{12}H_{21}O_{10}$ $[M]^-$, 325.1135. Elemental Analysis calculated ($C_{12}H_{22}O_{10}$): C, 44.17; H, 6.80. Found C, 44.00; H, 6.75.

Crystallization Experiments

Aqueous D-lactose solutions (10% w/w) were prepared, to which was added anhydrous methanol so that the final ratio of methanol to solution was 10:1.[26] For experiments without use of the additive, the solution was left to stand at room temperature for 24 h, after which crystals of α–D-lactose monohydrate were present. The crystals were filtered and allowed to dry in air at room temperature. In the experiments involving the additive, methanol was added to the aqueous D-lactose solution in the same

manner as described above. Then the additive was added to the solution, which was left to stand for 24 h. Different concentrations of additive were used, see Table 2. Crystalline material was filtered and allowed to dry in air at room temperature. Crystallization with 10% w/w of additive yielded very small crystals of αβ-D-lactose with needle habit (0.4799 g, 48 %), (m.p. 218-220 ^{o}C) ν_{max}/ cm^{-1} (KBr): 3387 (s), 2850 (s), 1450(m), 1095(s), 1032 (m).

Crystallographic Experimental Section

Crystals of 1-deoxy-D-lactose (**4**) are monoclinic, $P2_1$, $C_{12}H_{22}O_{10}$, M = 326.30, a = 4.69300(10) Å, b = 19.9373(4) Å, c = 7.5503(2) Å, β = 103.1590(10) º, U = 687.90(3) Å^3, F(000) = 348, μ(Mo-Kα) = 0.139 mm^{-1}, T = 120 K, R(F_o) = 0.0269 for 1528 observed reflections with I > 2σ(I), wR_2(F^2) = 0.0676 for all 1614 unique reflections. Data in the θ range 2.77–27.47° were collected on a Bruker-Nonius KappaCCD diffractometer, equipped with a Nonius FR591 rotating anode generator employing Mo-Kα graphite monochromated radiation, λ= 0.7107 Å. The crystal size was 0.35 x 0.30 x 0.10 mm.

Crystals of αβ-D-lactose are triclinic, $P1$, $C_{12}H_{22}O_{11}$, M = 342.30, a = 5.030(3)Å, b = 7.593(5) Å, c = 19.374(12) Å, α = 81.026(10) º, β = 85.044(9) º, γ = 74.247(9) º, U = 702.7(8) Å^3, F(000) = 364, μ(Mo-Kα) = 0.145 mm^{-1}, T = 120 K, R(F_o) = 0.0746 for 4292 observed reflections with I > 2σ(I), wR_2(F^2) = 0.2067 for all 4678 unique reflections. Data in the θ range 2.74–29.62° were collected on the Daresbury SRS station 9.8 diffractometer[27] using synchrotron silicon (111) monochromated radiation, λ= 0.6911 Å. The crystal size was 0.12 x 0.04 x 0.02 mm.

All data were corrected for Lorentz and polarisation effects. The structures were solved by direct methods and refined by full-matrix least-squares using all F^2 data. The SHELXS, SHELXL-97 and PLATON programs were used.[28-30] All non-hydrogen atoms were refined with anisotropic displacement factors. The C-H hydrogen atoms were placed in calculated positions and the hydroxyl hydrogen atoms were determined by difference maps. All hydrogen atoms were allowed to ride on the parent atom. For compound **4** it was necessary to merge the data and therefore the absolute structure is based on the synthetic procedure. The crystal of αβ-D-lactose was twinned and the absolute structure is based on the synthetic procedure. Full structural data have been deposited at the Cambridge Crystallographic Data Centre. CCDC reference numbers are 650837 and 650838.

RESULTS and DISCUSSION

1-deoxy-D-lactose, **4**, was designed as a tailor-made additive for the crystallization of D-lactose for three reasons: (i) it has structural similarity to D-lactose, (ii) it cannot exhibit mutarotation and (iii) it has no hydrogen-bonding capability at the anomeric position. The consequence of the first reason is that **4**

should be able to interact with D-lactose, either during nucleation or during crystal growth. The inability to display mutarotation should simplify the experiment and facilitate understanding of the effects of carbohydrate additives on lactose crystallization. The lack of hydrogen-bonding at the anomeric position may well disrupt the nucleation or growth of the most stable form of lactose, *viz* α-D-lactose monohydrate.

1-deoxy-D-lactose (**4)** was prepared as outlined in Scheme 1. 1-Bromo-1-deoxy-2,2',3,3',4',6,6'-*O*-hepta-acetyl-D-lactose (**2)** was prepared using a procedure reported by Koto *et al.*[25] where a binary AcBr-AcOH mixture was reacted with α-D-lactose monohydrate yielding the desired product in quantitative yields. The product was obtained in the α configuration at the anomeric position.[25] 1-Deoxy-2,2',3,3',4',6,6'-*O*-hepta-acetyl-D-lactose (**3**) was subsequently prepared *via* the tin hydride free radical reaction.[31] There are various conditions which can be employed for the deacetylation of sugars in alcoholic solutions of alkoxides, which include methanolic solutions of sodium methoxide, barium methoxide and 16% ammonia.[32] We tried several of these methods and found the method of Parrish *et al*. gave the best results i.e. 14 mmol potassium methoxide in methanolic solution. Using this method the product precipitated out of solution after 1 hr on all occasions. The crude **4** obtained was re-crystallized from boiling methanol to give single crystals, which showed excellent analytical data. These crystals, Figure 2, have a needle-like morphology and show a curved face, similar to α-D-lactose monohydrate.[11]

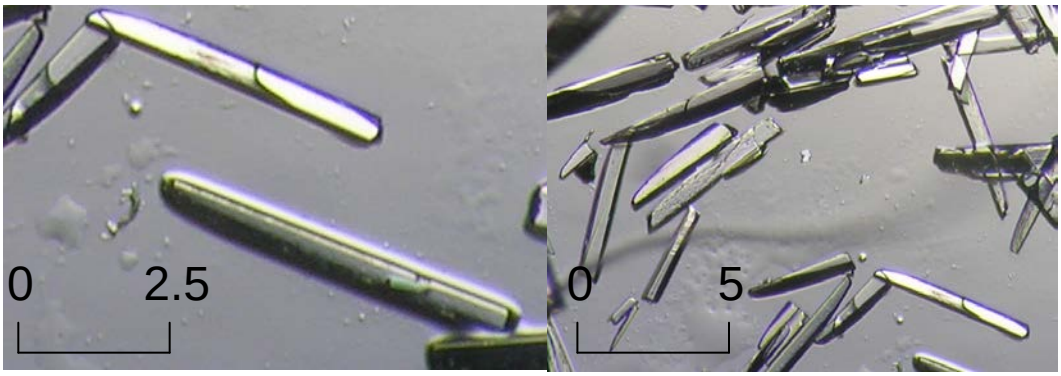


Figure 2. Representative crystals of **4**, obtained through re-crystallization from methanol. Scale bars indicate distance in millimetres.

The crystal structure of **4** reveals an extensive hydrogen-bonding network, Figure 3 and Table 1, with similarities to that seen for α-D-lactose monohydrate.[8] There are 2 intra-molecular hydrogen-bonds and six inter-molecular hydrogen-bonds. The two intra-molecular hydrogen-bonds O3-H3…O1' and O3-H3…O6' form S(7) and S(10) rings respectively, and the combination of the two leads to an $R_1^2(5)$ ring. The inter-molecular hydrogen bond O4'-H4'…O6 gives rise to C(11) chains along the y-axis, related by

the 2_1 screw axis. The O6'-H6'…O3' hydrogen bond gives rise to C(7) chains in the positive z-direction, with the O2'-H2'…O3 hydrogen bond forming C(8) chains in the negative z-direction. The O2-H2…O2' hydrogen bond leads to C(9) chains along the [101] direction. Both the O3'-H3'…O6' and the O6-H6…O2 hydrogen bonds form C(7) chains in the opposite sense, [$\bar{1}$ 0 $\bar{1}$]. Combination of these two lead to an $R_2^2(11)$ ring.

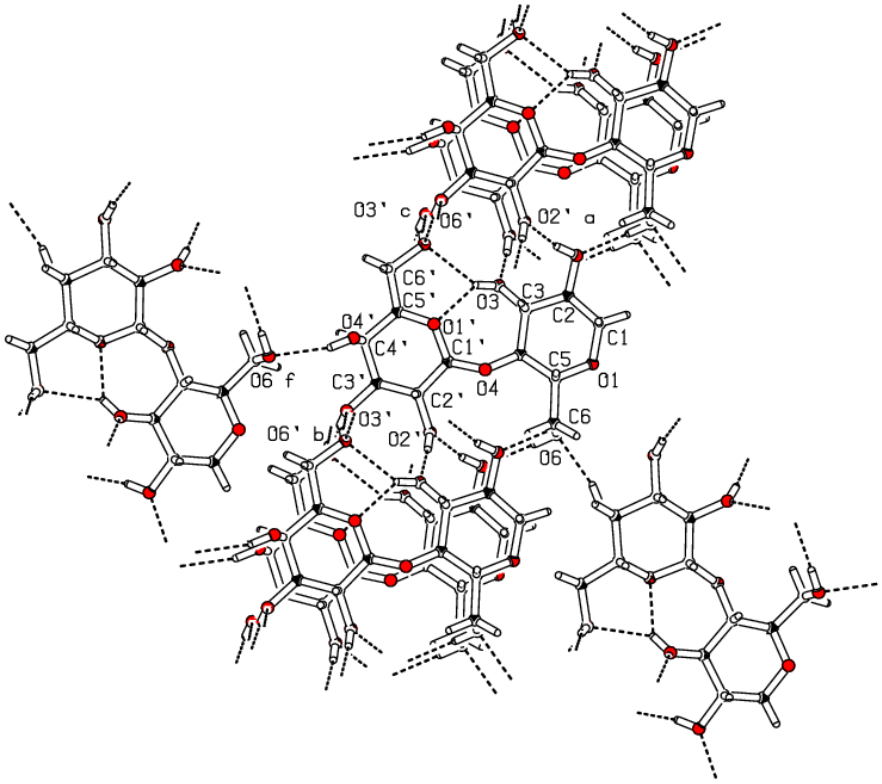


Figure 3. A view of **4** showing its numbering scheme and hydrogen bonding.

Donor -- H··Acceptor	[ARU]	H···A	D···A	D - H···A
O2' – H2'···O3	[x, y,-1+z]	1.87	2.7097(18)	174
O2 – H2···O2'	[1+x, y, 1+z]	1.88	2.6889(19)	162
O3 – H3···O1'		2.05	2.7430(15)	139
O3 – H3···O6'		2.21	2.9086(17)	140

O3’ – H3’⋯O6’	[-1+x, y, -1+z]	1.83	2.6646(14)	174
O4’ – H4’⋯O6	[-x, ½+y, -z]	2.07	2.8927(17)	167
O6 – H6⋯O2	[-1+x, y, -1+z]	1.93	2.7719(17)	176
O6’ – H6’⋯O3’	[x, y, 1+z]	1.85	2.6835(14)	172

Table 1. Hydrogen bonding information for **4**.

All crystallization experiments were undertaken in the conditions which normally produce exclusively α-D-lactose monohydrate. The products of the crystallization experiments both with and without the additive **4** began to show the production of crystalline material after 6 hr and were left for a further 18 hr. The results of the crystallisation experiments are shown in Table 2, and the associated PXRD data are shown in Figure 4.

Additive conc., w/w	Form obtained
0%	α-D-lactose monohydrate
1%	αβ-D-lactose / α-D-lactose monohydrate
5%	αβ-D-lactose / α-D-lactose monohydrate
10%	αβ-D-lactose

Table 2 Results from crystallizations carried out on D-lactose, using varying amounts of **4** as an additive.

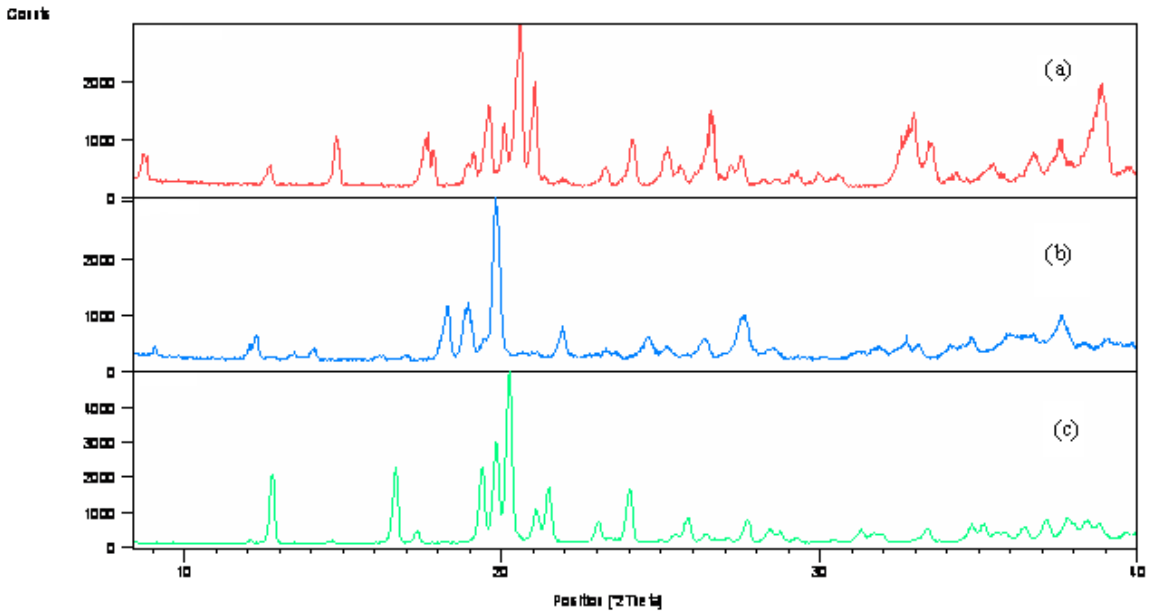


Figure 4. PXRD patterns of (a) 1-deoxy-D-lactose, **4** (b) αβ-D-lactose and (c) α-D-lactose monohydrate.

For the experiments using 0, 1 and 5% of additive, the presence of α-D-lactose monohydrate was confirmed by PXRD.[6] At concentrations of 10% w/w and higher it was found that another anhydrous crystalline form of D-lactose resulted. This form was also obtained, in a mixture with α-D-lactose monohydrate, from the other crystallization experiments involving lower quantities of additive **4**. The mixtures were determined by visual inspection since the crystals of this anhydrous material have a needle-like habit, Figure 5, and are significantly smaller than those of α-D-lactose monohydrate.

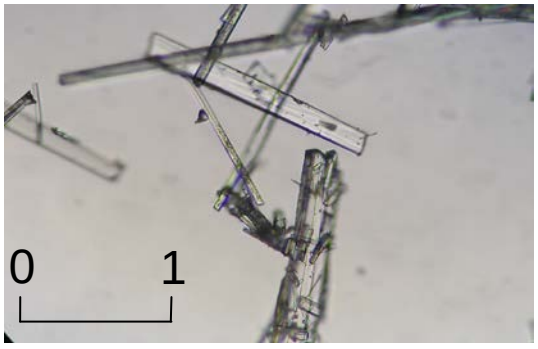


Figure 5. Representative crystals of αβ-D-lactose, obtained from crystallizations using **4** as an additive. Scale bars indicate distance in millimetres.

The PXRD pattern for the anhydrous material was similar to that of the mixed 1:1 αβ-D-lactose, which has previously been reported by thermal treatment of amorphous α-D-lactose.[33] The structure of αβ-D-lactose has been solved from powder data.[34] In order to confirm the identity of the anhydrous material

that we had obtained from the crystallisation experiments, we collected data at 120K for this compound using the Daresbury SRS station 9.8 diffractometer.[27] Analysis of the crystal revealed that the anhydrous material is αβ-D-lactose, Figure 6, with a structure similar to, but not identical to, that solved from powder data. This is the first time αβ-D-lactose has been produced at room temperature.

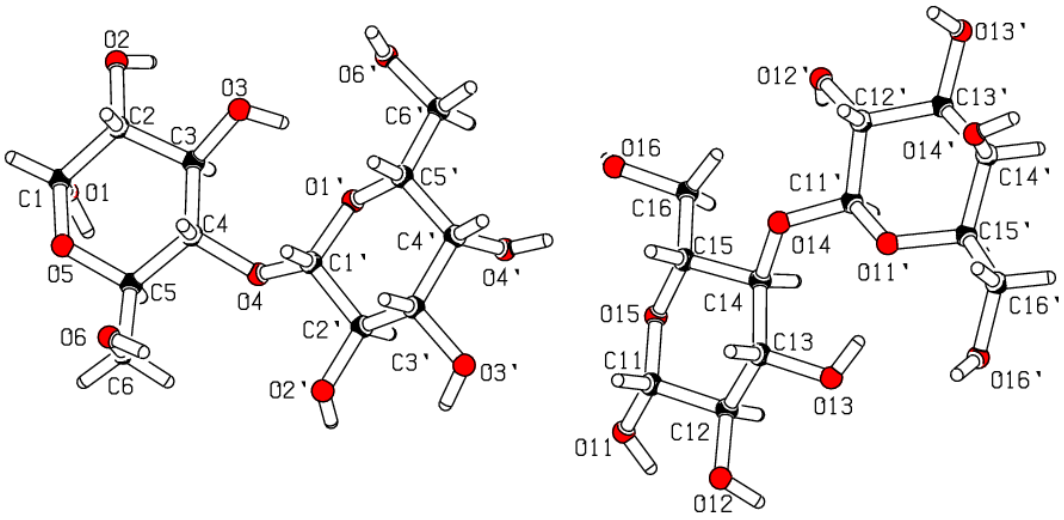


Figure 6 A view of αβ-D-lactose showing its numbering scheme. The α anomer is on the left.

The powder analysis had been undertaken at room temperature, and had significantly larger global isotropic displacement parameters for the glucosyl ring of the α anomer compared to the rest of the structure (B_{iso} is 8.7 cf. ≤ 3.3).[34] In the discussion of the powder data, it was postulated that this was due to orientational disorder of the glucosyl ring. Interestingly, the crystal used for our single crystal analysis was twinned, with the two components related by a 180 º rotation about c*.

Examination of the packing of the two structures, using the XPac approach and software,[35] shows that they have three dimensional isostructurality in terms of repeat motifs in the crystal structure. However simple overlay plots indicate that the oxygen of the CH_2OH group on the glucose ring of the α anomer is orientated in the opposite sense for our structure (see Electronic Supporting Information) and that there are positional differences between the two independent molecules. These effects give rise to significant differences in the hydrogen bonding between the two structural analyses. It is, therefore, meaningful to describe our structure, which, like all disaccharides, has an extensive hydrogen-bonding network, possessing five intra- and twelve inter-molecular hydrogen bonds, Figures 7 and 8 and Table 3. Two of these intra-molecular hydrogen bonds O3-H3A…O1' and O3-H3A…O6', form S(7) and S(10) rings respectively, and the combination of the two leads to an $R_1^2(5)$ ring. Two of the remaining intra-molecular hydrogen bonds are O3'-H3B…O2' and O13-H13A…O11', which form a S(5) ring and a S(7) ring respectively. The fifth intra-molecular hydrogen bond O11-H11A…O12 forms a S(5) ring.

Two of the inter-molecular hydrogen bonds, O2-H2A…O2' and O6-H6…O2, give rise to a C(9) chain along the [$\bar{1}$ 10] direction and a C(8) chain along the [1 $\bar{1}$ 0] direction respectively, with their combination giving rise to an $R_2^2(11)$ ring. Also forming hydrogen bonds in the [$\bar{1}$ 10] direction are O16-H16…O12 and O12'-H12A…O16', both of which give rise to C(8) chains. The hydrogen bond O16'-H16'…O12' gives rise to a C(8) chain in the [1 $\bar{1}$ 0] direction. The hydrogen bonds O11-H11A…O16 and O3'-H3B…O6' give rise to C(7) chains in the negative y-direction, while O2'-H2B…O3 forms a C(8) chain in the same sense. O6'-H6'…O3' and O13'-H13A…O16' form hydrogen bonding C(7) chains in the positive y direction. O4'-H4A…O15 forms a hydrogen bonded C(10) chain in the x-direction, while O14'-H14A…O5 gives rise to a C(10) chain in the [10 $\bar{1}$] direction.

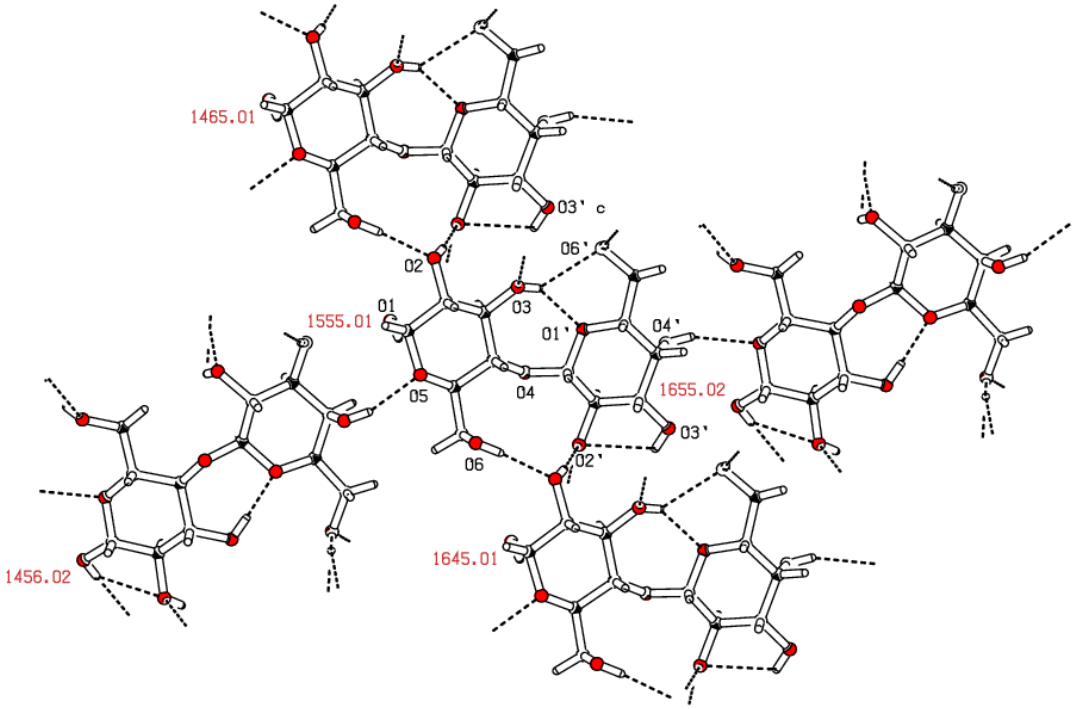


Figure 7 A view of αβ-D-lactose showing hydrogen bonding involving primarily the α-anomer. Symmetry codes are shown for clarity. The .01 and .02 extensions refer to the α and β anomers, respectively.

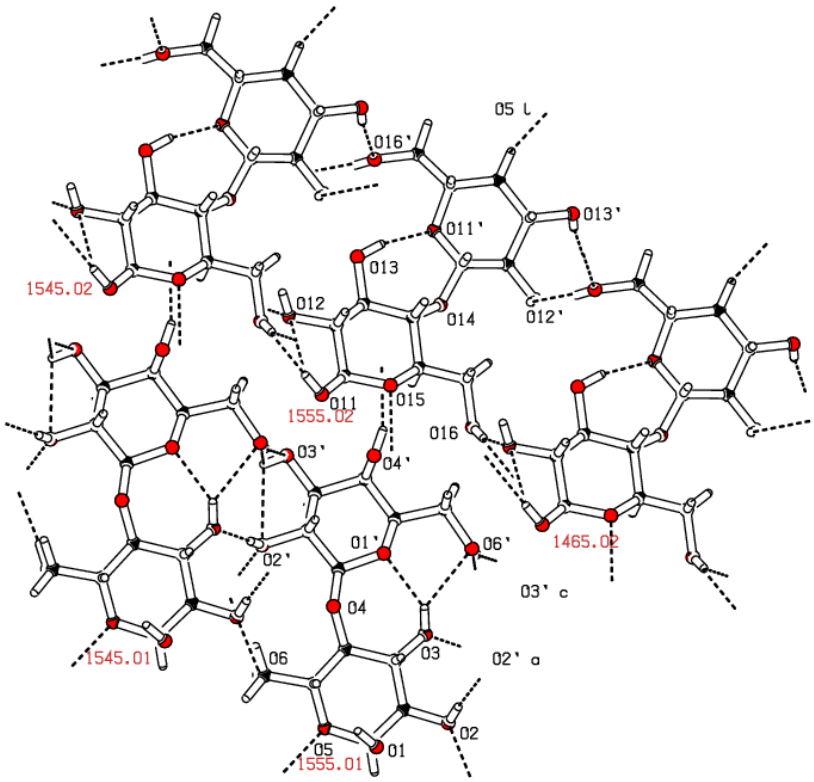


Figure 8 A view of αβ-D-lactose showing hydrogen bonding involving primarily the β-anomer. Symmetry codes are shown for clarity. The .01 and .02 extensions refer to the α and β anomers, respectively.

	This work			Powder analysis[34, a]		
Donor-H···Acceptor	H···A	D···A	D - H···A	H···A	D···A	D - H···A
α-anomer						
O1-H1A···O13'				2.74(4)	3.00(4)	95.6(21)
O2 – H2A···O2' [b]	1.89	2.707(6)	175	2.18(3)	2.55(3)	101.3(18)
O3 – H3A···O1'	2.03	2.730(5)	143			
O3 – H3A···O2'				2.74(4)	2.86(4)	87(3)
O3 – H3A···O6'	2.35	2.920(6)	127			
O6 – H6···O2 [c]	1.92	2.734(6)	176			
O2' – H2B···O3 [d]	1.92	2.726(6)	168	1.92(4)	2.86(4)	159.9(16)
O3' – H3B···O6' [d]	2.34	2.772(8)	114	2.07(3)	2.96(4)	148.5(21)

O3’ – H3B···O2’	2.42	2.847(6)	113			
O4’ – H4A···O15 [e]	2.16	2.943(6)	160	1.99(3)	2.60(3)	118.5(19)
O6’ – H6’···O3 [f]	1.96	2.772(8)	170	1.78(3)	2.68(3)	149.9(26)
O6-H6···O2				1.82(4)	2.48(4)	121.0(24)
β-anomer						
O11-H11A···O12	2.45	2.825(8)	109			
O11– H11A···O4’				2.82(3)	3.02(3)	91.7(19)
O11– H11A···O16 [d]	2.27	3.090(8)	179			
O12– H12A···O16				2.35(3)	3.09(3)	131.2(16)
O13 – H13A···O11’	2.06	2.813(5)	152	1.97(3)	2.81(3)	142.5(23)
O16 – H16···O12 [b]	1.91	2.710(6)	164	1.91(3)	2.52(3)	117.5(19)
O12’ – H12B···O13				1.97(4)	2.83(4)	144.1(21)
O12’ – H12B···O16’ [b]	2.29	2.689(6)	111			
O13’ – H13B···O16’ [f]	1.86	2.671(5)	168	2.04(4)	2.84(4)	136.6(21)
O14’ – H14A···O5 [g]	2.00	2.793(5)	163	2.15(3)	3.05(3)	151.9(20)
O16’ – H16’···O12’ [c]	1.88	2.689(6)	171	1.86(3)	2.54(3)	124.4(23)

[a] Note that the symmetry codes for the powder analysis are not given due to differences in reporting of the unit-cell; [b] x-1, y+1,z; [c] x+1, y-1, z; [d] x, y-1, z ; [e] x+1, y, z ; [f] x, y+1, z; [g] x+1, y, z-1.

Table 3 Hydrogen bonding information for αβ-D-lactose.

Table 3 also gives the data from the powder analysis in order to highlight the differences in the hydrogen-bonding networks between the two structures.[34]

There have been other reported forms of lactose containing mixtures of the two anomers: a 5α/3β form, a 3α/2β form and a 4α/1β form.[36-39] However, evidence for the different crystalline forms of lactose has recently been reviewed and critiqued,[6] in which it has been suggested that there is strong evidence for only four forms of lactose; namely α-D-lactose monohydrate,[7,8] a stable α-D-lactose anhydrate,[40] a hygroscopic α-D-lactose anhydrate[6] and β-D-lactose anhydrate.[41] There is no mention of the structure of αβ-D-lactose in this review and its structure, in addition to the four for which there is strong evidence, make a total of five crystalline forms of lactose. The structure of αβ-D-lactose, both from powder data

and this work, adopts a triclinic cell similar, but not identical, to that of the stable α-D-lactose anhydrate[40] and examination of the packing of the two structures, using the XPac approach and software, shows that they exhibit a very high level of three dimensional similarity.[35] This is not surprising given that thermal treatment of anhydrous α-D-lactose gives rise to αβ-D-lactose.[33]

α–D-lactose molecules are characterised by the presence of a symmetrical twist about the glycosidyl linkage and as a consequence have similar torsion angles, e.g. $|\psi_1| \approx |\psi_2|$ and $|\psi'_1| \approx |\psi'_2|$.[34,40] By contrast β–D-lactose molecules have a characteristic asymmetric twist which leads to a large difference between the respective torsion angles.[34,40] Table 4 shows the torsion angles around the β-1,4 glycosidyl linkage for the four of the crystalline forms of D-lactose. The structure of hygroscopic α-D-lactose anhydrate has not been included since there is some concern about its merit.[6] From our work the difference in the torsion angles, $||\psi_2| - |\psi_1||$ and $||\psi'_2| - |\psi'_1||$, for the α–D-lactose molecule are 3.3 ^{o} and 6.4 ^{o}, while the corresponding values from the powder data are 6.2 ^{o} and 14.1 ^{o}. The values based on this work are similar to those observed for the α-D-lactose monohydrate[7,8] and the stable α-D-lactose anhydrate.[40] The β–D-lactose molecule from this work exhibits similar differences in the torsion angles, 3.8 ^{o} and 7.2 ^{o}, compared to the values from powder data of 3.9 ^{o} and 11.0 ^{o}. Both structural analyses differ from β-D-lactose, for which the values are 37.3 ^{o} and 39.0 ^{o}.[40,41]

	ψ_1 [a]	ψ'_1 [b]	ψ_2 [c]	ψ'_2 [d]
αβ-Lactose, α [e]	-93.2 (5)	148.7 (4)	96.5 (5)	-142.3 (4)
αβ-Lactose, β [e]	-87.5	153.6 (4)	91.3 (5)	-146.4 (4)
αβ-Lactose, α[34]	-90.4 (10)	159.1 (8)	96.6 (10)	-145.0 (8)
αβ-Lactose, β[34]	-91.1 (17)	141.0 (13)	102.1 (15)	-137.1 (14)
α-Lactose, α[39]	-85.9 (13)	155.6 (11)	69.2 (14)	-160.9 (10)
α-Lactose, α[39]	-87.3 (10)	148.8 (8)	87.0 (10)	-153.0 (7)
α-Lactose. H_2O, α[39]	-92.6	146.2	94.6	-143.0
β-Lactose [39]	-70.7	170.3	108.0	-131.3

[a] O1'- C1'- O4- C4; [b] C2'- C1'- O4-C4; [c] C1'-O4- C4- C3; [d] C1'- O4- C4- C5; [e] this work

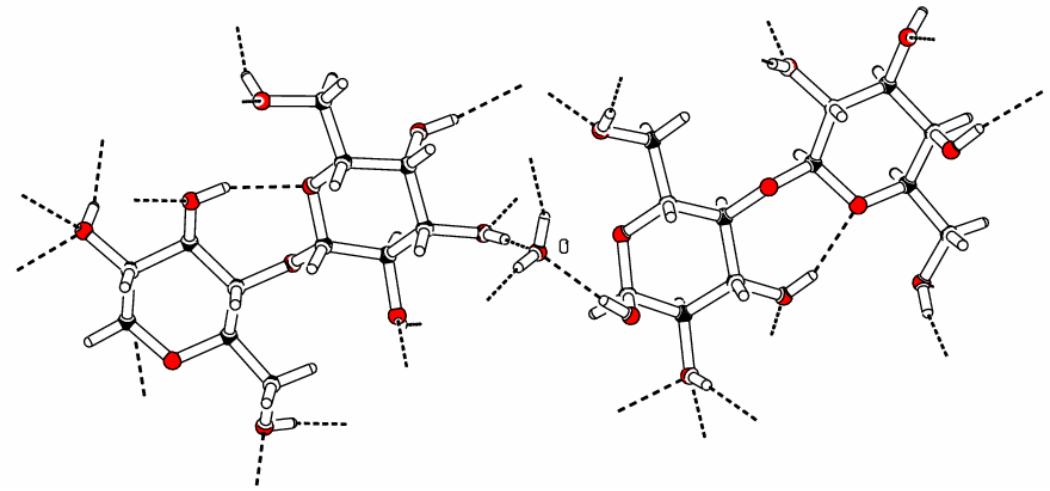
Table 4 Torsion angles for various crystalline forms of D-lactose.

There is no firm evidence available for the reason why it is αβ-D-lactose which forms even though the crystallization conditions are those which normally produce α-D-lactose monohydrate in the absence of additive. Indeed, obtaining meaningful insight into the molecular detail and pre-nucleation processes is a great challenge requiring detailed studies using a host of different techniques, as shown recently by

Harris.[42] One possibility is that the 1-deoxy-D-lactose is nucleating the growth of the αβ-D-lactose crystals. To help our understanding of this, we have compared the hydrogen bonding features present in both 1-deoxy-D-lactose and αβ-D-lactose, Tables 1 and 3 respectively. The inter-molecular hydrogen bonds O2'- H2'…O3, O2- H2…O2', O3'- H3'...O6', O6- H6...O2 and O6'- H6'...O3' and the intra-molecular hydrogen bonds O3- H3...O1' and O3- H3...O6' are present in both molecules and therefore this possibility is reasonable.

A second possibility is selective inhibition of the α-D-lactose monohydrate by the 1-deoxy-D-lactose molecules. Consideration of the structures of 1-deoxy-D-lactose and α-D-lactose monohydrate suggest that the galactose moiety in both materials is similar and therefore 1-deoxy-D-lactose can interact with the monohydrate during its nucleation and, as there is no group on the anomeric carbon which can act as a hydrogen bond acceptor, further growth of this form is blocked and the αβ-D-lactose form grows. Figure 9 shows (a) the unit-cell of α-D-lactose monohydrate (omitting the second water molecule) and (b) the same figure except that one of the α-D-lactose molecules has been replaced by one 1-deoxy-D-lactose molecule.[43]

The key features in Figure 9(b) are (i) the central water molecule is not able to hydrogen bond to the 1-deoxy-D-lactose molecule and (ii) there is plenty of other hydrogen bonding possible involving the 1-deoxy-D-lactose molecule. Therefore, we can speculate that the one or more molecules of 1-deoxy-D-lactose can interact with pre-critical nuclei via hydrogen bonding, but that the water molecules may not be required for formation of a stable lattice.



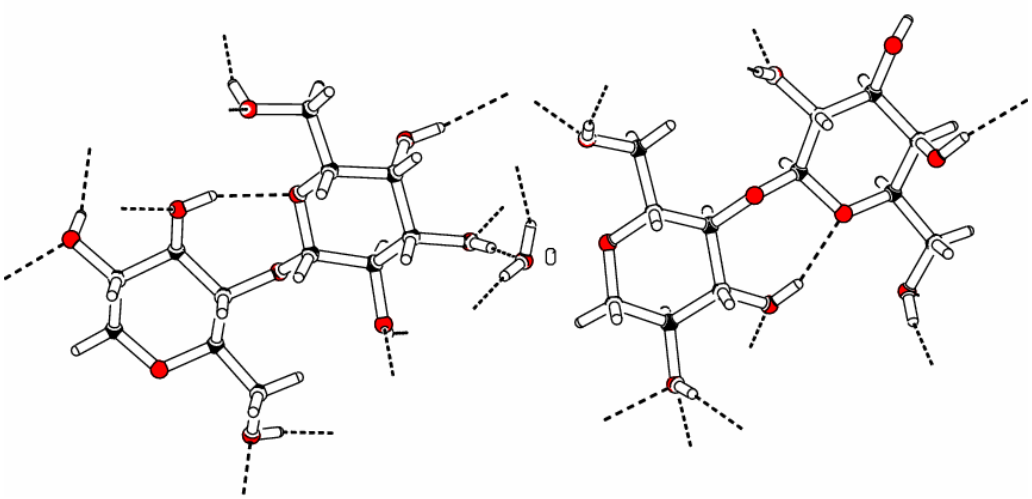


Figure 9 (a) Top: A view of the unit-cell of α-D-lactose monohydrate showing two α-D-lactose molecules hydrogen bonding with a water molecule. The second water molecule has been omitted for clarity. **(b)** Bottom: The same view, but with the right-hand α-D-lactose molecule replaced by a 1-deoxy-D-lactose molecule.

Another possibility is that the additive is simply perturbing the solubility phase diagram of lactose, allowing formation of the less commonly seen αβ-D-lactose. The fact that αβ-D-lactose is obtained by thermal treatment of amorphous α-D-lactose suggests that it must be relatively stable thermodynamically. It is possible that crystallisation from water, which usually results in formation of α-D-lactose monohydrate, is actually an example of Ostwald's rule of stages;[44] with α-D-lactose monohydrate being observed since it is the kinetic product; and αβ-D-lactose being the thermodynamic product which is not observed crystallising from aqueous solution. Whilst there are obvious differences between the crystallization methods used to make α-D-lactose monohydrate and αβ-D-lactose, it is interesting that the form which has been made in this study, a 1:1 mixture of anomers, can be considered as being "closest in character" to the equilibrium mixture of anomers in water, 37:63. It may well be that it is this similarity, rather than the lack of mutarotation in the additive, which is most significant in understanding the crystallization results obtained.

CONCLUSION

1-deoxy-D-lactose, **4**, has been successfully synthesised and the crystal structure reveals a complex hydrogen bonding network. The use of **4** in the tailor-made additive approach to the crystallization of D-

lactose has been undertaken and reveals for the first time the room temperature formation of αβ-D-lactose. This was analysed by low temperature single crystal X-ray diffraction, and its structure shows some differences, particularly in hydrogen-bonding patterns, to the room temperature structure obtained from powder data. There is some similarity in hydrogen-bonding between αβ-D-lactose and the tailor-made additive, 1-deoxy-D-lactose, indicating some role of the additive in the formation of αβ-D-lactose in experimental conditions which usually favour formation of α-D-lactose monohydrate.

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SUPPORTING INFORMATION AVAILABLE

Comparison of PXRD patterns for αβ-D-lactose and anhydrous α-D-lactose, and comparison of the single crystal analysis of αβ-D-lactose with that obtained from powder data. X-ray crystallographic information files are available for 1-deoxy-D-lactose and αβ-D-lactose. This material is available free of charge via the Internet at http://pubs.ac.org.

REFERENCES

1. Bolhuis, G.K. Armstrong, N.A. *Pharmaceutical Development and Technology* **2006**, 11, 111-124.

2. Nickerson, T.A. *Fundam. Dairy Chem.* **1965**, 224-260.

3. Hartel, R.W. in *Handbook of Industrial Crystallization, 2nd Ed;* Myerson, A.S. Ed.; Butterworth-Heinemann, Boston, 2002; pp 287-304.

4. Rodriguez-Hornedo, N. Murphy, D. *J. Pharm. Sci.* **1999**, 88, 651-660.

5. York, P. *Int. J. Pharm.* **1983**, 14, 1-28.

6. Kirk, J.H. Dann, S.E. Blatchford, C. G. *Int. J. Pharmaceut.* **2007**, 334, 103-114.

7. Fries, D.C. Roa, S.T. Sundaralingam, M. *Acta Crystallogr*. **1971**, B27, 994-1005.

8. Smith, J.H. Dann, S.E. Elsegood, R.J. Dale, S.H. Blatchford, C.G. *Acta Cryst.* **2005,** 61, 2499-2501.

9. Davey, R.J. Milislavjevic, B. Bourne, J.R. *J. Phys. Chem.* **1988**, 92, 2032-2036.

10. Michaels, A.S. Van Kreveld, A. *Neth. Milk Dairy J.* **1966**, 20, 163-181.

11. Raghavan, S.L. Ristic, R.I. Sheen, D.B. Sherwood, J.N. *J. Phys. Chem. B.* **2000**, 104, 12256-12262.

12. Halfpenny, P.J. Morrison, H. Ristic, R.L. Shepherd, E.E.A., Sherwood, J.N. Simpson, G.S. Yoon, C.S. *Proc. R. Soc.* **1993**, A440 683-689.

13. Clydesdale, G. Roberts, K.J. Telfer, G. Grant, D.W.J. *J. Pharm. Sci.* **1997**, 86, 135-141.

14. Garnier, S. Petit, S. Coquerel, G. *J. Crystal Growth* **2002**, 234, 207-219.

15. Davey, R.J. Blagden, N. Potts, G.D. Docherty, R. *J. Am. Chem. Soc.* **1997**, 119, 1767-1772.

16. Kelleher, J.M. Lawrence, S.E. Moynihan, H.A. *CrysEngComm*, **2006**, 8, 327-332.

17. Kelleher, J.M. Lawrence, S.E. McAuliffe, M.T. Moynihan, H.A. C*rystEngComm*, **2007**, 9, 72-77.

18. Guiry, K.P. Kelleher, J.M. Lawrence, S.E. McAuliffe, M.T. Moynihan, H.A. Ryan, A.L. *J. Enz. Inhib. Med. Chem.*, **2007**, 22, 550-555.

19. Berkovitch-Yellin, Z. van Mil, J. Addadi, L. Idelson, M. Lahav, M. Leiserowitz, L. *J. Am. Chem. Soc.* **1985**, 107, 3111-3122.

20. Addadi, L. Berkovitch-Yellin, Z. Domb, N. Gati, E. Lahav, M. Leiserowitz, L. *Nature (London)* **1982**, 296, 21-26.

21. Berkovitch-Yellin, Z. Addadi, L. Idelson, M. Lahav, M. Leiserowitz, L. *Angew. Chem. Suppl.* **1982,** 1336.

22. Berkovitch-Yellin, Z. Addadi, L. Idelson, M. Leiserowitz, L. Lahav, M. *Nature (London)* **1982**, 296, 27-34.

23. Addadi, L. Berkovitch-Yellin, Z. Weissbuch, I. Lahav, M. Leiserowitz, L. Weinstein, S. *J. Am. Chem. Soc.* **1982**, 104, 2075-2077.

24. Weissbuch, I. Addadi, L. Berkovitch-Yellin, Z. Gati, E. Weinstein, S. Lahav, M. Leiserowitz, L. *J. Am. Chem. Soc.* **1983,** 105, 6615-6621.

25. Koto, S. Hirooka, M. Tashiro, T. Sakashita, M. Hatachi, M. Kono, T. Shimizu, M. Yoshida, N. Kurasawa, S. Sakuma, N. Sawazaki, S. Takeuchi, A. Shoya, N. Nakamura, E. *Carbohydr. Res.* **2004**, 339, 2415-2424.

26. Ross, K.D. *J. Dairy Sci.* **1978**, 61, 152-158.

27. Cernik, R.J. Clegg, W. Catlow, C. R. A. Bushnell-Wye, G. Flaherty, J. V. Greaves, G. N. Hamichi, M. Burrows, I. Taylor D. J. Teat, S. J. *J. Synchrotron Radiat.* **1997**, 4, 279-286.

28. Sheldrick, G.M. *SHELXS*, University of Göttingen, Germany, 1986.

29. Sheldrick, G.M. *SHELXL-97*, University of Göttingen, Germany, 1997.

30. Spek, A.L. *PLATON*, University of Utrecht, The Netherlands, 2000.

31. Giese, B. Linker, T. Muhn, R. *Tetrahedron* **1989**, 45, 935-940.

32. Parrish, F.W. Ross, K.D. *Carbohydr. Res.* **1979**, 71, 322-326.

33. Lerk, C. F.; Andreae, A. C.; De Boer, A. H.; De Hoog, P.; Kussendrager, K.; Van Leverink, J. *J. Pharm. Sci.* **1984**, 73, 857-9.

34. Lefebvre, J. Willart, J. Caron, V. Lefort, R. Affouard, F. Danède, F. *Acta Cryst.* **2005**, B61, 455-463.

35. Gelbrich, T. Hursthouse, M.B. *Cryst. Eng. Comm.* **2005**, 7, 324-336.

36. Olana, A. Bernhard, R.A. Nickerson, T.A. *J. Food Sci.* **1977**, 42, 1066-1068.

37. Olana, A. Corzo, N. Martinez-Castro, I. *Milchwissenschaft*. **1983**, 38, 471-474.

38. Bushill, J. H. Wright, W.B, Fuller, H.F. Bell. A, V. *J. Sci. Food Agric*. **1965**, 16, 622-628.

39. Parrish, W. Brown, M. L. *J. Dairy Sci.* **1982**, 65, 1688-1691.

40. Platteau, C. Lefebvre, J. Affouard, F. Willart, J. Derollez, P. Mallet, F. *Acta Cryst.* **2005**, B61, 185-191.

41. Hirotsu, K. Shimada, A. *Bull. Chem. Soc. Jpn*. **1974**, 47, 1872-1879.

42. Hughes, C.E. Hamad, S. Harris, K.D.M. Catlow, C.R.A. Griffiths, P.C. *Faraday Discuss.* **2007**, 136, 71-89.

43. This has been done by generating the 2_1-related α-D-lactose molecule in the unit-cell of α-D-lactose monohydrate and changing the space group to *P*1. A hydrogen atom has replaced the hydroxyl group at the anomeric carbon. Figure 9(b) has been generated by PLATON, allowing a realistic assessment of the hydrogen bonding present. It assumes that the 1-deoxy-D-lactose molecule is adopting the same position as the 2_1-related α-D-lactose molecule in Figure 9(a).

44. Mullin, J.W. in *Crystallization*, *3rd Ed*; Butterworth-Heinemann, Oxford, 1997; pp 200-201.

SUPPORTING INFO

XPac comparison of the single crystal analysis of αβ-D-lactose with that obtained from powder data (green). Note the difference in orientation of the oxygen of the CH_2OH group on the glucose ring of the α anomer (circled).

