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Serendipity and Four Polymorphic Structures of Benzidine, $C_{12}H_{12}N_2$

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Abstract: The search for a co-crystal of benzidine as a bifunctional hydrogen-bond donor with potential hydrogen-bond acceptors has instead revealed four polymorphs of the source material benzidine for which, somewhat surprisingly, no structure has been reported as of the November 2005 update of the CSD. All four structures are characterized by a rather unusual number of molecules in the asymmetric unit ($Z' =$ 1.5, 3, and 4.5), which are found in only 0.25%, 0.4%, and 0.002% of structures in the CSD. Forms I and IV ($Z' = 4.5$) exhibit very similar crystal habits and are not distinguishable visually. In all forms except Form II ($Z' = 3$), one of the molecules lies on a crystallographic inversion center, requiring the molecule to be planar; other molecules are nonplanar. Spectroscopic and thermodynamic characterizations of the system, including at least two possible additional forms of benzidine obtained by HT polymorph screening are reported.

Introduction

This is truly a tale of chemical crystallographic serendipity. As part of a program to investigate and utilize the $R_4^2(8)$ hydrogen bonding motif[1,2] to design and prepare co-crystals,[3−6] we have been carrying out experiments to co-crystallize potential di-hydrogen bond donors (e.g., $-NH_2$) with potential di-hydrogen bond acceptors (e.g., O=X). One such hydrogen bond donor molecular building block in this program is benzidine, **B**, with benzophenone (**BP**) and diphenyl sulfoxide (**DPSO**) as potential acceptors.

In addition to co-crystals that were obtained,[7] the experiments also led to four polymorphs (I−IV) of pure **B**. A routine check of the CSD for the structure of **B**, a relatively simple, perhaps even "classic" molecule, revealed that no crystal structure has been reported to date, to say nothing of four polymorphs. Following traditional crystallization experiments (e.g., slow evaporation at room temperature), high throughput (HT) polymorphism and co-crystal screening[8] using solvent-drop grinding[9] and CRYSTALMAX technologies[10] were carried out at Transform Pharmaceuticals. At least two additional forms of **B** (either solvate or new polymorph), for which there may have been earlier literature reports (vide infra), were obtained and are being currently investigated.

In this context, the crystal chemistry of **B** has a long history. The synthesis of **B** was first reported in 1848 by Zinin[11] who observed peculiarities in its melting point. A drawing of a triclinic crystal with axial ratios (based on interfacial angles) is included in Groth's compendium,[12] citing a 1915 paper by Mieleitner.[13] Steinmetz[14] stated that the crystal was only apparently triclinic, being actually monoclinic. In 1937 Weygand[15] reported a melting point of 128 °C for the stable form; two other metastable forms have melting points of 125 °C and 122 °C. A great deal of Weygand's comprehensive discussion is dedicated to the conspicuous discrepancy of the literature-reported melting points prior to his investigation.[11,16−19] A year later Kofler[20] reported the hot stage microscopy (HSM) study of five crystal forms of **B** including their physical shape and thermal behavior. In 1978 Ahmed[21] reported thin monoclinic ($P2_1/c$) yellow plates

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obtained by slow evaporation from an ethanol solution with unit cell dimensions of $a = 5.55$ Å, $b = 7.644$ Å, and $c = 24.213$ Å, $\beta = 102°$. A lattice energy calculation using the atom−atom potential method and assuming one molecule at a general position in the asymmetric unit led to two crystal structures, both with nonplanar conformations. Finally, the 2001 edition of the Merck Index[22] lacks any specific reference to **B**'s polymorphic nature, reporting that it is a white or slightly reddish crystalline powder, which darkens upon exposure to air or light, with a melting point of 115−120 °C when slowly heated and 128 °C when anhydrous and rapidly heated.

For about 130 years, **B** and its derivatives had very wide industrial use, mainly as dyes and pigments in a variety of applications. By the middle the 1970s its use totaled 0.5−1 million kg. At that time the compound was found to be carcinogenic, and its commercial use has essentially been abandoned, apparently along with interest in its structure and properties.

B is a substituted biphenyl, compounds that have attracted the attention of many crystallographers (ref 23, for example), since in the gas phase the molecule has been shown to be nonplanar[24] and is a prototypical system for the study of the relationship between crystal forces and molecular conformations. We report here the preparation, crystal structures, characterization, and relative stability of Forms I−IV of **B**. All four crystal structures contain more than one molecule in the asymmetric unit ($Z' = 1.5$, 3, or 4.5), a rare phenomenon, occurring in only 0.25%, 0.4%, and 0.002% of the structures in the November 2005 version of the CSD. Even more surprising was the fact that Forms I and IV, which are indistinguishable on the basis of habit, both have the extremely rare $Z' = 4.5$. In all forms except Form II ($Z' = 3$), one molecule lies on a crystallographic inversion center (and therefore is planar). Other examples where the biphenyl lies both on a general position and on an inversion center are given in ref 25.

Experimental Section

Materials. A. Traditional Slow Evaporation Experiments: The solid materials **B** ≥ 98%, **BP** 99%, and **DPSO** 96% (Sigma-Aldrich) were used for the preparation of the co-crystals, as received. The solvents (toluene, chlorobenzene, dichloromethane, ethanol, chloroform, and acetone) were purchased from Frutarom except for amyl acetate (Acros), generally as analytical grade, and were used for the preparation of the co-crystals and the **B** polymorphs as received. Triply distilled water was also used for this purpose.

B. Solvent-Drop Grinding and HT CRYSTALMAX Experiments: The solid material **B** (Sigma-Aldrich) was used for the polymorph and co-crystal screening, as received. Different solvents (18) were used for the grinding experiments (acetone, toluene, chlorobenzene, dichloromethane, ethanol, nitromethane, dimethyl sulfoxide, ethyl acetate, chloroform, pyridine, water, methanol, *n*-methyl pyrolidine, dimethyl formamide, *n*-propyl acetate, cyclopentanone, acetophenone and methylphenyl sulfoxide); the first 11 of them were also used for the CRYSTALMAX experiments, in addition to 9 more (ethylene glycol, diethyl ether, 2-propanol, *n*-heptane, benzene, isobutyl alcohol, isopropyl acetate, acetic acid, and acetonitrile). All solvents were purchased from Sigma-Aldrich, generally as HPLC grade, and were used for the polymorph screen, as received. HPLC-grade distilled water (at pH > 5) was obtained from Fisher.

Methods. Solvent-Drop Grinding Crystallization. Mechanical grinding experiments were conducted by adding a small amount of solvent to **B**. Typical experiments contained 10 mg of **B**. A total of 18 solvents (10 μL) were used as additives in experiments designed to identify alternative polymorphs or co-crystals of **B**. The samples were ground for 20 min using a mechanical shaker, and the powders were characterized using XRPD.

HT CRYSTALMAX Crystallization. Crystallization of **B** was carried out in a 96-well aluminum block holding borosilicate tubes containing the crystallization mixtures, which were rendered supersaturated by heating to 75 °C for 2 h followed by a 0.5 °C/min cooling ramp to 5 °C. Each tube in a 96-tube array was sealed within 15 s of a combinatorial dispensing with a Teflon-coated crimp top to avoid evaporation of organic solvents. A selection of 20 diverse solvents was used as single solvent or as a 50% binary combination. The tubes contained 2 mg of **B**. The samples were incubated at 5 °C and monitored for crystallization over a 2-week period. The crystallization events are identified by an optical scanning station using automated image analysis. Samples that crystallized were removed from the original array.

Powder X-ray Diffraction (XRPD). X-ray powder data were collected on the following: (1) a Philips 1050 diffractometer, Cu $K\alpha$ radiation ($\lambda = 1.5406$ Å), graphite monochromator on diffracted beam, operated at 40 kV and 30 mA; (2) a Bruker AXS D8 Discover X-ray diffractometer. This instrument was equipped with GADDS (General Area Diffraction Detection System), a Bruker AXS HI-STAR area detector at a distance of 15.05 cm as per system calibration, a copper source (Cu $K\alpha_1$ radiation, $\lambda = 1.5406$ Å), automated $x-y-z$ stage, and 0.5 mm collimator. The sample was compacted into pellet form and mounted on the $x-y-z$ stage. A diffractogram was acquired under ambient conditions at a power setting of 40 kV and 40 mA in reflection mode while the sample remained stationary. The diffractogram obtained underwent a spatial remapping procedure to account for the geometrical pincushion distortion of the area detector then integrated along chi from −118.8° to −61.8° and 2-theta 2.1°−37° at a step size of 0.02° with normalization set to bin normalize.

Single-Crystal X-ray Diffraction. All single-crystal crystallographic data of the four forms (Table 2) were collected on a Bruker SMART 1000K diffractometer using Mo $K\alpha$ radiation ($\lambda = 0.710\,73$ Å) with a graphite monochromator. All the heavy atoms and most of the hydrogen atoms were located from the difference maps. The data were reduced by SAINT,[26] solved with SHELXS,[27] and then refined with SHELXL.[28]

FT-IR Microscopy. FT-IR measurements were preformed on a Jasco FT-IR-615 spectrophotometer, in the 400−4000 cm^{-1} region using a KBr disk.

Hot Stage Microscopy (HSM). All HSM examinations were performed on a Wagner and Munz Kofler Hot Stage equipped with digital video recorder facilities.

Differential Scanning Calorimetry (DSC). All DSC measurements were performed using a Q1000 differential scanning calorimeter (TA Instruments, New Castle, DE). Heating and cooling rates of 10 °C/min were employed under dry N_2 with a flow rate of 50 mL/min. The analysis software used was Universal Analysis 2000 for Windows 95/98/2000/NT, version 3.1E; Build 3.1.0.40 (2001 TA Instruments-Water LLC).

Results and Discussion

Preparation of the Crystals. All four forms were obtained by slow evaporation at room temperature from a toluene,

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Table 1. Summary of Results of Crystallization Experiments on **B** by Slow Evaporation at Room Temperature

solvent	material dissolved with **B**	Form(s)[a]
toluene	BP	I
	DPSO	
	–	
chlorobenzene	BP	I//I, III, IV
	DPSO	I
	–	I, IV
dichloromethane	BP	I//II
	DPSO	II
	–	I, II

[a] When more than one form is listed separated by a comma, the forms appear concomitantly.

Table 2. Crystallographic Data for Crystal Forms I–IV of **B**[a]

identification code	Form I	Form II	Form III	Form IV
crystal habit	prism	prism	plate	prism
temp (°C)	22	22	25	22
color	brown-red	brown-orange	orange-yellow	brown-red
crystal system	monoclinic	triclinic	monoclinic	monoclinic
space group	$P2_1/n$	$P\bar{1}$	$P2_1/c$	$P2_1/n$
a (Å)	11.318(1)	9.744(1)	14.735(4)	11.631(1)
b (Å)	22.552(3)	11.444(2)	5.533(2)	16.189(2)
c (Å)	18.076(2)	13.979(2)	19.200(6)	24.173(3)
α (deg)		104.847(3)		
β (deg)	99.966(4)	97.681(4)	106.827(9)	99.153(3)
γ (deg)		95.018(3)		
V (Å^3)	4544.0(10)	1481.2(3)	1498.4(8)	4493.7(9)
Z	18	6	6	18
d_{calcd} (Mg/m^3)	1.212	1.239	1.225	1.225
F(000)	1764	588	588	1764
reflections collected	39 535	12 939	12 048	39 061
reflections used	11 265	7337	3706	11 194
R; R_W	0.046; 0.118	0.047; 0.120	0.058; 0.141	0.048; 0.114

[a] Chemical formula of **B**: $C_{12}H_{12}N_2$. Formula weight: 184.24.

chlorobenzene, or dichloromethane solution containing 1:1 and 2:1 molar ratios of **B** with **BP** or **DPSO**. In some experimental vials the **B** polymorphs grew alone, and in others they grew concomitantly.[29] A summary of crystallization conditions and physical description of **B** polymorphs are given in Table 1 and Figure 1, respectively. In the case of **DPSO** and **B** in all three solvents we obtained a 2:1:1.5 co-crystal of **DPSO:B:H**$_2$O[7] in addition to **B** crystals. A routine check in which **B** was the only material that was dissolved was made to determine whether the potential acceptor component serves to enhance the growth of any or all of the **B** polymorphs (rather than to form a co-crystal). We conclude that the presence of the mixture of **B** with **BP** or **DPSO** or, for that matter, any substance may influence the crystal form obtained alone. For instance, Form III was obtained only when **B** was mixed with **BP**.

Solvent-drop grinding experiments were performed on neat **B**; XRPD was used to monitor the results. Forms I–IV and possibly two new crystal forms (solvate and/or new polymorph) or a mixture of a new form(s) with the known polymorph(s), were obtained (Figure 2); the additional new forms are currently under investigation.

CRYSTALMAX experiments carried out using **B** in solutions with a single solvent or 1:1 solvent mixtures yielded Forms I and III either concomitantly or separately. At least two crystal forms obtained repeatedly from ethylene glycol or water and their 1:1 mixtures with other solvents yielded XRPD patterns different from those of the **B** structures reported here. An example of a representative XRPD pattern is given in Figure 2. For ethylene glycol it appears that two new forms may have been obtained. A third suspected new form was obtained from a 1:1 mixture of toluene with dichloromethane.

Additional traditional and HT experiments in which **B** was dissolved in ethanol, water, chloroform, and acetone were also performed for comparison with the literature. According to the literature amyl acetate solution gave a triclinic product (prismatic habit, melting point of 122 °C) with axial ratios that do not match any of the four forms we obtained.[13] Ethanol solution gave a **B** form that was not obtained by us and was reported above.[21] Le Fevre[18] reported on a monohydrate of **B**. **B** "triclinic addition product" was obtained from chloroform and acetone solutions and may indicate the formation of solvates.[13] In contrast to the literature, we did not obtain a new form from amyl acetate,[13] ethanol,[21] or acetone[13] solutions in the crystallization methods that were used. The new form obtained from water and its mixtures with other solvents from HT CRYSTALMAX experiments could be a new polymorph of **B** or the previously reported but structurally uncharacterized monohydrate.[18] This is also the case for solvent-drop grinding with chloroform from which we obtained a new form, which may be an additional polymorph of **B** or a solvate.

Crystallographic Data. Crystallographic data of **B** I, II, III, and IV are given in Table 2.

In the course of the examination of crystals for the structure determination of Forms I–III, we observed a single crystal which was taken from a vial previously identified as Form I with a very similar habit and color. However, the cell dimensions were different from those of Form I. Solution of the structure surprisingly led to the fourth **B** polymorph, again with the extremely rare $Z' = 4.5$ and $P2_1/n$. Three additional crystals from the same batch gave identical results. A check for misassigned or additional symmetry (using the program PLATON[30]) did not generate any equivalence to Forms I–III or any other cell or space group assignment. Indeed the large difference (>6 Å) in the b axes of Forms I and IV is sufficient to define and confirm their uniqueness.

We attempted to correlate the literature description of the crystal chemistry of **B** with our observations reported here (the earlier data are summarized and available as Supporting Information); only two sources provide any semblance of structural data. Axial ratios (based on interfacial angles) and unit cell parameters of a **B** form are reported by Mieleitner[13] and Ahmed,[21] respectively. Neither of these reports is compatible with the data of the four **B** forms we describe here, which also suggests that there are potentially at least two additional polymorphs of **B** that we have not isolated and characterized. Additional supporting evidence for the existence of more than four **B** polymorphs is given by Kofler who reported on five polymorphs of **B** identified using HSM.

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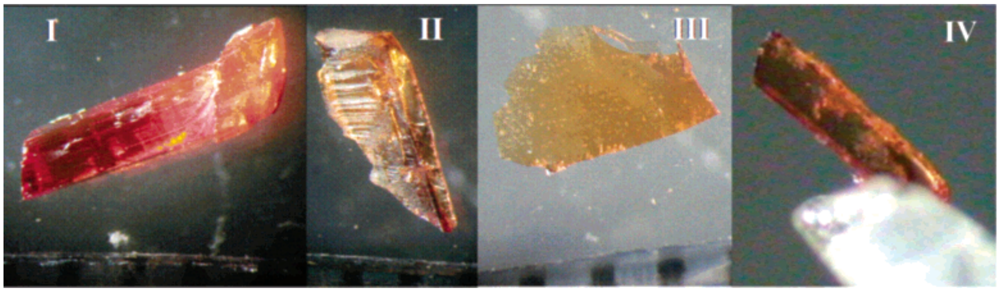

Figure 1. Sample crystals of the four polymorphs of **B**. The crystal photo of Form IV was taken on the goniometer.

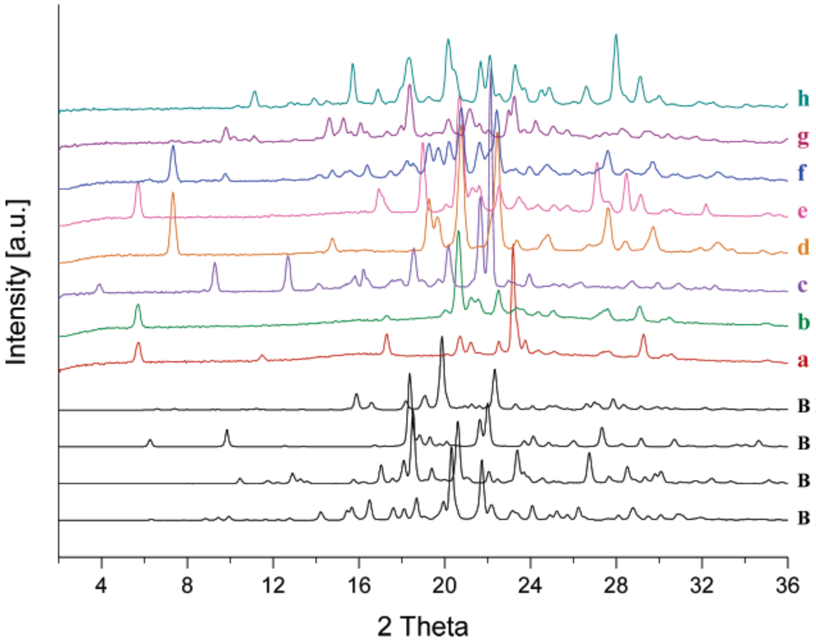

Figure 2. Overlay of representative calculated XRPD data for **B** I−IV (labeled **B**I, etc.) and eight samples obtained by CRYSTALMAX HT (a−f) and solvent-drop grinding (g and h) crystallization experiments of **B**, indicating the crystallization of new form(s) of **B** or a mixture of new form(s) with known polymorph(s); patterns (a) (ethylene glycol + *n*-heptane), (b) (ethylene glycol + methyl ethyl ketone), (c) (toluene + dichloromethane), and (d) (water + isobutyl alcohol) are significantly different from the reference patterns of the four forms of **B**. Patterns (e) (ethylene glycol + acetonitrile), (f) (water + isopropyl acetate), (g) (NMF) and (h) (chloroform) are suspected to be new **B** form(s) or a mixture of new form(s) with known one(s).

As noted above, the four **B** polymorphs have an unusual number of molecules in the asymmetric unit,[23b,31] with different conformations (the crystal packing of the four forms is represented in Figure 3, with symmetry-related molecules colored the same). In the monoclinic structures of Forms I and IV, there are four molecules in a general position which are nonplanar, while one molecule lies on a center of symmetry and therefore is planar in Forms I and IV.[23] Similarly in Form III, one molecule lies on a general position, and one, on a center of symmetry. Form II also crystallizes in a centrosymmetric space group, but no molecule lies on an inversion center.

Thermal Analysis (DSC, HSM). DSC measurements were carried out on Forms I, II, and III in three cycles: (1) Heating from room temperature (∼25 °C) to 150 °C; (2) Cooling from 150 °C to 25 °C; (3) Reheating from 25 °C to 150 °C. In all cycles, heating and cooling rates of 10 °C/min were used. Each sample was prepared in two ways: crushed and uncrushed crystals between the two aluminum pans; no significant differences were observed in DSC traces. The mean value results are given in Table 3. The thermodynamic events of Form II are illustrated in Figure 4. The first heating cycle of Form III is illustrated in Figure 5.

The results in Table 3 are compatible with observations from HSM measurements.

According to these results, we can conclude that Form I and its recrystallization product melt at ca. 125 °C. FT-IR measurements (Figure 6) indicate that the recrystallization product is Form I. Form II melts at ca. 129 °C. We believed that the recrystallization of the melt leads to Form I according to the melting point (ca. 125 °C). On measuring the FT-IR spectrum to verify this hypothesis we were surprised to obtain a spectrum that was neither that of Form I nor Form III, but rather of Form II. A possible explanation to the dissimilar recrystallization product melting point of Form II is that the recrystallization product is Form II and its apparent melting point decreases on the second heating cycle due possibly to better thermal contact between the sample and the pan (DSC) or slide (HSM). It could also be ascribed to a new **B** polymorph with IR similar to that of Form II[32] and a melting point similar to that of Form I. The thermogram of the thermodynamic events of Form II is given in Figure 4. Form III goes through an endothermic solid−solid phase change (Figure 5) between ca. 90−100 °C to Form I (confirmed by melting point and FT-IR measurements). During

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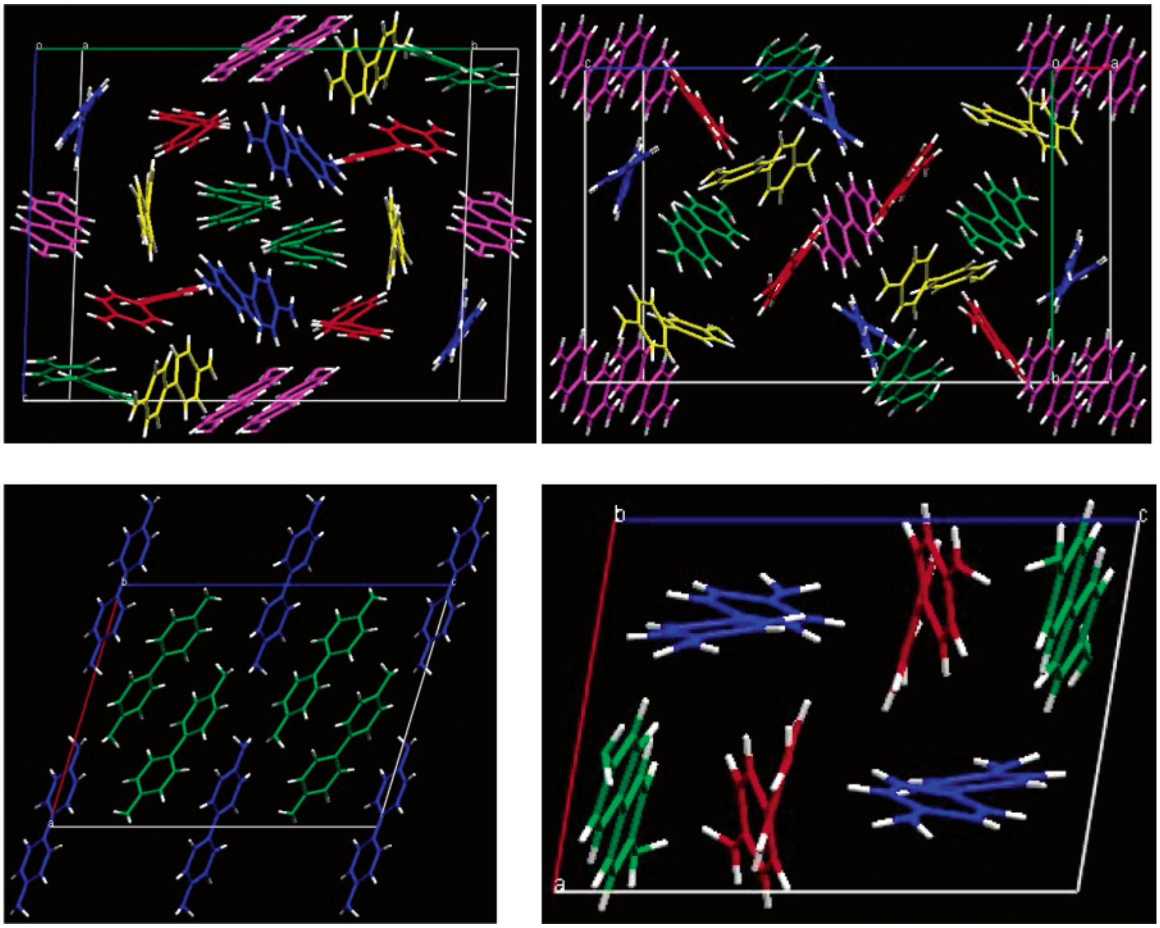

Figure 3. Crystal packing diagrams of Forms I and IV (top left and right, respectively) with $Z' = 4.5$, Form II (bottom right) with $Z' = 3$, and Form III (bottom left) with $Z' = 1.5$. The symmetry-related molecules are colored the same.

Table 3. Summary of DSC Measurements on Forms I−III of **B**

	cycle 1 – heating				cycle 3 – reheating	
	onset *t* [°C]	peak *t* [°C]	onset *t* [°C]	peak *t* [°C]	onset *t* [°C]	peak *t* [°C]
I	–	–	123.8	125.0	123.4	125.0
II	–	–	128.0	128.9	124.7	125.5
III	93.8	98.0	124.4	124.9	124.5	125.0

this transformation on the HSM, the yellow crystals became red. The system is enantiotropic according to Burger's Heat of Transition Rule.[33] These results are consistent with the forms Kofler marked as II, I, and III, respectively, but we could not obtain the melting point of Form III reported by Kofler.

Application of Burger's Density Rule[33] using the calculated densities of the three polymorphic forms (Table 2), together with the observed melting points and phase change, indicate that Form II is the thermodynamically favored form, followed by Form III, and then by Form I. An energy-temperature diagram[34] is given in Figure 7. Forms I and II tend to appear alone, while, in the few cases in which Forms III and IV appeared, they appeared concomitantly with Form I. The appearance of Form I, which appears to be the least thermodynamically stable among the three, must be due to kinetic reasons. In experimental practice, it is commonly observed that the thermodynamically metastable form appears instead of or concomitantly with the thermodynamically stable form (Ostwald's Rule of Stages[35,36]). The experiments show that Form III appears concomitantly with Form I at room temperature far below their transition temperature point, again most likely for kinetic reasons.

Discussion/Summary

The circumstances surrounding the serendipitous crystallization of these polymorphs of **B** warrant further comment. It has been long recognized that the crystallizing medium (i.e., solvent) can alter the crystal form obtained from crystallization.[37] Different crystal forms have also been obtained from different synthetic procedures.[38] The design of "tailor-made" additives to obtain a particular crystal form or to inhibit the growth of an unwanted form has proven to be a very successful strategy.[39] With the recognition of the important potential commercial value of generating new crystal forms (polymorphs and solvates),

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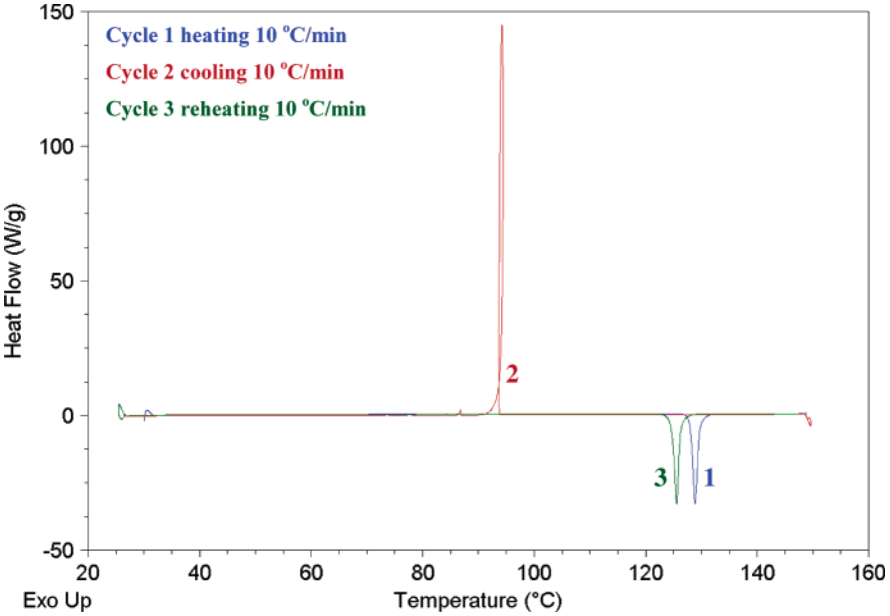

Figure 4. DSC thermogram of the heating (cycle 1, melting of Form II), cooling (cycle 2, recystallization of Form II?), and reheating (cycle 3, melting of Form II?). The starting polymorph is II.

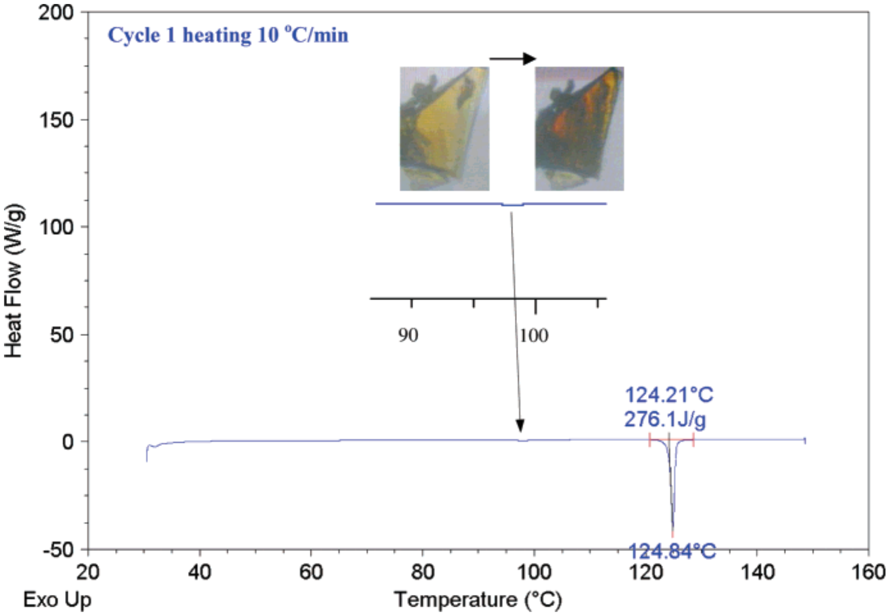

Figure 5. DSC thermogram of the heating (cycle 1, solid−solid phase change from Form III to Form I, which is barely but reproducibly detectable, and melting of Form I). The starting polymorph is III. The crystal photos were taken on the HSM before and after the phase change.

many new techniques have been developed to survey "crystal space" to prepare them.[40] The search for co-crystals is one of the tactics in this overall strategy. However, as we have shown here, the attempts to prepare co-crystals creates new crystallization media and can lead to new polymorphic forms of the intended components of the co-crystals.[41] This can be an unexpected but desirable result of such experiments, and the results of experiments to prepare co-crystals should be carefully analyzed for evidence of these new forms.

Specifically, in the present case four polymorphs of **B** have been serendipitously discovered during attempts to grow co-crystals of **B** with a number of potential acceptors. Polymorphs I and IV exhibit the same color and habit and are difficult to distinguish; they appear in the form of red-brown prisms, while Form II appears as light orange-brown prisms, and Form III, as orange-yellow plates. In subsequent experiments the various forms were obtained from different solvents with or without the presence of another component that was used as the acceptor, at room temperature by slow evaporation. Forms I and II tend to appear alone, while, in the few cases in which Forms III and IV appeared, they appeared concomitantly with Form I. They were also obtained by solvent-drop grinding and CRYSTALMAX experiments of **B** polymorph screens. In these HT experiments we obtained perhaps two or more new forms. These technologies have been developed to more rapidly and com-

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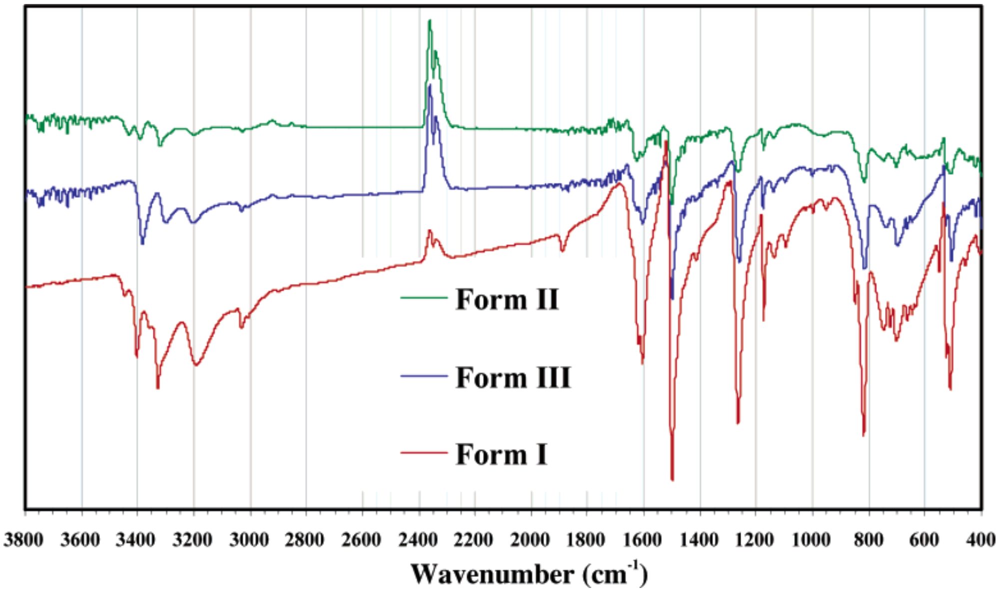

Figure 6. Solid-FT-IR spectra (KBr disk) of I−III **B** forms.

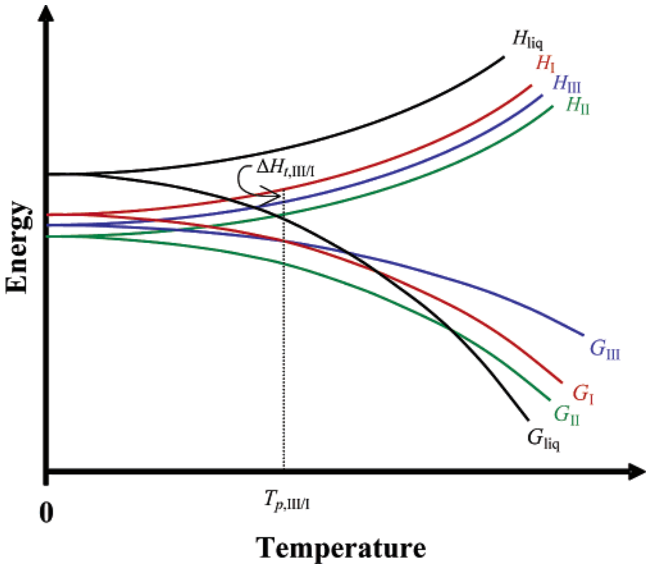

Figure 7. Suggested schematic energy vs temperature diagram for the I−III **B** forms.

prehensively explore solid form diversity. Investigation of the thermal behavior of Forms I, II, and III revealed that Form II is the most stable. The system is enantiotropic with relation to Forms I and III with a transition temperature at ca. 95 °C of Form III to I below which Form III is shown to be the most stable form after Form II.

Regarding this **B** system a number of questions remain to be investigated. We still cannot distinguish visually between Forms I and IV. In addition there is considerable experimental evidence for the existence of at least two more additional forms, for which there is also some suggestion in the literature.

As noted above, it is remarkable that the crystal structure has not been reported for any of the four polymorphs reported here (or others whose existence is suggested here). There are a number of possible reasons for this. First, as demonstrated in Figure 1, despite many crystallization experiments we did not obtain many high quality crystals in terms of well-defined faces. The early literature references indicate that **B** was difficult to crystallize. It is described in the forms of “scales”, “solid paste”,[11] “needles”,[42] “fine plates”, “twined triclinic crystal”,[13] and “plates”.[21] In one case it was clearly stated: “the material is hard to crystallize”.[13] The current diffractometer CCD technology is considerably less demanding in this regard than its predecessors, which allows facile examination and structure determination of samples that would not have been considered suitable two decades ago. Furthermore, more than 30 years ago when **B** played a more important role in chemistry, a crystallographer would have viewed a monoclinic structure with 4.5 molecules in the asymmetric unit or a triclinic structure with 3 molecules in the asymmetric unit as a daunting challenge. We suspect that one or more of these polymorphic forms are skeletons in some crystallographer’s closet.

This experiment points out the importance of paying careful attention to even small differences in habit or color for the possibility of different/new crystal forms. The possibility of screening crystal samples by full structure determination afforded by the CCD detector diffractometer greatly facilitated this investigation.

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Supporting Information Available: (1) X-ray crystallographic information files (CIF) for I−IV Forms of **B**. (2) The important literature sources on the crystal chemistry of **B**. (3) Complete ref 10. This material is available free of charge via the Internet at http://pubs.acs.org.

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