INVESTIGATIONS ON THE POLYMORPHISM AND PSEUDOPOLYMORPHISM OF CLOBETASONE BUTYRATE

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Dedicated to Dr. Zdeněk Havlas on the occasion of his 60th birthday.

Clobetasone butyrate was investigated for polymorphism and pseudopolymorphism. Solvent mediated conversion experiments reveal that the commercially available form I represent the thermodynamically most stable form at room temperature and DSC measurements shows that it should also be the most stable form until melting. Form I crystallizes in space group $P2_12_12_1$ with three crystallographically independent molecules of similar conformation. From methanol an additional pseudo polymorphic form was discovered. In the crystal structure (space group $P2_12_12_1$) the solvent molecules are connected to the clobetasone butyrate molecules by O–H···O hydrogen bonding. Investigations of the solvate using thermogravimetry, differential thermoanalysis as well as differential scanning calorimetry proves, that on solvent removal an amorphous form is obtained that crystallizes into form I on further heating.

Keywords: Clobetasone butyrate; Polymorphism; Pseudopolymorphism; Crystal structures; Thermoanalytical methods; Crystal growth; Calorimetry; X-ray diffraction.

Polymorphism, which is defined as the ability of a compound to exist in more than one crystalline modification is a widespread phenomenon and of particular importance in a number of areas like e.g. pharmaceutical development or material science[1–16]. In pharmaceutical sciences several aspects are of importance. This includes requests by the authority responsible for approval of a new drug, for which also investigations on the polymorphism of the active ingredient are needed, information on the influence of the corresponding phase onto the chemical, biological or physical properties of a drug and aspects of the patent law[2–4]. Moreover, this phenomenon is also of importance in academic research like e.g. for investigations on the structure properties relationships of a new compound because all differ-

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ences in physical properties can directly be traced back to their differences in crystal structures[1,5]. In this context also pseudo polymorphic forms, better be described as co-crystals are of importance, which includes compounds that contain additional solvent[10–11]. If pseudo polymorphic forms are identified, it has to be investigated in detail which solvent free modification is formed on the solvent removal[10]. Finally, during processing of a drug also amorphous forms can be generated, which in some cases can have advantages over crystalline forms because of their better solubility and faster dissolution rates[2,3].

One of the most versatile and effective drugs are still glucocorticoids, widely used in therapy because of their anti-allergic, anti-rheumatic, anti-inflammatory and immune repressive properties[12–18]. Although they are commercially available for several years, their polymorphism has not been investigated in detail but recently has become of increasing interest again. During the preparation of the dosage form a drug must be sterilized for which different methods exist. An elegant method is the sterile filtration, which is often used for the preparation of glucocorticoids as well as other drugs. In this procedure the drug is dissolved in a suitable solvent, filtered under pressure and afterwards the solvent is vaporized, the drug is dried and finally micronized. Different polymorphic modifications can be obtained directly or by decomposition of solvates formed by this procedure. Finally it must be guaranteed that the process provides only the form preferred by the manufacturer. This was one of our starting points for our investigations on glucocorticoids, in which we have discovered a large number of new forms, that were structurally characterized and investigated for their thermodynamic properties and transition behaviour[19–25]. In this contribution, we report on our investigations on clobetasone butyrate (Chart 1), which is frequently used in combination with clobetasone for the treatment of skin diseases, allergies and asthma[26–28]. For this drug, no polymorphic modifications have been described and no single crystal structure is available in the Cambridge Structure Database[29–31].

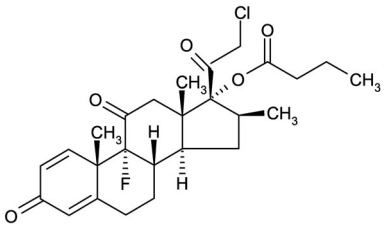

CHART 1

RESULTS AND DISCUSSION

Solvent Mediated Conversion Experiments

In the beginning of our investigations, solvent mediated conversion experiments were performed at room temperature in order to investigate if the commercially available form I of clobetasone butyrate represents the thermodynamically most stable form at room temperature or if other modifications or solvates exist. In these experiments an excess of crystalline powders of form I were stirred in saturated solutions of different solvents and the precipitates were investigated by X-ray powder diffraction after 14 days. In all solvents except methanol the X-ray powder patterns correspond to that of form I, which unambiguously shows that this form should be the thermodynamic most stable form at room temperature (Table I). Only in methanol a different powder pattern is obtained, which corresponds to that of a solvate (Table I).

TABLE I
Results of the solvent mediated conversion experiments

Solvent	Form	Solvent	Form
1-Butanol	I	Acetone	I
1-Propanol	I	Methyl acetate	I
2-Butanol	I	Methanol	solvate
Isopropanol	I	Water	I
Acetonitrile	I	HCl (0.1 M)	I
Chloroform	I	NaCl 150 mM, isotonic)	I
Ether	I	Methyl isobutyl ketone	I
DMF	I	Toluene	I
Ethyl acetate	I	Heptane	I
Ethyl methyl ketone	I	Tetrahydrofuran	I
Isoamyl alcohol	I	Acetone/Water	I
Methylene chloride	I	Ethanol/Water	I
Pentanol	I	Acetonitrile/Water	I
Carbon tetrachloride	I	Cyclohexane	I

Single Crystal Structures

In order to characterize the different forms by X-ray structure analysis single crystals of form I were grown as irregular polyhedral from ethyl acetate and crystals of the methanol solvate were obtained as needles from methanol (see Experimental).

Form I crystallizes orthorhombic in space group $P2_12_12_1$ with $Z = 12$ molecules in the unit cell (Table II). There are three crystallographically independent molecules in the asymmetric unit, which exhibit a similar conformation (Fig. 1). Only minor conformational differences are found in the side chains around the oxygen atoms O3 (O33, O63) and O5 (O35, O65) and around the carbon atoms C21 (C51, C81) and C22 (C52, C82) (Fig. 2).

In contrast to the structure of most other glucocorticoids, which are determined predominantly by intermolecular O–H···O hydrogen bonding interactions, in this compound no hydroxyl groups are present and

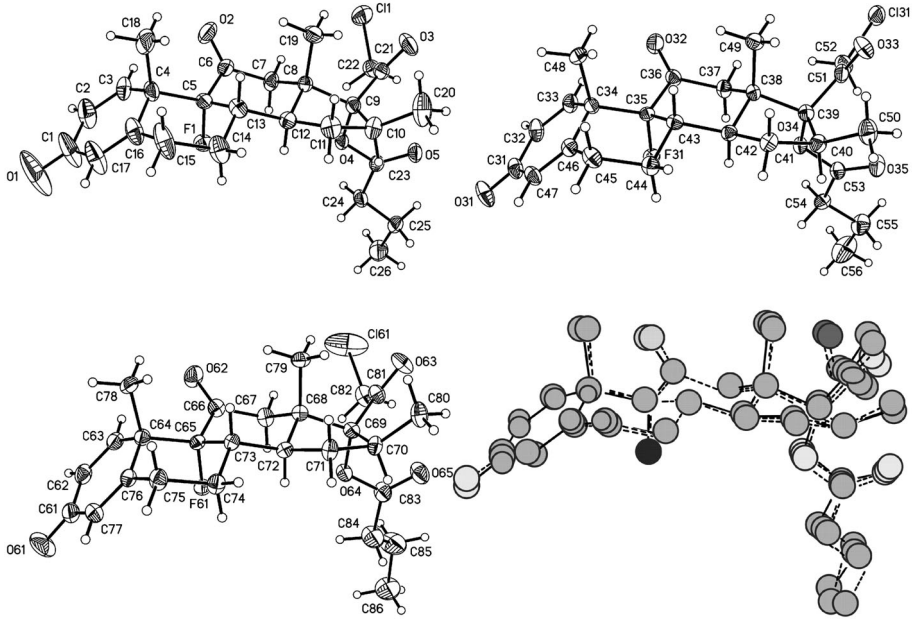

FIG. 1

Crystal structures of the three crystallographically independent molecules of form I with labeling and displacement ellipsoids drawn at the 50% probability level and all three molecular structures fitted onto each other (light grey, oxygen; mid grey, carbon; dark grey, chlorine; black, fluorine)

TABLE II
Selected crystal data and results of the structure refinement for form I and for the methanol solvate of clobetasone butyrate

Parameter	Form I	Methanol solvate
Empirical formula	$C_{26}H_{32}FClO_5$	$C_{26}H_{32}FClO_5 \cdot CH_3OH$
MW, g/mol	478.97	511.01
Crystal color	colorless	colorless
Crystal system	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$
a, Å	14.2345	7.4563
b, Å	18.8570	14.4312
c, Å	27.7142	23.7464
V, Å^3	7439.04	2555.19
Temperature, °C	–103	–103
Z	12	4
D_{calc}, mg/cm^3	1.283	1.328
$F(000)$	3048.0	1088.0
2θ-range, °	3.88 to 51.88	5.88 to 56.16
$h/k/l$ ranges	$-17 \le h \le 17$	$-8 \le h \le 9$
	$-17 \le k \le 23$	$-19 \le k \le 18$
	$-33 \le l \le 33$	$-31 \le l \le 27$
μ(MoKα), mm^{-1}	0.20	0.20
Reflection collection	35239	13091
R_{int}	0.0361	0.0394
Independent refl.	14312	6027
Refl. with $F_o > 4\sigma(F_o)$	12171	5028
Refined parameters	893	319
$R_1[F_o > 4\sigma(F_o)]$	0.0437	0.0400
wR_2 [all data]	0.1083	0.0986
Flack-x-parameter	–0.06 (4)	0.00 (6)
GOOF	1.016	1.018
Min./max. res., e/Å^3	0.880/–0.950	0.350/–0.350

therefore, its structure is determined predominantly by isotropic interactions. Consequently a complicated packing of the three crystallographically independent molecules is observed.

The methanol solvate of clobetasone butyrate crystallizes in the orthorhombic space group $P2_12_12_1$ with four molecules in the unit cell (Table II and Fig. 2). In this structure significant conformational changes in the side chains are found compared to those of the three crystallographically independent in form I (Fig. 2).

In the crystal structure the molecules are stacked in the direction of the crystallographic *c*-axis (Fig. 3, top). Between four adjacent stacks small channels are formed in which the methanol molecules are located (Fig. 3). The solvent molecules are connected to the clobetasone butyrate molecules via O–H···O hydrogen bonding between the hydroxyl group of the methanol molecule and the carbonyl oxygen atom of the clobetasone butyrate molecule (d(H···O3) = 2.223 Å, ∠O6HO3 = 159.55° and d(O6···O3) = 3.024 Å). Within the a/b plane, the molecules are arranged in layers in which the clobetasone butyrate molecules show a herringbone-like arrangement (Fig. 3, bottom).

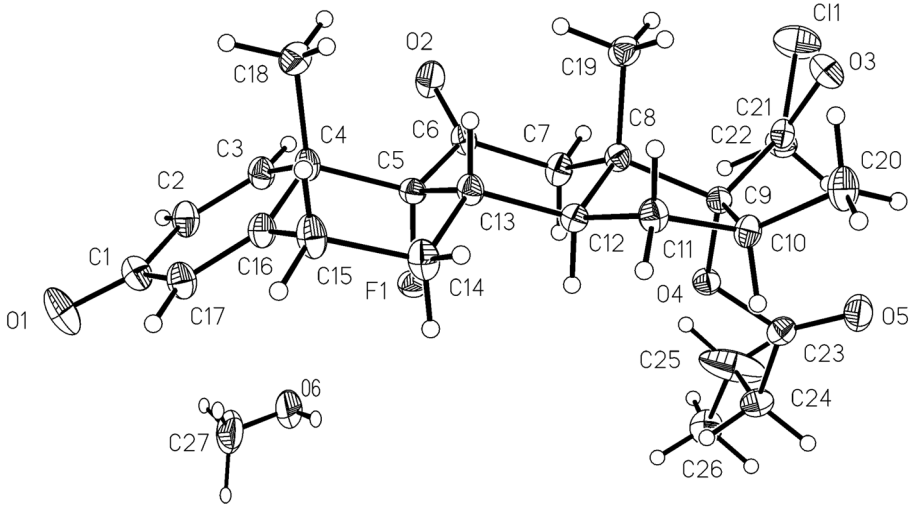

FIG. 2
Crystal structures of the methanol solvate with labeling and displacement ellipsoids drawn at the 50% probability level

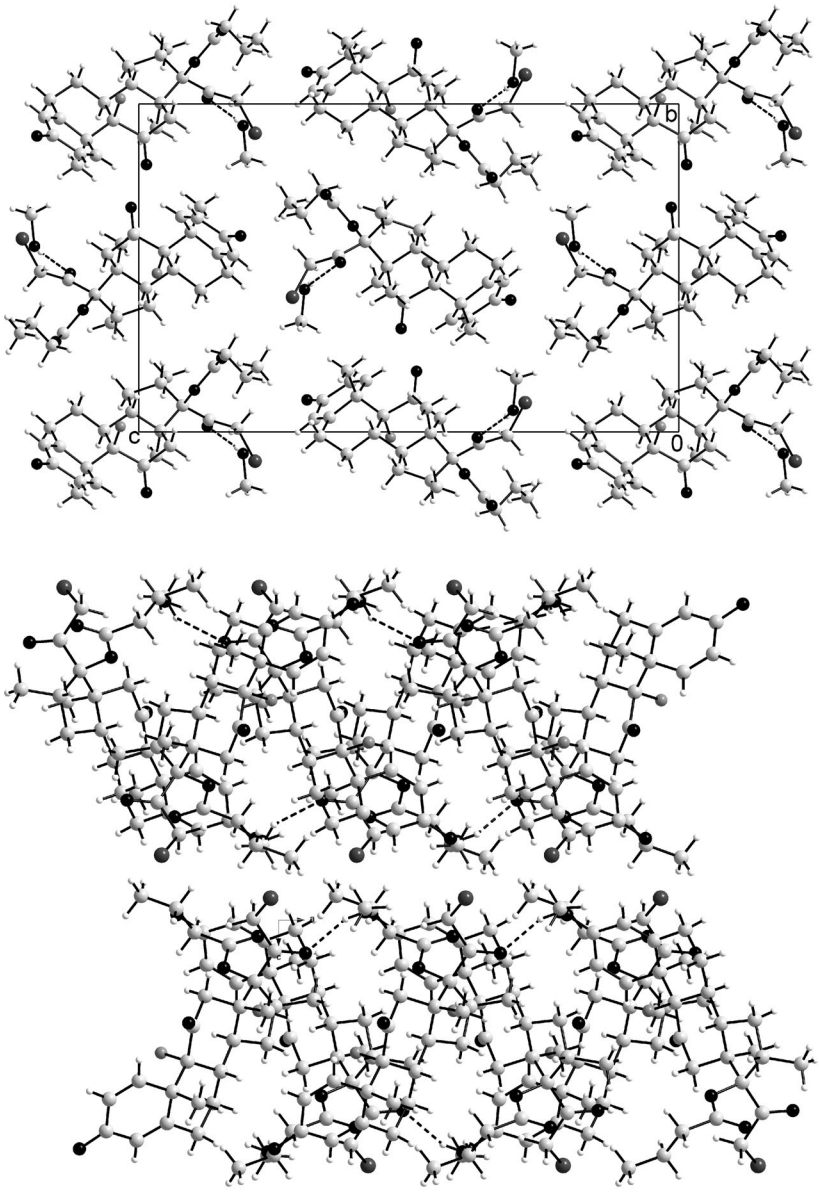

FIG. 3
Crystal structure of the methanol solvate of clobetasone butyrate with view along the *a*-axis (top) and the *b*-axis (bottom). O–H···O hydrogen bonding is shown as dashed lines

It must be noted, that this structure is very similar to that of the solvates from triamcinolone diacetate, in which different molecules like alcohols or ketones are embedded[19]. All of these solvates are isotypic crystallizing in space group $P2_12_12_1$ and exhibit similar cell parameters like the methanol solvate of clobetasone butyrate. Surprisingly our experiments clearly show that no solvates are formed if clobetasone butyrate is treated with other solvents. The void volume in the methanol solvate (Z = 4) was calculated using Platon to 238.2 Å^3 which is 9.3% of the complete unit cell volume[32]. In contrast, in triamcinolone diacetate ethanol solvate (Z = 4) the void volume is calculated to be 578.2 Å^3 which corresponds to 21.3% of the unit cell volume. This clearly shows that in clobetasone butyrate the voids are smaller and therefore, no larger guest molecules like e.g. ethanol can be incorporated.

However, based on the crystal structure of form I and the methanol solvate X-ray powder patterns can be calculated. If these powder pattern are compared with those measured, it is proven that both modifications are formed as phase pure materials.

Differential Thermoanalysis and Thermogravimetry (DTA-TG)

In order to investigate which modification is formed on solvent removal, the methanol solvate was investigated by simultaneous DTA-TG experiments. On heating, the mass of the sample decreases practically from room temperature and this mass step is finished at about 90 °C (Fig. 4). The experimental mass loss of this step of about –6.1%, corresponds to that calculated (Δm_{calc} = –6.3%) for the complete removal of the methanol molecules. On further heating there is an additional mass loss at about 200 °C, which corresponds to the vaporization or decomposition of the solvent free form.

The DTA curve shows a more complicated behavior. During desolvation two endothermic events at T_p = 75 and 90 °C, and one exothermic event are observed at T_p = 107 °C. The last peak at T_p = 180 °C corresponds to the melting of the form obtained by desolvation (Fig. 4).

In further DTA-TG experiments heating was stopped at 150 °C and the residue was investigated by X-ray powder diffraction. The experimental pattern of this residue is in perfect agreement with that calculated for form I, which clearly shows that this modification has formed after complete removal of the solvent (Fig. 5).

Differential Scanning Calorimetry (DSC)

In order to investigate the thermal behavior of form I and the methanol solvate in more detail additional measurements using differential scanning calorimetry were performed (Fig. 6).

On heating form I melts at T_o = 177 °C without any further transformation (Fig. 6, top). If the sample is cooled down with different cooling rates directly after melting a residue is obtained that is amorphous to X-rays. There are no hints for the formation of additional crystalline modifications.

The DSC curve of the methanol solvate is more complex. On heating a broad endothermic signal is observed at T_p = 55 °C that corresponds to the removal of the solvent (Fig. 6, bottom). On further heating one additional endothermic event at T_p = 89 °C and one exothermic event at T_p = 106 °C is observed. In a second DSC run heating was stopped after desolvation and

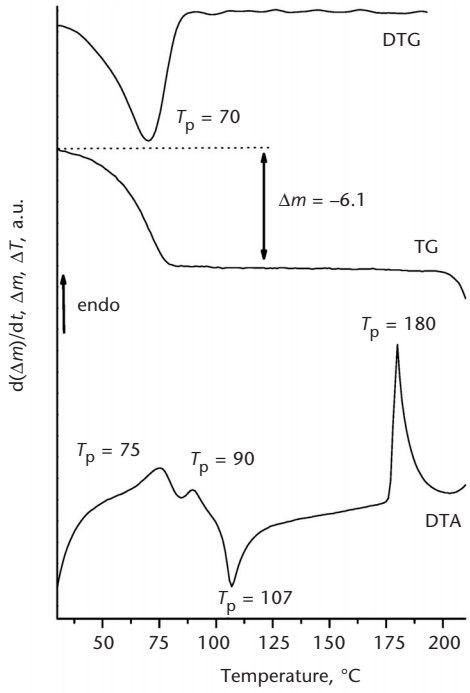

FIG. 4
DTA, TG and DTG curve for the methanol solvate (Al_2O_3 crucibles, nitrogen atmosphere, heating rate 4 °C/min, peak temperatures (T_p) given in °C and mass loss in %)

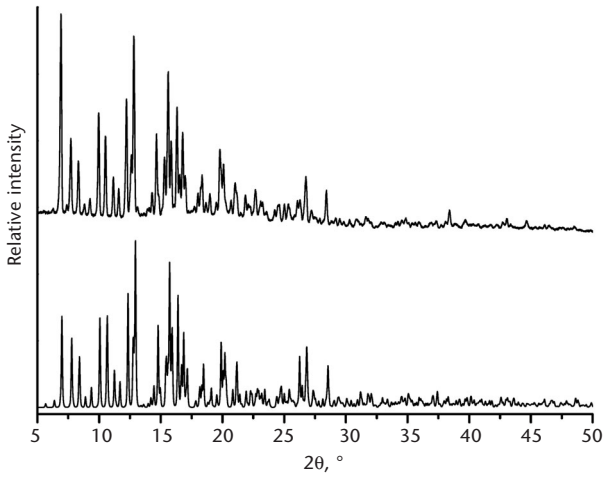

FIG. 5
Experimental X-ray powder pattern of the residue isolated after the removal of the solvent at 150 °C (top) and the calculated X-ray powder pattern for the commercially available form I (bottom). For clarity only the range between 5° ≤ 2θ ≥ 35° is shown

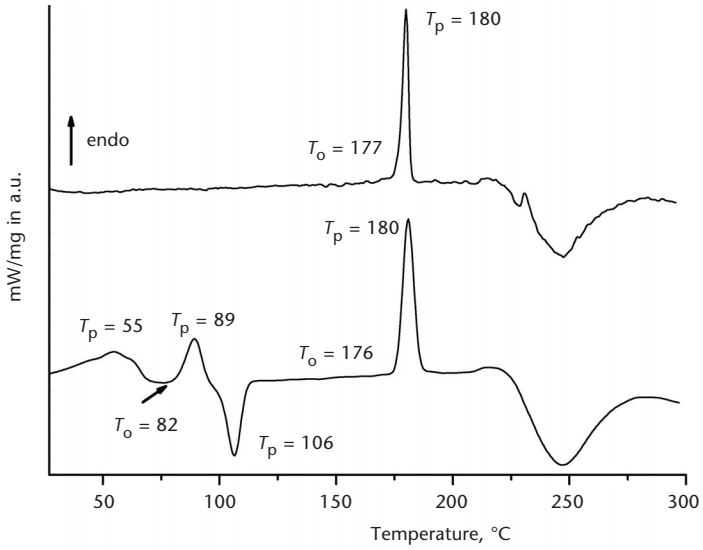

FIG. 6
DSC curves of form I (top) and the methanol solvate (bottom). (Al pans, nitrogen atmosphere, heating rate 3 °C/min, extrapolated onset (T_o) and peak (T_p) temperatures given in °C)

the residue was investigated by X-ray powder diffraction. This clearly shows that this residue is amorphous to X-rays. An amorphous residue is also obtained if heating is stopped after the second endothermic DSC signal at about 93 °C. In contrast, if the residue is isolated directly after the exothermic event at about 110 °C and investigated by X-ray powder diffraction it is clearly proven that modification I is formed. Moreover, if the crystalline form I and both amorphous forms obtained after the first and second DSC peak are investigated by IR and Raman-spectroscopy no differences are found in their spectra.

The results of these experiments clearly shows that the first thermal event correspond to the desolvation of the methanol solvate leading to the formation of an amorphous form. On further heating there is a second endothermic event that cannot unambiguously assigned to some definite reaction. The transformation of one amorphous form into a second in an endothermic reaction is unlikely. However, it might be that solvent removal takes place in two different steps which cannot be resolved using thermogravimetry. In any case the exothermic event at about 106 °C corresponds to the crystallization of the amorphous modification leading the formation of the thermodynamic most stable form I.

In this context, it is noted that the amorphous form is relatively stable. If this form is stored at 50 °C, no changes are observed for about three weeks. Afterwards a very slow transformation into form I is observed, which is even not completed after several weeks.

CONCLUSION

In this work, clobetasone butyrate was investigated for polymorphism. Solvent mediated conversion experiments clearly show that the commercial available form I should represents the thermodynamically most stable form at room temperature and the DSC measurements prove that this form might also be the most stable form until its melting point. From all of our investigations there are no hints for further crystalline forms. However, in methanol a pseudo polymorphic form is obtained, which transforms on solvent removal into a new amorphous modification that is stable over several weeks. If the amorphous form is heated to about 100 °C it transforms immediately into the most stable crystalline form I.

EXPERIMENTAL

Chemicals

Clobetasone butyrate is commercially available and was received from Symbiotec Pharmalab LTD, India. All solvents used for the crystallization experiments were of analytical grade.

Preparation of Single Crystals

Single crystals of form I were prepared by slow evaporation of the solvent from a solution of clobetasone butyrate in ethyl acetate. Single crystals of the methanol solvate were prepared by slow evaporation of the solvent from a solution of clobetasone butyrate in methanol.

Differential Thermal Analysis and Thermogravimetry

DTA-TG measurements were performed in Al_2O_3 crucibles using a STA-409CD thermo balance from Netzsch. Several measurements under nitrogen atmosphere (purity 5.0) and air with different heating rates (1, 2, 4, 8 and 16 °C/min) were performed. All measurements were performed with a flow rate of 75 ml/min and were corrected for buoyancy and current effects. The instrument was calibrated using standard reference materials from Netzsch.

Differential Scanning Calorimetry

DSC investigations were performed on the DSC 204/1/F device from Netzsch. The measurements were performed in Al pans with heating rates of 3, 10 and 20 °C/min. The instrument was calibrated using standard reference materials.

X-ray Powder Diffraction

X-Ray powder diffraction experiments were performed with a STOE STADI P transmission powder diffractometer using CuK_α radiation (λ = 1.540598 Å) that is equipped with a 2° to 130° IP-PSD (image plate position sensitive detector) and a 4° position sensitive detector.

X-ray Single Crystal Structure Analysis

All data were measured at using an Imaging Plate Diffraction system (IPDS-1) from STOE. Structure solutions were performed with direct methods using SHELXS97 and structure refinements were performed against F^2 using SHELXL97 [33]. All non-hydrogen atoms were refined using anisotropic displacement parameters. The C–H hydrogen atoms were positioned with idealized geometry (some of the methyl H atoms were allowed to rotate but not to tip) and refined with isotropic displacement parameters ($U_{iso}(C)$ = 1.2 × $U_{eq}(C_{methin/methylene})$ = 1.5 × $U_{eq}(C_{methyl})$ using a riding model with $C–H_{methine}$ = 0.95 Å, $C–H_{methylene}$ = 0.99 Å, $C–H_{methyl}$ = 0.98 Å. The O–H hydrogen atoms were located in difference map but positioned with idealized geometry allowed to rotate but not to tip and were refined isotropic using a riding model with O–H = 0.84 Å. The absolute structure was determined and is in agreement with the selected setting. Selected crystal data and details of the structure determinations can be found in Table II.

CCDC 681992 (form I of clobetasone butyrate) and 681993 (clobetasone butyrate methanol solvate) contain the supplementary crystallographic data for this paper. These

data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

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