Investigation of the Structure and Dynamics of Antiviral Drug Adefovir Dipivoxil by Site-Specific Spin–Lattice Relaxation Time Measurements and Chemical Shift Anisotropy Tensor Measurements

Krishna Kishor Dey and Manasi Ghosh*

Cite This: ACS Omega 2020, 5, 29373–29381

ABSTRACT: Adefovir is regarded as a potential antiviral agent. However, it cannot be considered as a valuable drug candidate due to its high polarity that limits its permeability across the human intestinal mucosa. When the ribose phosphate group of adefovir is replaced by the isopolar phosphonomethyl ether functionality, it neutralizes the negative charge of the drug. This makes the drug lipid-soluble and potent to diffuse across the cell membrane. The prodrug adefovir dipivoxil is regarded as a potent antiviral drug against hepatitis B virus (HBV), human immunodeficiency virus (HIV), Rauscher murine leukemia virus (R-MuLV), murine cytomegalovirus (MCMV), herpes simplex virus (HSV), simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV). The correlation between the structure and the dynamics of adefovir dipivoxil is determined by measuring the principal components of chemical shift anisotropy (CSA) tensor, site-specific spin–lattice relaxation time, and molecular correlation time at crystallographically different carbon nuclei sites. The CSA parameters, spin–lattice relaxation time, and molecular correlation time of phosphorous nucleus of the organophosphate group of adefovir dipivoxil molecule are also determined. The spin–lattice relaxation time of carbon nuclei varies from 1 to 107 s. The range of molecular correlation time also varies from $10^{-4}$ to $10^{-8}$ s. These remarkable diversities of motional dynamics of the molecules imply that there exist various motional degrees of freedom within this valuable drug and these motional degrees of freedom are independent of each other, which may be the reason for the biological activities exhibited by the drug. The correlation between structure and dynamics of such an important antiviral drug adefovir dipivoxil can be visualized by these types of extensive spectroscopic measurements, which will enlighten the path of inventing advanced medicine in the pharmaceutical industry, and it will also illuminate the understanding of the structure–activity relationships of antiviral drug.

1. INTRODUCTION

Adefovir dipivoxil is regarded as a stable protected monophosphate nucleoside prodrug that can efficiently cross the biological barrier and reach the targeted cell. It is fabricated by replacing the ribose phosphate group of adefovir by the isopolar phosphonomethyl ether functionality. The acyclic nucleoside phosphonate (ANP) compounds constructed to overcome the first phosphorylation step, which is indispensable for the foundation of the nucleoside analogues such as acyclovir (ACV) or ganciclovir (GCV). The activation of human immunodeficiency virus (HIV) inhibitors zidovudine (AZT), stavudine (D4T), zalcitabine (DDC), didanosine (DDI), and lamivudine (3TC) depends on cellular enzymes. Within the liver cell, adefovir dipivoxil is bisected into adefovir by intracellular esterases. It is phosphorylated by adenyate kinases and then transformed into adefovir diphosphate by nucleoside diphosphatase. Adefovir diphosphate is an analogue of deoxyadenosine triphosphate (dATP) and competitive inhibitors of the viral DNA polymerases. It binds with the viral DNA polymerase enzymes of hepatitis, herpes viruses, retroviruses (including HIV), Epstein–Barr virus, human immunodeficiency virus, cytomegalovirus, and other DNA viruses. It assimilates into the growing DNA strand. In this way, it causes chain termination and blocks the replication of viral DNA.1–4

Adefovir dipivoxil is composed of 9-{2-[bis-(pivaloyloxymethoxy)phosphinylmethoxy]ethyl}-adenine and falls in the class of acyclic nucleoside phosphonates.5,6,54 It degrades via two pathways: hydrolysis of the pivaloyloxymethyl moiety and formaldehyde-catalyzed dimerization of the adenine ring. The pharmacokinetic of adefovir is altered substantially with moderate and severe renal impairment.8 Adefovir dipivoxil structure is associated with 13 hydrogen...
bond acceptors and 2 hydrogen bond donors, which makes the structure favorable for cocystal formation.5,7,8 Adefovir dipivoxil dehydrate is formed by intermolecular interactions via N−H···N, O−H···O, and O−H···N hydrogen bondings. Two adefovir dipivoxil molecules connect by N−H···N hydrogen bonding between adenine rings to form a dimer synthon. Two hetero synthons are also fabricated by O−H···O and O−H···N hydrogen bonding between adefovir dipivoxil and water molecules. Due to the formation of the synthons, adefovir dipivoxil molecules form cocrytal with saccharin and water molecules. Due to the formation of the synthons, nucleus site is calculated by considering that the spin relaxation mechanism is mainly dominated by chemical shift anisotropy (CSA) and site-specific spin−lattice relaxation time, and molecular correlation time at crystallographically different carbon sites and phosphorous site of organophosphate group of the molecule. CSA tensor has a huge impact on determining the molecular conformation and electronic distribution surrounding the nucleus. Relaxometry measurements can explore the dynamics of a molecule at an atomic scale. The principal components of the CSA tensor can be also determined by two-dimensional magic-angle spinning (MAS)/CSA NMR experiment;13 separation of undistorted powder patterns by effortless recoupling (SUPER);14 recoupling of chemical shift anisotropy (ROCSA);15 γ-encoded RNcs−symmetry-based chemical shift anisotropy (RNCSA);16 two-dimensional magic-angle flipping (2DMAF) experiment;1719 two-dimensional magic-angle turning (2DMAT) experiment;20 and 2DPASS CP-MAS (two-dimensional phase-adjusted spinning sideband cross-polarization magic-angle spinning) SSNMR experiment.21,22 Structure and dynamics of biopolymer, drugs molecules, and glass compounds are determined by CSA and site-specific relaxation measurements.24−31 13C-CSA parameters of adefovir dipivoxil are calculated by 2DPASS CP-MAS SSNMR experiment.30 13C CSA parameters are extracted by the deconvolution of 31P MAS NMR spectrum at the MAS speed of 6 kHz by dmfit.42 Site-specific spin−lattice relaxation time of the carbon-13 nucleus is calculated by the Torchia CP method.43 The spin−lattice relaxation time of phosphorous nucleus is determined by the inversion recovery method. Molecular correlation time of chemically different carbon nuclei sites and phosphorous nucleus site is calculated by considering that the spin−lattice relaxation mechanism is mainly dominated by chemical shift anisotropy interaction and dipole−dipole interactions for spin 1/2 nucleus. These types of in-depth measurements will illuminate the path of inventing advanced antiviral drugs and establishing the structure−activity relationship of drug molecules.

2. EXPERIMENTAL SECTION

2.1. NMR Measurements. The active pharmaceutical ingredient of adefovir dipivoxil was purchased from Sigma-Aldrich.13C CP-MAS SSNMR and 31P MAS SSNMR experiments were performed on a JEOL ECX 500 NMR spectrometer with a 3.2 mm JEOL double-resonance MAS probe. The MAS speed for the 13C CP-MAS NMR experiment was 10 kHz. 31P MAS NMR experiments were performed at the MAS speeds of 3, 5, 6, 10, and 13 kHz. The contact time for cross-polarization (CP) was 2 ms, with a repetition interval of 30 s, and SPINAL-641H decoupling at 3072 accumulation time. 13C-spin−lattice relaxation experiment was performed using the Torchia CP method with a contact time of 2 ms and a relaxation time of 10 s. The spin−lattice relaxation time of the phosphorous nucleus was measured by the inversion recovery method. All solid-state experiments were performed at room temperature.

2.2. CSA Measurements. The information regarding the three-dimensional structure of a molecule and spin dynamics is encoded in anisotropic interactions. The pulse sequences for the 2DPASS CP-MAS SSNMR experiment is associated with five π pulses of the variable pitch but constant duration. It generates spectra with sidebands of different phases. The time interval among five π pulses is chosen by following the PASS equations. The spacing among the π pulses was reported by Antzutkin et al.21 The 90° pulse length for the 13C nucleus was 3.3 μs. The relaxation delay was 10 s. The number of scans for the 2DPASS CP-MAS NMR experiment was 4030 (integral multiple of 13). The coherence transfer pathway for the 2DPASS CP-MAS NMR experiment is given by Ghosh et al.24 Thirteen steps cogwheel phase cycling was used. As the number of sidebands was less than 16, 16 data points were acquired in the indirect dimension. The anisotropic part of the chemical shift interaction evolves during the PASS sequence. The variable of this evolution is called “pitch,” which varies from 0 to 2π. The principal values of the chemical shift anisotropy tensor can be extracted by evaluating the sideband intensity for few sidebands by the graphical method.33 13C 2DPASS CP-MAS SSNMR experiments were performed at MAS frequencies of 600 Hz and 2 kHz.

3. RESULTS AND DISCUSSION

3.1. Determination of CSA Parameters. Figure 1a shows that adefovir dipivoxil is fabricated by adenine ring, organophosphate group, and two pivaloyloxymethyl groups. Through a phosphorous−carbon−oxygen bond, the adenine ring is attached to the organophosphate group, which enables the drug to achieve higher degrees of the active metabolites in the cell and to possess antiviral activity against most of the DNA viruses like hepadnaviruses, retroviruses, and herpes viruses. Nucleotides of the nucleic acids are formed by two purine nucleobases adenine and guanine. Adenine binds with thymine of DNA via two hydrogen bonds to bring stability to the structure of nucleic acid. Pivaloyloxymethyl group is a protecting group used to synthesize the produgs. The adenine ring exhibits planar conformation. Two pivaloyloxymethyl groups are attached to a negatively charged organophosphate group to neutralize the negative charge of the drug. This is essential to make the drug lipid-soluble and potent to diffuse across the cell membranes into the cell. Hence, two pivaloyloxymethyl ester groups enhanced the bioavailability of the drug. The organophosphate group is a key functional group of DNA, RNA, and ATP. It helps to recognize the viral kinases for phosphorylation and bind with the viral DNA polymerase.57−59

Figure 1b represents the 13C CP-MAS SSNMR spectrum of adefovir dipivoxil. The resonance peak position is assigned by following Swain et al.54−56 The isotropic chemical shift of C18 and C24 carbon nuclei of two pivaloyloxymethyl groups is maximum. The electronegative oxygen atoms at the immediate neighborhood of C18 and C24 attract the electron cloud around the carbon nucleus. As a consequence, the nuclear shielding effect is decreased and the effective magnetic field experienced by these nuclei is increased. The Larmor precession frequency corresponding to these nuclei is shifted
toward the higher-frequency side. The isotropic chemical shift of carbon nuclei C2, C4, C5, C9, and C7 residing on the adenine ring is substantially high. The isotropic chemical shift is lowest for the methyl group carbon nuclei C28, C30, C31, C32, C33, and C34 residing on the pivaloyloxymethyl group. Figure 2 shows the $^{13}$C 2DPASS CP-MAS SSNMR spectrum of adefovir dipivoxil. The isotropic chemical shift is correlated with the anisotropic chemical shift by this measurement. The direct dimension shows the pure isotropic spectrum, which is also referred to as the infinite spinning speed spectrum. The indirect dimension represents the anisotropic spectrum. Figure 3 shows the spinning CSA sideband pattern at crystallographically different carbon sites of adefovir dipivoxil.

In solid-state NMR spectroscopy, chemical shift interaction, magnetic dipole–dipole interaction, and electrical quadruple interaction are taken into consideration. Each of these interactions has an isotropic and an anisotropic part. The Larmor precession frequency of the nucleus depends on the orientation of the molecular moiety with respect to the direction of the applied magnetic field and the electronic distribution surrounding the nucleus. The shift of the Larmor precession frequency compared to the bare nucleus can be expressed as $\omega(\theta, \phi) = \omega_0 + (\Delta\delta/2)(3\cos^2 \theta - 1 - \eta \sin^2 \theta \cos 2\phi)$, where $\Delta\delta$ is the anisotropy parameter, which measures the deviation of the electron cloud from the spherically symmetric charge distribution, and $\eta$ is the asymmetry parameter, which measures the deviation from the axially symmetric distribution. $\theta$ and $\phi$ are the polar and the azimuthal angles with respect to the direction of the applied magnetic field ($B_0$) in the principal axis system (PAS). It is called the chemical shift anisotropy (CSA) tensor. It is diagonalized in the principal axis system (PAS), and the expressions of the principal components of CSA tensor are

$$
\delta_{33} = \frac{e^2}{2m} \begin{pmatrix} 0 & x^2 + y^2 & 0 \\ 0 & r^3 & 0 \\ -\left(\frac{\hbar}{2m}\right)^2 \sum_n \left\langle 0 \left| \frac{nL_n}{r} \right| nL_n \right| \langle nL_n | 0 \rangle \right\rangle \\
+ \left\langle 0 \left| \frac{nL_n}{r} \right| nL_n \right| \langle nL_n | 0 \rangle \right\rangle \right\rangle \\
- \left(\frac{\hbar}{2m}\right)^2 \sum_n \left\langle 0 \left| \frac{nL_n}{r} \right| nL_n \right| \langle nL_n | 0 \rangle \right\rangle \end{pmatrix}
$$

$$
\delta_{11} = \frac{e^2}{2m} \begin{pmatrix} 0 & y^2 + z^2 & 0 \\ 0 & r^3 & 0 \\ -\left(\frac{\hbar}{2m}\right)^2 \sum_n \left\langle 0 \left| \frac{nL_n}{r} \right| nL_n \right| \langle nL_n | 0 \rangle \right\rangle \\
+ \left\langle 0 \left| \frac{nL_n}{r} \right| nL_n \right| \langle nL_n | 0 \rangle \right\rangle \right\rangle \\
- \left(\frac{\hbar}{2m}\right)^2 \sum_n \left\langle 0 \left| \frac{nL_n}{r} \right| nL_n \right| \langle nL_n | 0 \rangle \right\rangle \end{pmatrix}
$$

Figure 1. (a) Adefovir dipivoxil is composed of an adenine ring, an organophosphate group, and two pivaloyloxymethyl groups. (b) $^{13}$C CP-MAS NMR spectrum of adefovir dipivoxil.

Figure 2. $^{13}$C 2DPASS CP-MAS SSNMR spectrum of adefovir dipivoxil. The direct dimension shows the isotropic spectrum, which is also referred to as an infinite spinning speed spectrum. The indirect dimension shows the anisotropic spectrum.
Figure 3. Spinning CSA sideband pattern at crystallographically different carbon nuclei sites of adefovir dipivoxil. For C18, C24, C7, C5, C2, and C9, the CSA pattern is determined by the 2DPASS CP-MAS SSNMR experiment at the MAS frequency of 2 kHz, and for the rest of the carbon nuclei, the spinning CSA sideband pattern is determined by performing the same experiment at the MAS frequency of 600 Hz.

Table 1. Principal Components of the CSA Tensor of Adefovir Dipivoxil at Crystallographically Different Carbon Sites

| CSA parameters of adefovir dipivoxil | carbon nuclei | δ_{11} (ppm) | δ_{22} (ppm) | δ_{33} (ppm) | span (ppm) | Ω = δ_{11} − δ_{33} | skew \( k = \frac{δ_{11}−δ_{33}}{2} \) | δ_{iso} (ppm) | anisotropy \( (\frac{δ_{11}+δ_{22}}{2})−δ_{iso} \) | asymmetry \( η = \frac{δ_{11}−δ_{iso}}{δ_{33}−δ_{iso}} \) |
|--------------------------------------|---------------|---------------|---------------|---------------|-------------|----------------------|-------------------|------------------|----------------------|-------------------|
| C18                                  | 225           | 225           | 82.2          | 142.8         | 1           | 177.4                | −142.8            | 0                | 0.3                  | 0.3               |
| C24                                  | 227.6         | 225.5         | 75.4          | 152.2         | 1           | 176.2                | −151.1            | 0.02             | 0.02                 | 1                 |
| C4                                   | 252.3         | 109.5         | 109.5         | 142.8         | −1          | 157.1                | 142.8             | 0                | 0.6                  | 0.6               |
| C7                                   | 239.8         | 176.1         | 43.6          | 196.2         | 0.3         | 153.2                | −164.3            | 0.6              | 0.6                  | 0.6               |
| C5                                   | 241           | 132.4         | 81            | 159.9         | −0.3        | 151.5                | 134.2             | 0.6              | 0.6                  | 0.6               |
| C2                                   | 206.1         | 142           | 74.9          | 131.2         | 0.02        | 141                  | −99.2             | 1                | 1                    | 1                 |
| C9                                   | 188.4         | 87.2          | 79.2          | 109.2         | −0.8        | 118.3                | 105.2             | 0.1              | 0.1                  | 0.1               |
| C16                                  | 102.9         | 86.1          | 60.3          | 42.6          | 0.2         | 83.1                 | −34.2             | 0.7              | 0.7                  | 0.7               |
| C22                                  | 105.6         | 85.1          | 56.4          | 49.2          | 0.2         | 82.4                 | −38.9             | 0.8              | 0.8                  | 0.8               |
| C11                                  | 112.8         | 60.3          | 48.5          | 64.3          | −0.6        | 73.8                 | 58.4              | 0.3              | 0.3                  | 0.3               |
| C13                                  | 111.4         | 54.9          | 40.5          | 70.9          | −0.6        | 69                   | 63.7              | 0.3              | 0.3                  | 0.3               |
| C10                                  | 110           | 50.8          | 41.7          | 68.3          | −0.7        | 67.5                 | 63.7              | 0.2              | 0.2                  | 0.2               |
| C27                                  | 60.9          | 42.6          | 22.8          | 38            | 0.04        | 42.1                 | −28.9             | 0.9              | 0.9                  | 0.9               |
| C29                                  | 53.3          | 32.6          | 30.1          | 23.2          | −0.8        | 38.7                 | 22                | 0.2              | 0.2                  | 0.2               |
| C28, C30, C31, C32, C33, C34         | 34.4          | 30.8          | 16.4          | 18            | 0.6         | 27.2                 | −16.2             | 0.3              | 0.3                  | 0.3               |
adenine ring. The adenine ring exhibits a planar conformation and magnetic susceptibility parallel and perpendicular to the molecule is perpendicular to the axes of the CSA tensor. The CSA parameter is determined using the equation:

$$\delta_{22} = \frac{1}{2m} \left( \langle 0 | \sum_n \left( \frac{2 | L_x | n}{r^3} \right) \langle n | \frac{2 | L_z | 0}{r^3} | 0 \rangle \right)$$

where $L_x$, $L_y$, and $L_z$ are the components of the angular momentum along the $x$, $y$, and $z$-axes, respectively. The first part of these three equations arises from the spherical distribution of the electronic changes when the electrons are in the s-orbital state. The second term arises from the distortion of spherical charge distribution when electrons are in the p-orbital state. In liquid-state NMR spectroscopy, due to the tumbling motion of the molecule, the orientation-dependent term of the CSA tensor is averaged out and only the isotropic component of the chemical shift survives. The resonance frequency of numerous carbon nuclei differs according to the orientation of the molecular moiety with respect to the external magnetic field. The largest chemical shift (deshielding effect) in the resonance frequency ($\delta_{11}$) occurs when the narrowest part of the electron distribution is oriented along the direction of the external magnetic field ($B_0$), whereas the smallest chemical shift (nuclear shielding effect) in the resonance frequency ($\delta_{33}$) occurs when the widest part of the electron cloud is oriented along the direction of the external magnetic field. The third principal value ($\delta_{22}$) of the CSA tensor arises when the orientation of the molecular moiety is perpendicular to the axes of $\delta_{11}$ and $\delta_{33}$. The left and right edges of the spinning CSA sideband pattern correspond to the largest ($\delta_{11}$) and smallest ($\delta_{33}$) chemical shifts, respectively, whereas $\delta_{22}$ corresponds to the position of the maximum intensity of the CSA sideband pattern when $\delta_{11} \geq \delta_{22} \geq \delta_{33}$.

Table 1 shows the principal components of the CSA tensor at crystallographically different carbon nuclei sites of adefovir dipivoxil. The CSA parameters of C18 and C24 residing at the pivaloyloxymethyl group are substantially high. As the carbonyl group carbon has no directional symmetry, there arises magnetic anisotropy $\delta_{anis} = (\Delta X_H - 1) / 3R^2$, where $\theta_1$ and $\theta_2$ are the angles subtended by the radius vector with $x$-axis and $z$-axis, respectively, and $\Delta X_H = X_z - X_x$ and $\Delta X_L = X_y - X_x$ are the components of susceptibility parallel and perpendicular to the applied magnetic field, respectively. The higher degree of directional specificity due to the presence of magnetic anisotropy leads to the higher values of CSA parameters for C18 and C24 nuclei. The CSA parameters of C2, C4, C5, C7, and C9 residing on the adenine ring are largely due to the presence of $\pi$ electrons. This induced polarization in the neighborhood region of the carbon nuclei and there induced magnetic anisotropy. The intermolecular and intramolecular hydrogen bonds O–H···N, O–H···O, and O–H···N are associated with the adenine ring. The adenine ring exhibits a planar conformation because of the orbitals of the nitrogen atoms connected with hydrogen bonds in sp² hybridization. Two adjacent molecules of adefovir dipivoxil are connected via intermolecular hydrogen bonding through adenine rings. These intermolecular hydrogen bonds reduce the polarity of the molecule and bring stability to the crystal structure. The existence of these hydrogen bonds is another reason for the higher values of the CSA parameters of the adenine ring.

The isotropic chemical shift ($\delta_{iso} = (\delta_{11} + \delta_{22} + \delta_{33})/3$) represents the center of gravity of the spinning CSA sideband pattern. The magnitude of the anisotropy parameter ($\Delta \delta = \delta_{11} - (\delta_{11} + \delta_{22} + \delta_{33})/2$) indicates the largest separation of the spinning CSA sideband pattern from the center of gravity. The sign of the anisotropy parameters signifies on which side of the center of gravity, the separation is the maximum. Figure 5 shows the variation of asymmetry and anisotropy parameters at numerous carbon sites of adefovir dipivoxil.

The asymmetry parameter is defined as $\eta = (\delta_{22} - \delta_{11})/(\delta_{33} - \delta_{11})$. If $\delta_{22} = \delta_{11}$ or $\delta_{22} = \delta_{33}$, then the spinning CSA sideband pattern is axially symmetric. Table 1 and Figure 5a indicate that the spinning CSA sideband pattern is axially symmetric for C18, C24, and C4. The CSA parameter is nearly axially symmetric for C9, C10, C11, C13, C28, C29, C30, C31, C32, C33, and C34 carbon nuclei when $\eta \leq 0.3$. The CSA pattern is highly asymmetric for C2, C22, and C27 when the asymmetry parameter $\eta \geq 0.8$. Skew $k = (\Delta \delta - \delta_{iso}) / \Omega$ represents the orientation of the asymmetric pattern.

Figure 4 shows the 31P MAS SSNMR spectrum of adefovir dipivoxil at the MAS speed of 6 kHz. The line shape is a CSA powder pattern originating from the orientation dependence of the chemical shift, described by the expression $\delta(\alpha, \beta) = \delta_{11} \sin^2 \beta \cos^2 \alpha + \delta_{33} \sin^2 \beta \sin^2 \alpha + \delta_{22} \cos^2 \beta$, where $\alpha$ and $\beta$ are the Euler angles, and $\delta_{11}$, $\delta_{22}$, and $\delta_{33}$ are the principal components of the CSA tensor in PAS. The CSA parameters are determined using dmfit. The isotropic chemical shift of phosphorous is 26.69 ppm. The values of asymmetry and anisotropy parameters are $-115$ ppm and 0.12, respectively. The spinning CSA sideband pattern of phosphorous nuclei residing on the organophosphate group is nearly axially symmetric. The organophosphate functionality allows the drug to penetrate the cell membrane. Within the cell, the bioconversion of adefovir dipivoxil is performed by esterases, and it is transformed into adefovir. Adefovir is then
phosphorylated to adefovir monophosphate by various kinases, one of which in lymphoid cells is identified as adenylase kinase. The adefovir monophosphate is then transformed to adefovir diphos-3.2. Spin–Lattice Relaxation Time and Molecular Correlation Time phate by second phosphorylation. Adefovir diposph-ate is an aggressive inhibitor of hepatitis B virus (HBV) DNA polymerase (reverse transcriptase) in addition to other viral DNA polymerases. This inhibition results in DNA chain termination and the destruction of viral replication. These make adefovir dipivoxil a highly efficient drug in the treatment of human hepatitis B virus (HBV) infection (Figure 5).

Figure 5. Bar diagram of (a) asymmetry (η = (δ_{C2} - δ_{C1})/(δ_{C3} - δ_{Cm})) and (b) anisotropy (Δδ = δ_{C3} - (δ_{C1} + δ_{C2})/2) parameters at crystallographically different carbon nuclei sites of adefovir dipivoxil.

The role of dipole–dipole interaction in the spin–lattice relaxation mechanism is expressed as

\[
\frac{1}{T_1^{DD}} = \frac{1}{10} \left( \frac{\gamma X \hbar}{r_{CX}} \right)^2 \left[ \frac{3}{1 + \omega C^2 r_{CX}^2} + \frac{1}{1 + (\omega_C - \omega)^2 r_{CX}^2} + \frac{1}{1 + (\omega_C + \omega)^2 r_{CX}^2} \right]
\]

By keeping only the first term

\[
\frac{1}{T_1^{DD}} = \frac{1}{10} \left( \frac{\gamma X \hbar}{r_{CX}} \right)^2 \left[ \frac{3}{1 + \omega C^2 r_{CX}^2} \right]
\]

where X represents hydrogen, oxygen, and nitrogen, phosphorous atoms. r_{CX} is the distance between carbon and neighboring atoms hydrogen, oxygen, and fluorine, which is determined by X-ray crystal structural studies. The Larmor precession frequency of carbon nucleus is \(\omega_C = 2 \gamma f_C = 2 \times 3.14 \times 125.758 \text{ MHz} = 789.76024 \text{ MHz}. The Larmor precession frequency of phosphorous nucleus is \(\omega_P = 2 \gamma f_P = 2 \times 3.14 \times 202.457 \text{ MHz} = 1271.42996 \text{ MHz}, B = 11.74 \text{ T}, T_1 = 10.7084 \text{ MHz/T}, f_H = 42.577 \text{ MHz/T}, \gamma_C = 17.235 \text{ MHz/T}, \gamma_P = 1.054 \times 10^{-21} \text { Js). The spin–lattice relaxation rate for^{13}C can be articulated as

\[
\frac{1}{T_1} = \frac{1}{T_{CSA}} + \frac{1}{T_{DD}}
\]

where \(T_{CSA} = \omega \tau, T_{DD} = \omega \tau\) and \(B\) is the applied magnetic field. Where \(S^2 = (\Delta \delta)^2 (1 + \eta^2/3)\) and \(\Delta \delta = \delta_{C3} - \left(\delta_{C1} + \delta_{C2}\right)/2\), \(\eta = \delta_{C2} - \delta_{C1}\). The molecular correlation time is calculated by this equation. Figure 7 represents the bar diagram of the molecular correlation time at crystallographically different carbon sites of adefovir dipivoxil.

Table 2 shows the spin–lattice relaxation time and the molecular correlation time of adefovir dipivoxil at crystallographically different sites of carbon nuclei and one phosphorous nucleus. Figure 6 shows the longitudinal magnetization decay curves at (a) C4, (b) C5, and (c) C7 carbon nuclei sites of adefovir dipivoxil. The spin–lattice relaxation time of carbon nuclei residing on the adenine ring is comparatively longer than those residing on the pivaloyloxymethyl ester group. The spin–lattice relaxation time at chemically different carbon nuclei sites varies from 1 to 107 s. Figure 6e shows the bar diagram of the spin–lattice relaxation time at crystallographically different carbon sites. The spin–lattice relaxation time is maximum at the C5 nuclei site on the adenine ring, and it is minimum for C28, C30, C31, C32, C33, and C34 methyl group carbon nuclei residing on the pivaloyloxymethyl group. The molecular correlation time varies in the range of 10^{-4} to 10^{-8} s. For C5, it is 1.7 \times 10^{-4} s and for C28, C30, C31, C32, C33, and C34 methyl group carbon nuclei, it is 2.1 \times 10^{-8} s. Remarkable variation in the spin–lattice relaxation time and the molecular correlation time at different functional groups of the molecule implies that various motional degrees of freedom exist within the molecule and different motional degrees of freedom are independent of each other.
Within the adenine ring, the molecular correlation times of C2 and C9 nuclei are 1 order faster ($10^{-8}$ s) than those of the C4, C5, and C7 nuclei ($10^{-4}$ s). The molecular correlation time of phosphorus nuclei of the organophosphate group varies in the range of $10^{-4}$ to $10^{-8}$ s. As mentioned before that the pivaloyloxymethyl group plays a crucial role in neutralizing the organophosphate group and makes the drug lipid-soluble and potent to diffuse across the cell membranes. The existence of different degrees of motional freedom within this molecular moiety may be the reason for the biological activity exhibited by this group. The CSA parameters (which reflect the molecular conformation and electronic distribution observed in the pivaloyloxymethyl group) are highly correlated with the electronic bonding and molecular conformation (Figure 7).

4. CONCLUSIONS

Adefovir dipivoxil is a prodrug of the antiviral agent, adefovir (PMEA). The degradation kinetics of adefovir dipivoxil is administered by two distinct but inter-related degradation pathway hydrolysis of the pivaloyloxymethyl moiety and formaldehyde-catalyzed dimerization of the adenine ring. Formaldehyde is a reactive and cross-linking agent for nucleic acid. Adefovir dipivoxil shows antiviral activity when administered intravenously, intraperitoneally, or intramuscularly against the hepatitis B virus (HBV), human immunodeficiency virus (HIV), Rauscher murine leukemia virus (R-MuLV), murine cytomegalovirus (MCMV), herpes simplex virus (HSV), simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV). The spinning CSA sideband pattern of phosphorous nucleus residing on the organophosphate group, which allows the drug to penetrate the cell membrane, is nearly axially symmetric. The pivaloyloxymethyl group plays a crucial role in neutralizing the organophosphate group and making the drug lipid-soluble. The existence of two various motional degrees of freedom within this molecular moiety. For C18 and C24 carbon double bonded with the oxygen atom, the molecular correlation time is $3.3 \times 10^{-5}$ and $2.7 \times 10^{-5}$ s, respectively, but for C16 and C22, the molecular correlation times are $3.3 \times 10^{-7}$ and $4.4 \times 10^{-7}$ s, respectively. For methyl group carbon nuclei C28, C30, C31, C32, C33, and C34, the molecular correlation time is $2.1 \times 10^{-8}$ s and for C27 and C29 it is $6.1 \times 10^{-8}$ and $4.6 \times 10^{-7}$ s, respectively. Hence, within this pivaloyloxymethyl group, the molecular correlation time varies from $2.7 \times 10^{-5}$ to $2.1 \times 10^{-8}$ s. As mentioned before that the pivaloyloxymethyl group plays a crucial role in neutralizing the organophosphate group and makes the drug lipid-soluble and potent to diffuse across the cell membranes. The existence of different degrees of motional freedom within this molecular moiety may be the reason for the biological activity exhibited by this group. The CSA parameters (which reflect the molecular conformation and electronic distribution observed in the pivaloyloxymethyl group) are highly correlated with the electronic bonding and molecular conformation (Figure 7).
The remarkable variation of the CSA parameters implies a significant difference in the electronic environment and molecular conformation within this molecular moiety. The anisotropy \[ \Delta \delta = \delta_{33} - \frac{(\delta_{22} + \delta_{11})}{2} \] and span \( \Omega = \delta_{11} - \delta_{33} \) of C18 and C24 nuclei are substantially higher than those of C16, C22, C27, and C29 nuclei and methyl group carbon nuclei C18 and C24. On the other hand, the CSA parameters of the methyl group carbons C28, C30, C31, C32, C33, and C34 residing on the pivaloyloxymethyl group are the lowest. The remarkable variation of the CSA parameters implies a significant difference in the electronic environment and molecular conformation within this molecular moiety. The molecular correlation time is of the order 10^{-5} s for C18 and C24; 10^{-7} s for C16 and C22; and 2.1 \times 10^{-8} s for the methyl group carbon nuclei C28, C30, C31, C32, C33, and C34. The molecular correlation time is 6.1 \times 10^{-6} and 4.6 \times 10^{-7} s for C27 and C29, respectively. This huge variation of the electronic structure and the motional degrees of freedom within this molecular moiety may be the reason for the biological activity exhibited by this group.

The CSA parameters of the carbon nuclei residing on the adenine rings C2, C4, C5, C7, and C9 are substantially high due to the presence of \( \pi \) electrons and hydrogen bonding. Adenine derivatives possess antiviral activity against most of the double-stranded DNA viruses. The adefovir dipivoxil molecules form a chain structure via intermolecular hydrogen bonding through adenine rings. These intermolecular hydrogen bonds reduce the polarity of the molecule and bring stability to the crystal structure. The molecular correlation time of the carbon nuclei of these adenine rings varies in the range of 1.3 \times 10^{-4}–6.5 \times 10^{-5} s. The motional degrees of freedom are highly correlated with electronic bonding and molecular conformation. These types of investigations about the structure and dynamics of this valuable antiviral drug will help to illuminate the path of inventing the advanced antiviral drug and to understand the structure–activity relationship of antiviral drugs. These types of studies are also useful in NMR crystallography.

**AUTHOR INFORMATION**

**Corresponding Author**

Manasi Ghosh – Physics Section, MMV, Banaras Hindu University, Varanasi 221005, UP, India; orcid.org/0000-0002-8472-0288; Email: manasi.ghosh@bhu.ac.in

**Author**

Krishna Kishor Dey – Department of Physics, Dr. Harisingh Gour Central University, Sagar 470003, MP, India

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c04205

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The author Manasi Ghosh is grateful to the Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Government of India (file no. EMR/2016/000249) for financial support. The authors are thankful to SIC, Dr. Harisingh Gour Central University for providing solid state NMR facility.

**REFERENCES**

(1) Littler, E.; Stuart, A. D.; Chee, M. S. Human cytomegalovirus UL97 open reading frame encodes a protein that phosphorylates the antiviral nucleoside analogue ganciclovir. Nature 1992, 358, 160–162.

(2) Naessens, L.; Snoeck, R.; Andrei, G.; Balzarini, J.; Neys, J.; De Clercq, E. HPMPC (cidofovir), PMEA (adefovir) and related acyclic nucleoside phosphate analogues: a review of their pharmacology and clinical potential in the treatment of viral infections. Antiviral Chem. Chemother. 1997, 8, 1–23.

(3) Qaqish, R. B.; Mattes, K. A.; Ritchie, D. J. Adefovir dipivoxil: A new antiviral agent for the treatment of hepatitis B virus infection. Clin. Ther. 2003, 25, 3084–3099.

(4) Julander, J. G.; Sidwell, R. W.; Morrey, J. D. Characterizing antiviral activity of adefovir dipivoxil in transgenic mice expressing hepatitis B virus. Antiviral Res. 2002, 55, 27–40.

(5) Jung, S.; Ha, J.-M.; et al. Bis[2,2-dimethoxypropanoyl]methy] [[1-(6-amin-9H-purin-9-yl)ethoxy]-methyl]phosphonate–succinic acid (2/1). Acta Crystallogr., Sect. E: Struct. Rep. Online 2012, 68, o809–o810.

(6) Chang, Y.; Zheng, Qi-Tai.; Lu, Y. 9-{2-[Bis-(pivaloyloxymethoxy)phosphinylmethoxy]ethyl}adenine dehydrate. Acta Crystallogr, Sect. E: Struct. Rep. Online 2007, 63, o1014–o1015.

(7) Jung, S.; Lee, J.; Won Kim, I.; Structures and physical properties of the coocrystals of adefovir dipivoxil with dicarboxylic acids. J. Cryst. Growth 2013, 373, 59–63.

(8) Gusheiko, G. Adefovir dipivoxil for the treatment of HBAg-positive chronic hepatitis B: a review of the major clinical studies. J. Hepatol. 2003, 39, 116–123.

(9) Yuan, L.; Dahl, T. C.; Oliyai, R. Degradation kinetics of oxycarbonyloxymethyl prodrugs of phosphonates in solution. Pharm. Res. 2001, 18, 234–237.

(10) Yuan, L.; Dahl, T. C.; Oliyai, R. Effect of carbonate salts on the kinetics of acid-catalyzed dimerization of adefovir dipivoxil. Pharm. Res. 2000, 17, 1098–1103.

(11) Starrett, J. E., Jr.; Tortolani, D. R.; Hitchcock, M. J. M.; Martin, J. C.; Mansuri, M. M. Synthesis and in vitro evaluation of a phosphonate prodrug: bis(pivaloyloxymethyl)-9-(2-phosphonylhexethoxy)adenine. Antiviral Res. 1992, 19, 267–273.

(12) Ji-Hun, An.; Kiyonga, A. N.; Yoon, W.; Ryu, H. C.; Kim, J.; Kang, C.; Park, M.; Yun, H.; Jung, K. Crystal Structure Analysis of the First Discovered Stability-Enhanced Solid State of Tenofovir Disoproxil Free Base Using Single Crystal X-ray Diffraction. Molecules 2017, 22, No. 1182.

(13) Tycko, R.; Dabbagh, G.; Mirau, P. A. Determination of chemical shift anisotropy lineshapes in a two-dimensional magic angle spinning NMR experiment. J. Magn. Reson. 1989, 85, 265–274.

(14) Liu, S. F.; Mao, J. D.; Schmidt-Rohr, K. A robust technique for two-dimensional separation of undistorted chemical shift anisotropy powder patterns in magic angle spinning NMR. J. Magn. Reson. 2002, 155, 15–28.

(15) Chan, J. C. C.; Tycko, R. Recoupling of chemical shift anisotropies in solid state NMR under high speed magic angle spinning and in uniformly \(^{13}C\) labelled systems. J. Chem. Phys. 2003, 118, 8378–8389.

(16) Hou, G.; Byeon In-Ja, L.; Ahn, J.; Gronenborn, A. M.; Polenova, T. Recoupling of chemical shift anisotropy by R-symmetry sequences in magic angle spinning NMR spectroscopy. J. Chem. Phys. 2012, 137, 134201–134210.

(17) Bax, A.; Szeveiereni, N. M.; Maciel, G. E. Chemical shift anisotropy in powdered solids studied by 2D FT NMR with flipping of the spinning axis. J. Magn. Reson. 1983, 55, 494–497.

(18) Bax, A.; Szeveiereni, N. M.; Maciel, G. E. Correlation of isotropic shifts and chemical shift anisotropies by two-dimensional...
Fourier-transform magic angle hopping NMR spectroscopy. J. Magn. Reson. 1983, 52, 147–152.
(19) Bax, A.; Szeverenyi, N. M.; Maciel, G. E. Chemical shift anisotropy in powdered solids studied by 2D FT CP/MAS NMR. J. Magn. Reson. 1983, 51, 400–408.
(20) Gan, Z. High-resolution chemical shift and chemical shift anisotropy correlation in solids using slow magic angle spinning. J. Am. Chem. Soc. 1992, 114, 8307–8309.
(21) Antzutkin, O. N.; Shkar, S. C.; Levitt, M. H. Two-dimensional sideband separation in magic angle spinning NMR. J. Magn. Reson., Ser. A 1995, 115, 7–19.
(22) Dixon, W. T. Spinning-sideband-free and spinning-sideband-only NMR spectra in spinning samples. J. Chem. Phys. 1982, 77, 1800–1809.
(23) Torchia, D. A. The measurement of proton-enhanced carbon-13 T1 values by method which suppresses artifacts. J. Magn. Reson. 1978, 30, 613.
(24) Ghosh, M.; Sadhukhan, S.; Dey, K. K. Elucidating the internal structure and dynamics of α-chitin by 2D PASS-MAS-NMR and spin-lattice relaxation measurements. Solid State Nucl. Magn. Reson. 2019, 97, 7–16.
(25) Ghosh, M.; Prajapati, B. P.; Kango, N.; Dey, K. K. A comprehensive and comparative study of the internal structure and dynamics of natural β-keratin and regenerated β-keratin by solid state NMR spectroscopy. Solid State Nucl. Magn. Reson. 2019, 101, 1–11.
(26) Ghosh, M.; Kango, N.; Dey, K. K. Investigation of the internal structure and dynamics of cellulose by 13C-NMR relaxometry and 2D PASS-MAS-NMR measurements. J. Biomol. NMR 2019, 73, 601–616.
(27) Dey, K. K.; Ghosh, M. Understanding the effect of decactylation on chitin by measuring chemical shift anisotropy tensor and spin lattice relaxation time. Chem. Phys. Lett. 2020, 738, No. 1367812.
(28) Dey, K. K.; Gayen, S.; Ghosh, M. Investigation of the detailed internal structure and dynamics of itraconazole by solid-state NMR measurements. ACS Omega 2019, 4, 21627–21635.
(29) Dey, K. K.; Gayen, S.; Ghosh, M. Understanding the correlation between structure and dynamics of diclofenac pivalate by solid state NMR measurements. RSC Adv. 2020, 10, 4310–4321.
(30) Ghosh, M.; Gayen, S.; Dey, K. K. An atomic resolution description of folic acid by solid state NMR measurements. RSC Adv. 2019, 10, 24973–24984.
(31) Walder, B. J.; Dey, K. K.; Kaseman, D. C.; Baltisberger, J. H.; Grandinetti, P. J. Sideband separation experiments in NMR with phase incremented echo train acquisition. J. Chem. Phys. 2013, 138, No. 174203.
(32) Fayon, F.; Bessada, C.; Douy, A.; Massiot, D. Chemical bonding of lead in glasses through isotropic vs anisotropic correlation: PASS shifted Echo. J. Magn. Reson. 1999, 137, 116–121.
(33) Herzfeld, J.; Berger, A. E. Sideband intensities in NMR spectra of samples spinning at the magic angle. J. Chem. Phys. 1980, 73, 6021–6030.
(34) Swan, D.; Vijay Prasad, K. V. S.; Karumuri, C.; Srinivas, K. S.; Samanthula, G. Structural Characterisation of the Stress Degradation Products of Adefovir Dipivoxil by LCMS and NMR. Anal. Chem. Lett. 2018, 8, 379–392.
(35) Ramsey, N. F. Magnetic Shielding of Nuclei in Molecules. Phys. Rev. 1950, 78, 699–703.
(36) Ramsey, N. F. Chemical effects in nuclear magnetic resonance and in diamagnetic susceptibility. Phys. Rev. 1952, 86, 243–246.
(37) McConnell, H. M. Theory of Nuclear Magnetic Shielding in Molecules: Long-Range Dipolar Shielding of protons. J. Chem. Phys. 1957, 27, 226–229.
(38) Orendt, A. M.; Facelli, J. C. Solid state effects on NMR chemical shifts. Annu. Rep. NMR Spectros. 2007, 62, 115–178.
(39) Tjandra, N.; Szabo, A.; Bax, Ad. Protein backbone dynamics and 15B chemical shift anisotropy from quantitative measurement of relaxation interference effects. J. Am. Chem. Soc. 1996, 118, 6986–6991.
(40) Dais, P.; Spyros, A. 13C nuclear magnetic relaxation and local dynamics of synthetic polymers in dilute solution and in the bulk state. Prog. Nucl. Magn. Reson. Spectros. 1995, 27, 555–639.
(41) Nicholas, M. P.; Eryilmaz, E.; Ferrage, F.; Cowburn, D.; Ghose, R. Nuclear spin relaxation in isotropic and anisotropic media. Prog. Nucl. Magn. Reson. Spectros. 2010, 57, 111–158.
(42) Massiot, D.; Fayan, F.; Capron, M.; King, I.; Calve, S. L.; Alonso, B.; Durand, J. O.; Bujoli, B.; Gan, Z.; Hoatsson, G. Modelling one- and two-dimensional solid-state NMR spectra. Magn. Reson. Chem. 2002, 40, 70–76.
(43) Sen, S. Dynamics in Inorganic Glass-Forming Liquids by NMR Spectroscopy. Prog. Nucl. Magn. Reson. Spectros. 2020, 116, 155–176.
(44) Antzutkin, O. N. Sideband manipulation in magic-angle-spinning nuclear magnetic resonance. Prog. Nucl. Magn. Reson. Spectros. 1999, 35, 203–266.
(45) Ando, I.; Kurosu, S.; Kurosou, H.; Yamanobe, T. NMR chemical shift calculations and structural characterizations of polymers. Prog. Nucl. Magn. Reson. Spectros. 2001, 39, 79–133.
(46) de Dios, A. C. Ab initio calculations of the NMR chemical shift. Prog. Nucl. Magn. Spectros. 1996, 29, 229–278.
(47) Shao, L.; Titman, J. J. Chemical shift anisotropy amplification. Prog. Nucl. Magn. Reson. Spectros. 2007, 51, 103–137.
(48) Sitkoff, D.; Case, D. A. Theories of chemical shift anisotropies in proteins and nucleic acids. Prog. Nucl. Magn. Reson. Spectros. 1998, 32, 165–190.
(49) Veeman, W. S. Carbon-13 Chemical shift anisotropy. Prog. Nucl. Magn. Reson. Spectros. 1984, 16, 193–235.
(50) Wylie, B. J.; Rienstra, C. M. Multidimensional solid state NMR of anisotropic interactions in peptides and proteins. J. Chem. Phys. 2008, 128, No. 052207.
(51) Kaseman, D. C.; Endo, T.; Sen, S. Structural disorder and the effects of aging in a phosphates glass: Results from two-dimensional 31P PASS NMR spectroscopy. J. Non-Cryst. Solids 2013, 359, 33–39.
(52) Kaseman, D. C.; Gullben, O.; Ariken, B. G.; Sen, S. Isotropic rotation vs. shear relaxation in supercooled liquids with globular cage molecules. J. Chem. Phys. 2016, 144, No. 174501.
(53) Laws, D. D.; Bitter, H.-M. L.; Jeschow, A. Solid-State NMR Spectroscopic Methods in Chemistry. Angew. Chem., Int. Ed. 2002, 41, 3096–3129.
(54) Tycko, R. Nuclear Magnetic Resonance Probes of Molecular Dynamics; Springer Science and Business Media, 2003.
(55) Hadziyannis, S. J.; Tassopoulos, N. C.; Heathcote, E. J.; Chang, T. T.; Kitis, G.; Rizzetto, M.; Marcellin, P.; Lim, S. G.; Goodman, Z.; Wulfsbo, M. S.; Xiong, S.; Fry, J.; Brosch, C. L. Adefovir dipivoxil for the treatment of hepatitis B and aneget-negative chronic hepatitis B. N. Engl. J. Med. 2003, 348, 800–807.
(56) Gao, Y.; Zu, H.; Zhang, J. Enhanced dissolution and stability of adefovir dipivoxil by cocystal formation. J. Pharm. Pharmacol. 2011, 63, 483–490.
(57) Naesens, L.; Balzarini, J.; Bischofberger, N.; De Clercq, E. Antiretroviral Activity and Pharmacokinetics in Mice Of Oral Bis(pivaloyloxymethyl)-9-(2-methoxethyl)adenine, the Bis-(pivaloyloxymethyl) Ester Prodrug of 9-(2-Phosphonylmethoxethyl)adenine. Antimicrob. Agents Chemother. 1996, 40, 22–28.
(58) Robbins, B. L.; Greenhaw, J. J.; Connelly, M. C.; Fridland, A. Metabolic Pathways for Activation of the Antiviral Agent 9-(2-Phosphonylmethoxethyl)adenine in Human Lymphoid Cells. Antimicrob. Agents Chemother. 1995, 39, 2304–2308.
(59) Prohens, R.; Barbas, R.; Portell, A.; Font-Bardia, M.; Alcobe, X.; Puigjaner, C. Expanding the crystal form landscape of the antiviral drug adefovir dipivoxil. Crystal Growth and Design 2015, 15, 475–484.