In recent years, associated (H-bonded) systems forming supramolecular aggregates, as well as the van der Waals liquids, have been the subject of intensive studies.\textsuperscript{1,−4} The main aim of these studies is to capture the differences between both types of compounds or to formulate universal patterns/regularities governing their behavior at varying external conditions. The intriguing class of substances are compounds (including numerous active pharmaceutical ingredients (APIs), such as ibuprofen, ketoprofen, indomethacin, and acetaminophen) with a single hydroxyl moiety in the structure. Importantly, these systems, which can create mainly dimeric structures connected by H-bonds (HBs), share the properties of associated substances, as well as the van der Waals liquids.\textsuperscript{1} In this context, it should be mentioned that their structural dynamics is strongly sensitive to compression, which is reflected in the high value of the pressure coefficient of the glass transition temperature \((dT_g/dp)\).\textsuperscript{1,5−8} Moreover, for these systems, the phenomenological temperature pressure superpositioning rule (TPS), which does not work in the highly associated liquids,\textsuperscript{1,9−11} is often satisfied.\textsuperscript{6−8}

It is well established that the type of intermolecular interactions in the system not only determines its physical and dynamical properties but also influences the progress of
many processes (e.g., crystallization), their mechanisms, and kinetics.\textsuperscript{12–14} Therefore, the other problem that is worthy of discussion in the context of the considered classes of materials is the crystallization tendency. In general, in most associated systems, the presence of HBs favors the transition to the glassy state and improves the stability of this phase.\textsuperscript{2,12,13} However, this statement is valid mainly for substances that can form extensive H-bonds and create supramolecular structures, such as saccharides (e.g., glucose)\textsuperscript{12} or alcohols.\textsuperscript{1,16–19} In contrast, the compounds capable of forming dimers/trimers are usually characterized by a lower glass-forming ability (GFA) and simultaneously stronger tendency to crystallization.\textsuperscript{12} Such behavior has been shown for many APIs, classified as nonsteroidal anti-inflammatory drugs (NSAIDs), e.g., celecoxib,\textsuperscript{20} naproxen,\textsuperscript{21,22} flurbiprofen,\textsuperscript{23} or acetylsalicylic acid.\textsuperscript{24} The impact of dimers on the crystallization rate was also reported for ibuprofen,\textsuperscript{7,25} ketoprofen,\textsuperscript{9} and indomethacin.\textsuperscript{20} However, it should be stressed that according to the classification of amorphous pharmaceuticals proposed by Baird et al.,\textsuperscript{27} these three APIs belong to the classes with low crystallization tendency. The fact that dimeric structures decrease the GFA has also been confirmed in our previous work,\textsuperscript{22} where it was demonstrated that the esterification of the carboxylic group of naproxen (NAP) significantly increases its GFA by inhibiting the formation of HBs between the NAP molecules.

Hence, the chemical structure, and thereby the type of intermolecular interactions, is one of the most significant factors determining the glass-forming tendency/physical stability of the given material. This is especially important in the case of amorphous pharmaceuticals (mainly from II and IV groups of the Biopharmaceutical Classification System (BCS)), which are characterized by improved solubility and bioavailability in comparison to their crystalline counterparts. However, the problem with the prediction of their physical stability, due to the change in temperature, humidity, irradiation, and time, seems to be a limiting step toward the better use of these systems in commercial applications.\textsuperscript{13,28,29} Therefore, studies on active substances forming dimeric H-bonded structures are crucial not only to better understand their nonintuitive molecular dynamics but also to improve the long-term stability of these amorphous pharmaceuticals. Interestingly, the impact of varying populations of HBs in anti-HIV agent ritonavir (RTV) due to sub-\(T_g\) annealing has recently been examined by Tominaka and co-workers.\textsuperscript{30} The authors showed that the applied procedure significantly stabilizes this highly associated compound and protects it against crystallization. In this paper, we have selected gemfibrozil (GEM), which was further chemically modified (by replacing the hydrogen from the carboxyl group by the deuterium atom, as well as the methyl moiety) to change the interactions from H-bonded to purely van der Waals ones. It should be mentioned that GEM is an antihyperlipidemic API belonging to the II class of BCS, which is characterized by relatively low GFA, manifested as a strong tendency to crystallization from the amorphous state. In the next step, we performed similar experiments to those carried out by Tominaka et al. to probe the impact of annealing in the vicinity of \(T_g\) on the kinetics of the crystallization process in the examined systems.

\section*{EXPERIMENTAL SECTION}

\subsection*{Materials and Methods. Materials.} Gemfibrozil, GEM (IUPAC name: 5-(2,5-dimethoxy)-2,2-dimethyl-penta-
noic acid, \(\text{C}_{15}\text{H}_{22}\text{O}_3\), \(M_w = 250.33\) g/mol), having purity \(>98\%\) was supplied by TCI Europe and used as received. Deuterated gemfibrozil (dGEM, \(\text{C}_{15}\text{H}_{21}\text{D}_3\), \(M_w = 251.33\) g/mol) and methylated derivative (metGEM, methyl 5-(2,5-dimethoxy)-2,2-dimethyl-pentanoate, \(\text{C}_{19}\text{H}_{26}\text{O}_3\), \(M_w = 264.33\) g/mol) have been prepared for the purpose of this paper. dGEM was obtained by the replacement of the hydrogen from the hydroxyl group by deuterium from heavy water, whereas metGEM was synthesized using the esterification procedure of this group. Details of both methods are presented in the Supporting Information (SI). It should be added that GEM and dGEM are white crystalline powders, while metGEM is a clear oily liquid. The chemical structures of investigated compounds are illustrated in Scheme 1.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Scheme1}
\caption{Chemical Structure of Gemfibrozil and Its Derivatives}
\end{figure}

\subsection*{Methods. Differential Scanning Calorimetry (DSC).} Thermodynamic properties of the examined substances have been investigated using the DSC technique. Calorimetric measurements were carried out using a Mettler Toledo DSC apparatus (Mettler Toledo International, Inc., Greifensee, Switzerland) equipped with a liquid nitrogen cooling accessory and an HSS8 ceramic sensor (heat flux sensor with 120 thermocouples). Temperature and enthalpy calibrations were performed using indium and zinc standards. Each sample was placed in an aluminum crucible (40 \(\mu\)L) and measured at the rate of 10 K/min. The crystalline compounds (GEM, dGEM) were heated inside the DSC apparatus over the melting temperature, next immediately cooled to vitrify the liquid samples, and subsequently scanned to above the respective melting points. In turn, metGEM (a liquid) was cooled to 180 K and then heated to 280 K.

\subsection*{Broad-Band Dielectric Spectroscopy (BDS).} Isobaric complex dielectric permittivity measurements \((\varepsilon^* (\omega) = \varepsilon' (\omega) - i \varepsilon'' (\omega))\) were taken using the Novocontrol Alpha dielectric spectrometer (Novocontrol Technologies GmbH & Co. KG, Hundsangen, Germany) with the control of temperature provided by a Quatro system, using a nitrogen gas cryostat and stability better than 0.1 K. The data were collected at ambient pressure over the frequency range from \(10^2\) to \(10^6\) Hz. The sample was placed between two stainless steel electrodes of the capacitor (diameter 10 mm, gap 0.1 mm) and mounted on a cryostat. The molecular dynamics studies were carried out in the following temperature ranges: from 173 K up to 279 K (GEM), from 163 K up to 275 K (dGEM), as well as from 163 K up to 229 K (metGEM) after fast cooling to the glassy state. The crystallization kinetics studies (isothermal measurements) were performed at \(T = 279, 273, 268\), and 265 K (GEM, dGEM), as well as at \(T = 231, 227, 223\), and 219 K (metGEM). The annealing experiments were carried out at temperatures above \((T = 242 \text{ K for GEM, } T = 240 \text{ K for dGEM, } T = 203 \text{ K for metGEM})\) and below \((T = 232 \text{ K for GEM, } T = 230 \text{ K for} \)
dGEM, $T = 187$ K for metGEM) the glass transition points and were continued for about 1 h.

Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectra were measured using the Nicolet iS50 FTIR spectrometer (Thermo Scientific) with a spectral resolution of 4 cm$^{-1}$. They were recorded in the 4000–800 cm$^{-1}$ frequency range. The bands located below 800 cm$^{-1}$ were not taken into account due to the absorption of CaF$_2$ windows. The 32 scans were co-added for each spectrum. The glassy/supercooled GEM was obtained by the cooling (30 K/min) of the molten API in a Linkam THMS 600 heating/cooling stage (Linkam Scientific Instruments Ltd., Surrey, U.K.) mounted inside the sample stage of the IR spectrometer. The temperature stabilization accuracy was equal to 0.1 K. The IR spectra were recorded at equal intervals (every 15 min) for 1 h after the temperature stabilization at 253 K. The cell, consisting of the two CaF$_2$ windows, separated by a 15 μm thick spacer, was used to produce the glassy/supercooled sample of uniform thickness and warrants the constant geometry of the system.

■ RESULTS AND DISCUSSION

At first, we performed calorimetric measurements to characterize the thermodynamic properties as well as phase transitions in the examined compounds. In Figure 1a, DSC thermograms obtained during heating the crystal and glassy GEM and dGEM with a heating rate of 10 K/min (a). The inset presents a single scan for metGEM, which is a liquid sample. Dielectric loss spectra measured for all studied substances above and below the $T_g$ (b–d).

![Figure 1. DSC thermograms obtained during heating the crystal and glassy GEM and dGEM with a heating rate of 10 K/min (a). The inset presents a single scan for metGEM, which is a liquid sample. Dielectric loss spectra measured for all studied substances above and below the $T_g$ (b–d).](https://dx.doi.org/10.1021/acs.molpharmaceut.9b01244)
ibuprofen ($\Delta C_p = 0.362 \text{ J/g K}$)\textsuperscript{35} and indomethacin ($\Delta C_p = 0.417 \text{ J/g K}$).\textsuperscript{35} However, it should be noted that for some pharmaceuticals forming H-bonded dimers, e.g., celecoxib and ketoprofen, as well as strongly H-bonded associates, such as ritonavir, the determined values were slightly higher ($\Delta C_p = 0.440$,\textsuperscript{35} 0.471,\textsuperscript{35} and 0.470 J/g K,\textsuperscript{35} respectively) or lower ($\Delta C_p = 0.298 \text{ J/g K}$—the case of probucol). Moreover, it can be stated that the heat capacity jump at $T_g$ obtained for the deuterated derivative of GEM is practically the same as $\Delta C_p$ calculated for neat GEM, despite the weakening of the H-bonds. In turn, the $\Delta C_p$ for metGEM (van der Waals system) is somewhat higher than that of GEM and dGEM. Interestingly, the higher value of this thermodynamic parameter in comparison to the neat API was also determined for the methyl, isopropyl hexyl, and benzyl derivative of IBU (thermograms presented in our recent paper)\textsuperscript{37}: $\Delta C_p^{\text{IBU}} = 0.362 \text{ J/g K}$, while $\Delta C_p^{\text{IBU}}$ varies within the range 0.487–0.526 J/g K. Based on the above, one can suppose that the change in the intermolecular interactions (from H-bonded to van der Waals) results in the increase in the value of heat capacity jump at $T_g$ for the systems characterized by the very similar backbone.

Having determined the thermal properties of GEM and its two derivatives, we carried out dielectric measurements at ambient pressure and in a wide temperature range. In Figure 1b–d, the dielectric loss spectra collected for GEM, dGEM, and metGEM, above and below the $T_g$ are presented. As illustrated, in the supercooled liquid phase ($T > T_g$) of all examined compounds, the structural ($\alpha$) relaxation, followed by dc conductivity connected to the charge transport, can be observed. Both processes move toward lower frequencies ($f$) with decreasing temperature. Moreover, at higher $f$, a significant decrease in the amplitude of $\alpha$-loss peak (indicating the undergoing crystallization) is detected. On the other hand, in the glassy state ($T < T_g$), two secondary relaxation processes ($\beta$ and $\gamma$) with smaller amplitude dominate the spectra of GEM and its two derivatives. The $\beta$-process in GEM and dGEM is less visible than the $\gamma$ one. In the case of metGEM, both secondary relaxations ($\beta$, $\gamma$) are not well separated from each other and, thus, form one broad peak.

To comprehensively characterize the molecular dynamics of the investigated compounds, the dielectric loss spectra shown in Figure 1b–d were fitted to the one (above the $T_g$) or superposition of two (below the $T_g$) Havriliak–Negami (HN) functions with an additional term describing the dc conductivity $\varepsilon_0$:

$$
\varepsilon^*(\tilde{\omega}) = \varepsilon_\infty + \frac{\Delta\varepsilon}{1 + (i\tilde{\omega}\tau_H)^{\gamma_H}} + \frac{\Delta\varepsilon_0}{1 + (i\tilde{\omega})^{\gamma_0}} + \varepsilon_\infty \frac{\Delta\varepsilon f_0}{\varepsilon_\infty f_0}\text{Im}\left(e^{-\tilde{\omega}f_0}\right) + \frac{\Delta\varepsilon_j}{1 + (i\tilde{\omega}\tau_j)^{\gamma_j}}
$$

(1)

where $\tau_H$ is the HN relaxation time, $\varepsilon_\infty$ is the vacuum permittivity, $\tilde{\omega}$ is the angular frequency ($\tilde{\omega} = 2\pi f$), $\Delta\varepsilon$ is the dielectric relaxation strength, and $\gamma_H$ and $\gamma_0$ are the shape parameters, representing the symmetric and asymmetric broadening of the given relaxation peaks, respectively. Next, $\tau_{\text{dGEM}}$ and $\tau_{\text{metGEM}}$ were recalculated from $\tau_H$ using the formula given in ref 39. The obtained values were plotted as a function of temperatures scaled to $T_g$ in Figure 2b. As can be seen, the relaxation times of the two secondary relaxation processes ($\beta$, $\gamma$) in metGEM deviate from the respective values determined for the two other compounds. It is especially noticeable in the case of $\tau_\beta$ of methylated derivatives, which are much shorter than those obtained for GEM and dGEM.

To describe the temperature dependencies of $\tau_\alpha$, the Vogel–Fulcher–Tammann (VFT) equation\textsuperscript{40–42} was applied (Figure 2b, solid red lines)

$$
\tau_\alpha = \tau_{\text{VFT}} \exp\left(\frac{D_T T_0}{T - T_0}\right)
$$

(2)

where $\tau_{\text{VFT}}$ is the relaxation time at finite temperature, $D_T$ is the strength parameter, and $T_0$ represents the temperature at which the structural times tend to infinity. Using the VFT fits,
the glass transition temperatures (defined herein as temperatures at which $\tau_\alpha = 100$ s) for GEM, dGEM, and metGEM were determined—see Figure 2b. It can be noted that the obtained values are slightly lower than the calorimetric $T_g$s (see Figure 1a).

Moreover, for three examined compounds, isobaric fragility ($m$), which describes the sensitivity of the structural process to the temperature changes, was calculated according to the following equation\(^43\)

$$m = \frac{d \log \tau_\alpha}{d(T_g/T)} \bigg|_{T=T_g}$$

(3)

Based on the determined values ($m = 68–80$; Figure 2b), one can classify GEM, dGEM, and metGEM as moderately fragile materials.

In turn, the temperature dependences of secondary ($\gamma$ and $\beta$) relaxation times were fitted to the Arrhenius equation (the red dotted lines in Figure 2b)

$$\tau = \tau_\infty \exp \left( \frac{E_x}{RT} \right), \quad x = \beta, \gamma$$

(4)

where $\tau_\infty$ is a pre-exponential factor, $R$ is a gas constant, and $E_x$ is the activation energy. We found that the activation energies of both secondary modes obtained for the van der Waals system, metGEM ($E_\beta = 53$ kJ/mol, $E_\gamma = 32$ kJ/mol), are slightly higher than those determined for GEM ($E_\beta = 46$ kJ/mol, $E_\gamma = 28.5$ kJ/mol) and dGEM ($E_\beta = 48.5$ kJ/mol, $E_\gamma = 26$ kJ/mol), which are capable of forming H-bonded dimeric structures.

To gain a better insight into the molecular origin of the slower ($\beta$) and faster ($\gamma$) processes observed in the dielectric spectra of the examined compounds, the coupling model (CM) proposed by Ngai\(^44-46\) was applied. It is worth mentioning that this approach is commonly used to distinguish the secondary relaxations having an intermolecular character (called the Johari–Goldstein (JG) type) from those originating from intramolecular motions of some parts of the molecules (the non-JG type). In particular, it links the relaxation time of the given (JG) secondary process ($\tau_{\text{JG}}$) and the primitive relaxation time ($\tau_0$) of the CM by the following relation

\begin{align*}
\tau = \frac{\tau_0}{\tau_{\text{JG}}} & = \frac{\tau_0}{\tau_{\text{CM}}} = \frac{\tau_0}{\tau_{\text{CM}}} \\
\end{align*}

Figure 3. Time evolution of the imaginary (a, c, e) and real (b, d, f) parts of complex dielectric permittivity plotted versus frequency for GEM (a, b), dGEM (c, d), and metGEM (e, f). The isotherms were measured at the indicated temperatures.

\(994\)
\[ \tau_{\text{CM}}(T, p) \approx \tau_{0}(T, p) \]  

The value of \( \tau_0 \) can be determined from the parameters \( \tau_{\text{CM}} \) and \( \beta_{\text{KWW}} \) (eq 5), which is a coupling parameter of the Kohlrausch–Williams–Watts (KWW) function\(^{37,38}\):

\[ \phi_{\text{KWW}}(t) = \exp[-(\tau/\tau_{\text{CM}})^{\beta_{\text{KWW}}}] \]  

used to fit the \( \alpha \)-loss peak at the same temperature, by the CM equation

\[ \tau_0 = (\tau_c)^n(\tau_{\text{CM}})^{-n} \]  

where \( \tau_c = 2 \text{ ps} \) for small molecular glass-forming liquids. From the analysis of the data (i.e., the normalized dielectric loss spectra of the investigated compounds, measured at selected temperatures close to the \( T_g \)) using KWW function (eq 6), the fractional exponent, \( \beta_{\text{KWW}} \), equal to 0.58 (GEM, dGEM) and 0.53 (metGEM—see Figure 2a), which corresponds to \( n = 0.42 \) and 0.47, respectively, was obtained. Subsequently, we calculated primitive relaxation times \( (\tau_0) \) at four temperatures close to the \( T_g \), and from the corresponding \( \tau_{\text{CM}} \) (VFT fit) of all investigated compounds. As illustrated, the determined values of \( \tau_0 \) (the crossed symbols in Figure 2b) are very close to the experimental \( \beta \)-relaxation times. Therefore, one can postulate that the slower secondary \( (\beta) \)-relaxation in GEM and its derivatives is a true JG process originating from the local motions of the entire molecules. The faster \( (\gamma) \)-relaxation has the most likely intramolecular character (it is probably related to the rotations of the side alkyl chain in the examined systems).

Besides studying the relaxation dynamics of GEM and its derivatives, we have also performed comprehensive crystallization kinetics studies. Interestingly, we applied two different procedures to measure the progress of this process. The first one was a standard approach—the substances were cooled and next heated to the appropriate crystallization temperatures \( (T_c) \). During the second path, the samples were cooled to the two temperatures close to the \( T_g \) (one below and one above the \( T_g \)) annealed at these \( T_c \), and finally heated up to the \( T_c \). Dielectric data for all examined samples (the standard procedure) were collected at four \( T_c \) mentioned in the Methods section. In turn, the postannealing isotherms (second approach) were registered at one selected \( T_c \) in which the first (standard) measurements were also performed. Representative dielectric loss and dispersion spectra obtained during the standard isothermal crystallization of GEM, dGEM, and metGEM are presented in Figure 3a–f, while the analogical data collected before and after annealing at \( T = 273 \text{ K} \) (GEM and dGEM) and \( T = 223 \text{ K} \) (metGEM) are given in the SI (Figures S1–S3). As can be seen, in all cases, the amplitude of the \( \alpha \)-relaxation process, as well as the static permittivity, systematically decreases with time, which is a result of freezing out the molecular mobility during crystallization. It should be mentioned that we have compared the dielectric loss spectra measured for three examined compounds before and after annealing at \( T \sim T_g \) (see Figure S4 in the SI) and found that there are no differences between them.

To analyze the progress of the crystallization in the examined API and its derivatives (standard and annealing procedures), the measured static permittivity \( (\varepsilon') \) (Figures 3 and S1–S3) was renormalized using the following equation

\[ \varepsilon'_N(t) = \frac{\varepsilon'(0) - \varepsilon'(t)}{\varepsilon'(0) - \varepsilon'(\infty)} \]  

where \( \varepsilon'(0) \) is the dielectric permittivity at the beginning of the crystallization, \( \varepsilon'(\infty) \) is the long-time limiting value, and \( \varepsilon'(t) \) is the value at the time, \( t \). In Figure 4a–c, the values of \( \varepsilon'_N \) have been plotted as a function of time. The data obtained during the standard isothermal crystallization are shown in the main panels, whereas those collected for the samples after annealing are presented in the insets and compared with those measured for the samples without annealing. The solid red lines represent the Avrami fits in terms of eq 9.
where $k$ is a rate constant, and $n_{(A)}$ is the Avrami exponent, which depends on the crystal morphology. The solid red lines in Figure 4 represent the best fits of eq 9 to experimental data, and, as illustrated, the Avrami model describes them in a satisfactory way. Interestingly, the values of $n_{(A)}$ obtained for GEM, dGEM, and metGEM from the global fitting using eq 9 were as follows: 1.75, 1.93, and 3.48, respectively. This simple comparison indicates the formation of crystals of different morphologies in the methylated derivative of GEM with respect to two other substances. It should also be noted that the Avrami exponents determined from the analysis of the data after annealing of GEM, $n_{(A)} = 1.42$ ($T_{\text{ann}} = 242$ K) and $n_{(A)} = 1.41$ ($T_{\text{ann}} = 232$ K), as well as dGEM: $n_{(A)} = 1.60$ ($T_{\text{ann}} = 240$ K) and $n_{(A)} = 1.71$ ($T_{\text{ann}} = 230$ K)—see Table S1, were lower than those obtained from fitting the kinetic curves constructed from the standard isothermal measurements. On the other hand, the values of $n_{(A)}$ for metGEM before ($n_{(A)} = 3.48$) and after annealing ($n_{(A)} = 3.60$ ($T_{\text{ann}} = 203$ K) and $n_{(A)} = 3.57$ ($T_{\text{ann}} = 187$ K)) were very close to each other. Based on the above, one can conclude that the annealing does not influence the morphology of the growing crystals in the studied systems.

As a next step, the parameters $k$ obtained from the above-mentioned fitting procedure (standard isothermal data) were plotted versus inverse temperature ($1/T$) in Figure 5. To estimate the activation barrier for crystallization ($E_k$) in the examined systems, the dependencies $\ln(k)$ versus $1/T$ were analyzed using the following form of the Arrhenius equation

$$k = k_0 \exp\left(\frac{-E_k}{k_B T}\right)$$  

where $k_0$ is the pre-exponential factor and $k_B$ is a Boltzmann constant. We found that the values of $E_k$ obtained for GEM ($E_k = 120$ kJ/mol) and metGEM ($E_k = 106$ kJ/mol) are close to each other, whereas those determined for dGEM are clearly lower ($E_k = 77$ kJ/mol). Additionally, for all examined samples, we calculated the activation energy of the $\alpha$-process ($E_\alpha$) in the temperature range at which the crystallization was carried out.

The obtained values were higher than $E_k$ determined for the given compound and changed within the ranges: 161–193, 167–194, and 107–138.0 kJ/mol for GEM, dGEM, and metGEM, respectively. Hence, one can conclude that there is no close relationship between $E_k$ and $E_\alpha$ in the investigated systems. This finding is in line with the data showing the decoupling between the viscosity or reorientational structural dynamics and the rate of the overall crystallization or crystal growth, reported in the literature for several APIs.32–35

From Figure 5, one can notice that the crystallization rate of GEM is much lower when compared to those of dGEM and metGEM. It is also evident when we plot $\ln k$ versus $T_k/T$ (see the inset of Figure 5). As illustrated, the differences in the speed of crystallization between GEM and other samples (at the same degree of supercooling) are larger than an order of magnitude. Importantly, the data presented in Figure 5 (the crossed symbols) also confirmed that the annealing at $T_k$ affects the progress of crystallization of the nonmodified API in a significant way, while in the other samples the thermal history does not have as much influence on the pace of this process. To explain the obtained results, two possible scenarios can be considered. First, during the isothermal annealing, the nucleation process could have been initiated. Second, the observed behavior might be associated with some equilibration/reorganization in the H-bonding pattern, as has recently been suggested by Tominaka et al.30 In their paper, the authors showed that the variation in the H-bonding scheme in ritonavir (RTV) due to the sub-$T_k$ annealing inhibits/delays the crystallization. Herein, the situation is much different since crystallization rates of GEM and dGEM increased after the annealing procedure. The discrepancy between the results reported in this work and the one published earlier by Tominaka et al.30 is probably connected to the fact that RTV has many centers capable of forming extensive HBs, while in the case of GEM there are mainly small dimeric structures. Therefore, due to the annealing, there might be some reorganization in the hydrogen bond pattern or within dimers of API. In this context, it is worth mentioning our previous paper, in which we have explicitly shown that in naproxen (NAP), which also forms dimers, these small structures are responsible for the weak glass-forming ability and the enhanced crystallization of the examined pharmaceutical.

To verify which of the two hypotheses is correct, first we analyzed the representative absorption spectra collected during the annealing of GEM at $T \sim T_k$—see the main panel in Figure 6a. As illustrated, at the time of annealing at $T = 242$ K, only subtle changes in the position of the structural ($\alpha$) peak can be detected (the maximum is just slightly shifted toward lower frequencies). By fitting the collected data to the one Havriliak–Negami function (eq 1), we determined the evolution of the dielectric strength ($\Delta\varepsilon$) of the structural process and plotted it as a function of time ($t$)—see the inset of Figure 6a. As can be seen, the values of $\Delta\varepsilon$ (amplitude of $\alpha$-peak) slightly increase with time. In this context, it is worthwhile to mention the studies on glycerol carried out by Sanz and Niss.56 The authors observed that, in contrast to our data, the dielectric permittivity (which is related to the dielectric strength of $\alpha$-process) decreases with the time during annealing. This effect was related to the nucleation phenomenon. However, it should be stressed that the procedure applied in paper 56 lasted 200 h, whereas GEM and its derivatives were annealed by 1–1.5 h (the short time was used intentionally to avoid the nucleation process—the
Theoretical results, the bands observed at 3045 and 3024 cm\(^{-1}\) related to the C\(_{\text{H}}\) stretching vibrations of the aliphatic CH\(_3\) groups are assigned to the C\(_{\text{H}}\) deformation vibrations of the aliphatic CH\(_3\) groups. The symmetric CH\(_2\) stretching vibration, while the one at 1586 cm\(^{-1}\) is ascribed to the ring-breathing vibration. The band at 1367 cm\(^{-1}\) originates from the CH\(_2\) scissoring vibration. The medium intense bands at 1413 and 1286 cm\(^{-1}\) correspond to the C–H in-plane-bending vibrations. The CH\(_2\) twisting vibrations are detected at 1266 cm\(^{-1}\). Finally, the bands at 1215 and 1158 cm\(^{-1}\) correspond to the C–H in-plane-bending vibrations.

In panels (c) and (d) of Figure 6, the representative infrared spectra measured for the crystalline and supercooled GEM—see Figure S5 in the SI. It was found that the subtle structure and spectral parameters, including the position, full width at half-maximum (FWHM), shape, etc., characterizing the band corresponding to the stretching vibration of the hydroxyl moiety, are very similar in both cases. Therefore, considering the crystallographic data reported for this API in ref 58, one can clearly state that in both crystalline and glassy/supercooled states GEM forms dimers connected via medium-strong H-bonds (the average donor–acceptor distance is in the range of 2.5–3.2 Å).

**Figure 6.** Dielectric loss spectra measured during the annealing procedure at temperatures higher than the \(T_g\) for GEM (a). The inset shows the dielectric strength of the structural relaxation plotted as a function of the time of annealing; the kinetic analysis of the absorbance changes for the selected difference bands of GEM (the asterisk symbol in the legend indicates a new component of the C\(_{\text{O}}\) stretching vibration band observed at ca. 1720 cm\(^{-1}\)) (b); FTIR difference spectra of GEM in the 3500–2000 cm\(^{-1}\) (c) and 1800–1100 cm\(^{-1}\) (d) frequency ranges, recorded with a 15 min interval and obtained after subtracting the initial spectrum from the time-dependent measurements carried out at 253 K.

Dielectric loss spectra measured during the annealing procedure at temperatures higher than the \(T_g\) for GEM (a). The inset shows the dielectric strength of the structural relaxation plotted as a function of the time of annealing; the kinetic analysis of the absorbance changes for the selected difference bands of GEM (the asterisk symbol in the legend indicates a new component of the C\(_{\text{O}}\) stretching vibration band observed at ca. 1720 cm\(^{-1}\)) (b); FTIR difference spectra of GEM in the 3500–2000 cm\(^{-1}\) (c) and 1800–1100 cm\(^{-1}\) (d) frequency ranges, recorded with a 15 min interval and obtained after subtracting the initial spectrum from the time-dependent measurements carried out at 253 K.
annealing (see Figure 6b). Furthermore, the applied data treatment revealed the complex behavior in the spectral frequency regime connected to the C=O stretching vibration, where a significant negative difference band appears at 1698 cm⁻¹, indicating a loss of absorption in this region (Figure 6d). Simultaneously, a new band component emerges at ca. 1720 cm⁻¹. It is worthwhile to notice that the observed changes within the collected spectra are not due to the variation in the sample size since we have used a 15 μm spacer in the sample holder to warrant the constant sample thickness/geometry during time-dependent measurements. Moreover, the intensity of the absorption peaks did not change monotonically in the whole spectral range over time, since some peaks revealed the increase or decrease in intensity (see Figure 6b). Additionally, the lack of frequency shifts of the selected bands over time indicated that the variation in the FTIR spectra is not related to the undergoing phase transition (nucleation) or conformational change of GEM. It is also worth stressing that, generally, the intensity of the vibrational band is simply proportional to the probability of the transition from the vibrational ground state to the excited state, which in turn depends on the population of the initial state involved in the transition and the change in the dipole moment vector with the vibrational coordinate. Considering these facts, one can hypothesize that the above-described changes in the intensity of the selected bands are most likely related to some reorganization within the dimeric structures of GEM upon annealing at T ~ T_g.

This experimental observation, together with the data reported by Tominaka et al.,³⁰ seems to be very interesting and in some way question the common belief that annealing above the T_g erases the thermal history of the sample. Thus, as far as this procedure is performed in purely the van der Waals liquids (having no tendency to form associates), for which nucleation is avoided, as well as the system geometry and experimental conditions are preserved, it should not make any significant impact on the physical properties or crystallization tendency of these compounds. This hypothesis is somehow confirmed by the data reported herein for metGEM. The situation becomes far more complex in the case of the associating substances. For this particular class of materials, the degree of association and population of more or less complex supramolecular structures, as well as the strength of the H-bonds, may change during the sample annealing in the vicinity of the T_g. Such rearrangements in the H-bonding pattern may have a strong impact on the behavior or crystallization ability of the associating liquids. Furthermore, in the compounds forming self-assemblies of varying architecture and complexity, this effect might be completely different. The above hypothesis is confirmed by the data reported by Tominaka et al.,³³ and by us, although, for sure, further thorough studies are required to better understand this quite interesting issue.

### CONCLUSIONS

In this paper, the results of the molecular dynamics and crystallization studies (performed using the BDS technique), supported by calorimetric and infrared data for gemfibrozil (GEM) and its two derivatives, deuterated (dGEM) and methylated (metGEM), have been presented. Dielectric measurements showed that besides the dc conductivity and structural (α)-relaxation (observed for all examined compounds at T > T_g), two secondary relaxations (β and γ) dominate the loss spectra of glassy samples (T < T_g). Analysis using the coupling model (CM) suggested that the slower secondary process, labeled as β, originates from the intermolecular motions of the whole molecules (is a true JG-relaxation), whereas the faster mode (γ) has a rather intramolecular character. The most interesting results were obtained from the crystallization kinetics measurements carried out after applying two procedures: (1) the standard cooling and heating to crystallization temperature (T_c) and (2) the annealing at T ~ T_g just prior to the start of the crystallization process at T_c. These studies showed that the activation barrier of crystallization (E_a) for GEM and metGEM is similar (120 and 106 kJ/mol, respectively), while that determined for dGEM is clearly lower (77 kJ/mol). Moreover, and most importantly, they revealed that the annealing significantly increases the crystallization rate of GEM (H-bonded system), while it has a very weak impact on the pace of this process in the case of dGEM and metGEM (compounds that are characterized by weaker H-bonds and van der Waals interactions, respectively). To explain the observed behavior, we analyzed the changes of the dielectric strength (Δε) of the α-process during the annealing of GEM, as well as the evolution of respective bands (connected to H-bonds) in the infrared spectra. The first analysis excluded the early nucleation as a reason for the enhanced crystallization rate of GEM. In turn, infrared studies suggested that some kind of reorganization within dimers is responsible for this effect. The results obtained herein are in contrast to those published by Tominaka et al.,³⁰ who suggested that the sub-T_g annealing inhibits the rate of crystallization in H-bonded API ritonavir (RTV), which has many moieties capable of forming extensive HBs. Based on these contradictory reports, one can conclude that the annealing close to the T_g may have a completely different impact on the behavior or crystallization ability of the materials forming H-bonds and supramolecular structures of varying architecture.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.molpharmaceut.9b01244.

Description of the determination of dGEM, the procedure of the synthesis of metGEM, crystallization data and comparison of the spectra obtained before and after annealing for all studied substances, as well as the FTIR spectra of crystalline and supercooled GEM (PDF)

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Notes
The authors declare no competing financial interest.

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