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Vacuum Ultraviolet Photoionization and Dissociative Photoionization of Capecitabine, 5′-Deoxy-5-fluorocytidine, and 5′-Deoxy-5-fluorouridine

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Vacuum ultraviolet (VUV) photoionization and dissociative photoionization of capecitabine and its metabolites, 5′-deoxy-5-fluorocytidine (5′-DFCR) and 5′-deoxy-5- fluorouridine (5′-DFUR), were investigated with infrared laser desorption/tunable synchrotron VUV photoionization mass spectrometry. Molecular ions (M^+) with small amounts of fragments can be found for these compounds at relatively low photon energies, while more fragment ions would be produced by increasing the photon energies. $(M\text{-}H_2O)^+$, $(base+H)^+$, $(base+2H)^+$, $(base+30)^+$, $(base+60)^+$, and sugar moiety were proposed for these nucleoside drugs with similar backbones. Decomposition channels for the major fragments were discussed in detail. Moreover, *ab initio* calculations were introduced to study the dehydration pathways of three fluoro-nucleosides. Corresponding appearance energies for the $(M\text{-}H_2O)^+$ ions were computed.

Key words: Capecitabine, 5′-Deoxy-5-fluorocytidine, 5′-Deoxy-5-fluorouridine, Photoionization, Mass spectrometry, Synchrotron radiation

I. INTRODUCTION

Fluorinated pyrimidines and related nucleosides are found to have significant anticancer activities, among which 5-fluorouracil (5-FU) is one of the most widely used anticancer drugs [1]. However, 5-FU exhibits noticeable toxicity due to the lack of selectivity towards tumor cells. In such circumstance, capecitabine (N^4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine) was designed as an oral prodrug of 5-FU for the treatment of patients with some types of cancers like breast cancer [2, 3], metastatic colorectal cancer [4, 5], gastrointestinal cancer, and so on [6]. Capecitabine is readily absorbed and can undergo enzymatic conversion into the metabolites 5′-deoxy-5-fluorocytidine (5′-DFCR) and 5′-DFUR. Then 5′-deoxy-5- fluorouridine (5′-DFUR) will be converted into 5-FU by thymidine phosphorylase. The bioactivation pathway of capecitabine to 5-FU is shown in Fig.1 [7, 8]. Capecitabine, 5′-DFCR and 5′-DFUR are all nucleoside compounds, which lead to similar bio-behaviors [9, 10]. Their metabolism and mechanisms of action are based on interaction with membrane transporters, kinases and intracellular enzymes usually transporting, phosphorylating and transforming endogenous/physiological nucleosides and nucleotides [11]. Under certain conditions, they can convert each other [12].

Like other nucleosides, capecitabine and its metabolites can be characterized by mass spectrometric methods, such as chemical ionization (CI) and traditionally used electron impact mass spectrometry (EIMS). CI MS produces less information-bearing fragments. EIMS can offer plentiful structural information of nucleosides. However, due to the thermal nonvolatile characteristics, structural identification of some nucleosides is difficult. Electrospray ionization (ESI) combined with liquid chromatography (LC) is ideal for the qualification of nucleoside drugs. This method has been widely used for capecitabine and its metabolites in biological matrices [13, 14]. In addition, trimethylsilylation before MS analysis is a complementary method for the characterization of naturally occurring nucleosides [15]. Zambonin *et al.* have simultaneously determined 5′-DFUR and 5-FU in human plasma by trimethylsilylation and subsequent GC-MS analysis [1].

Vacuum ultraviolet (VUV) photoionization and dissociative photoionization mass spectrometry have been successfully applied to the studies of bases and nucleosides [16, 17]. To the best of our knowledge, fluorinated pyrimidines and related nucleosides have not been investigated by photoionization mass spectrometric technique. In this work, we implemented infrared laser desorption/tunable synchrotron VUV photoionization mass spectrometry (IR LD/VUV PIMS) to study the photoionization and dissociative photoion-

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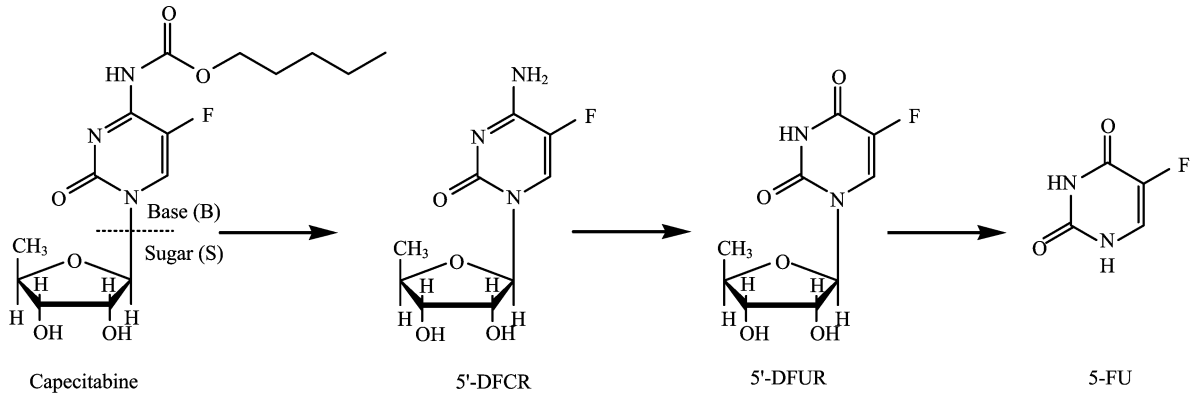

FIG. 1 The bioactivation pathway of capecitabine to 5-FU.

ization of capecitabine, 5′-DFUR and 5′-DFCR. Major fragments were characterized with the help of a commercial EI-TOFMS, and corresponding dissociation channels were proposed. *Ab initio* calculations were used to explain the possible dehydration pathways of capecitabine, 5′-DFCR, and 5′-DFUR. The lowest appearance energies (AE) values of $(M\text{-}H_2O)^+$ ions were obtained.

II. MATERIAL AND METHODS

A. Material

Capecitabine, 5′-DFCR and 5′-DFUR (>97% purity) were purchased from JK Chemical Ltd., and were used without further purification.

B. MS measurements

The experiments were fulfilled at National Synchrotron Radiation Laboratory (NSRL) USTC in Hefei, China. The IR LD/VUV PIMS apparatus has been reported elsewhere in detail [16–18]. Briefly, the experiments utilized the 1064 nm output of a pulsed Nd:YAG laser (Surelite I-20, Continuum, USA) with a duration of 7 ns and repetition rate of 10 Hz for IR laser desorption. Laser power was controlled at 6−8 mJ/pulse to generate neutral molecules. Tunable VUV light beam from a synchrotron perpendicularly overlapped with the desorption plume in the photoionization region. VUV photoionization took place at a distance of 2−4 mm from the substrate surface, where the plume of molecules traveled back and was ionized by VUV light. Ions were analyzed by a reflectron TOF mass spectrometer [19]. The pulsed voltage with a frequency of 10 kHz worked with a delay of 150 μs after the laser fires with a frequency of 10 Hz, which was controlled by a homemade pulse/delay generator.

Synchrotron VUV radiation from an undulator beamline of 800 MeV electron storage ring was monochromatized by a 1 m Seya-Namioka monochromator with a laminar grating (1500 grooves/mm, Horiba Jobin Yvon, France), covering the photon energy range of 7.8−24 eV with the energy resolution ($E/\Delta E$) of about 10^3. The average photon flux was 10^{13} photons/s at the ionization region. A silicon photodiode (SXUV-100, International Radiation Detectors Inc., USA) was used to monitor the photon flux for normalizing the ion signals. The higher order harmonic radiation was eliminated by a gas filter filled with argon or neon.

A commercial available EI (70 eV) TOF mass spectrometer (Micromass GCT, Manchester, UK) was used for the exact mass measurements. The samples were volatilized from a heated direct insertion probe in the source. The instrument was calibrated at a mass resolution of 8×10^3 (FWHM) using heptacosafluorotributylamine as internal reference and the single point lock-mass was at m/z=218.9856. Sample analysis, exact mass measurements and elemental composition determination were performed automatically using the OpenLynx software within MassLynx.

C. Computational methods

The theoretical calculations were carried out using Gaussian 03 program [20]. The geometries were fully optimized at the hybrid density functional B3LYP/6-311G(d,p) level. The vibrational frequencies were calculated at the same level for characterizing the nature of structures and used for computing zero-point energy (ZPE) corrections. Optimized structures of ionized capecitabine, 5′-DFCR, and 5′-DFUR are shown in Fig.2.

III. RESULTS AND DISCUSSION

A. Capecitabine and 5′-DFCR

The photoionization mass spectra of capecitabine at 9.0 and 10.5 eV are shown in Fig.3. At 9.0 eV, nearly

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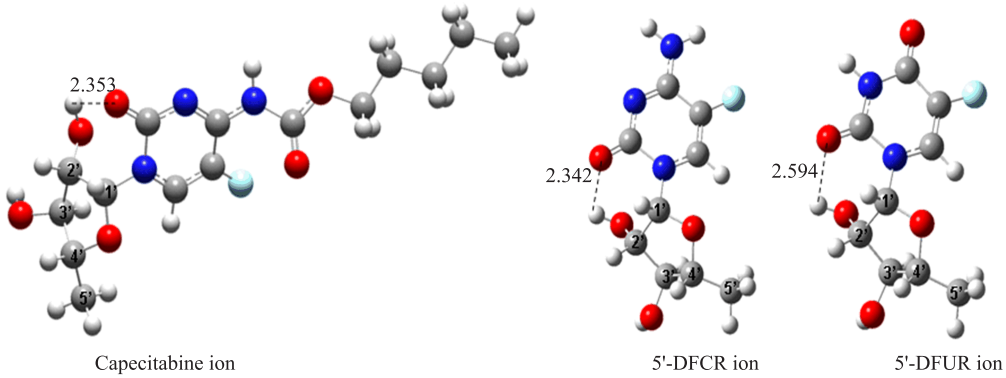

FIG. 2 Structures of capecitabine, 5′-DFCR, and 5′-DFUR ions. Bond lengths are in Å.

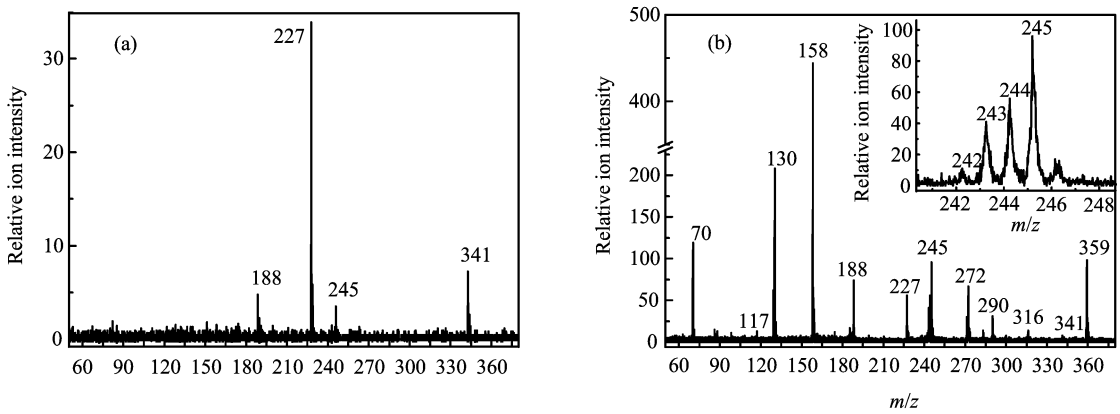

FIG. 3 Photoionization mass spectra of capecitabine at the photon energies of (a) 9.0 eV and (b) 10.5 eV.

no molecular ion at m/z=359 can be detected except for several fragments at m/z=227, 188, 245, and 341. When the photon energy was increased to 10.5 eV, more fragments at m/z=290, 272, 158, 130, and 70 together with M^+ of capecitabine were formed, as shown in Fig.3(b). The detection of M^+ at higher photon energy may be due to the increase of photoionization cross section. As a metabolite of capecitabine [7, 8], 5′-DFCR presents a pronounced M^+ at m/z=245 at 9.0 eV, and displays some similar peaks to those of capecitabine's at higher photon energy (Fig.3).

It is well known that the *N*-glycosidic bond connecting the sugar ring (S) and the base (B) of a nucleoside is expected to break easily during the interaction with electron, producing the fragments of B^+, $(B+H)^+$, $(B+2H)^+$, and S^+ [21, 22]. On the mass spectrum of capecitabine in Fig.3(b), the weak fragment at m/z=242 is assigned to B^+, deriving from the direct bond cleavage. Peaks at m/z=243 and 244 are assigned to $(B+H)^+$ and $(B+2H)^+$ ions formed in rearrangement involving one or two hydrogen atoms, which has also been found in the positive mode of ESI and APCI-MS [23, 24]. It should be mentioned that the weak fragment at m/z=245 in Fig.3(b) has the same structure of 5′-DFCR, arising from the C−N bond fission on base moiety and subsequent loss of $COOC_5H_{10}$. For 5′-DFCR (Fig.4(b)), the fragments at m/z=129 and 130 are attributed to $(B+H)^+$ and $(B+2H)^+$, which have also been found in the ESI and EIMS [23]. Interestingly, the $(B+H)^+$ of 5′-DFCR (and capecitabine) is less abundant than its protonated base cation $(B+2H)^+$, which is contrary to that of 2′-deoxyribosides. This difference has been used to distinguish between ribosides and 2′-deoxyribosides [21]. The B^+ (at m/z=242 for capecitabine and m/z=128 for 5′-DFCR) is less abundance than its protonated counterpart, like the usual behaviors of other nucleosides in EI experiments [25]. The sugar moiety ions of capecitabine and 5′-DFCR can be observed at expected positions in Fig.3(b) and Fig.4(b), but the relative abundances are very weak.

The fragment at m/z=188 in Fig.4(b) is assigned to the decomposition product from the m/z=245 ion of 5′-DFCR, known as $(B+60)^+$. This ion has been found on the spectra of other 5′-deoxyadenosine, but is absent in most 2′-deoxypentosides. As denoted in Scheme 1, C2′−C3′ bond cleavage is the first step to form this ion, followed by proton transfer and the C4′−O bond fission. Since the stabilization of $(B+60)^+$ depends on the oxygen atom on C2′, 2′-deoxypentosides generally don't produce this ion [25]. The $(B+60)^+$ of capecitabine can

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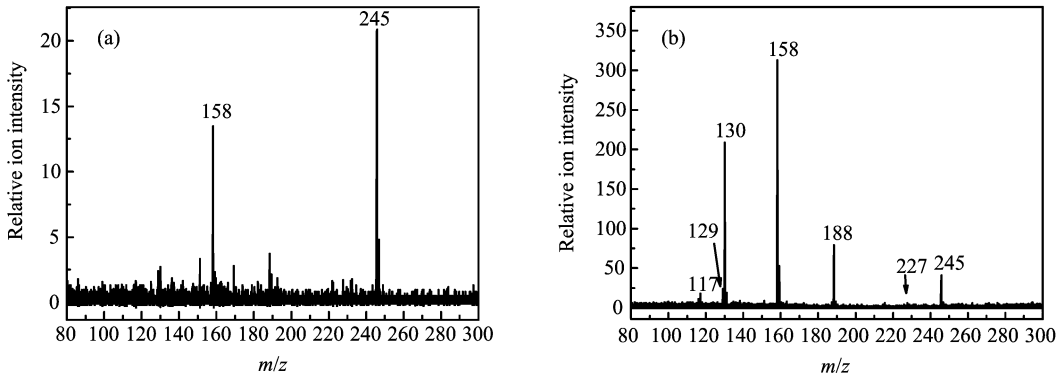
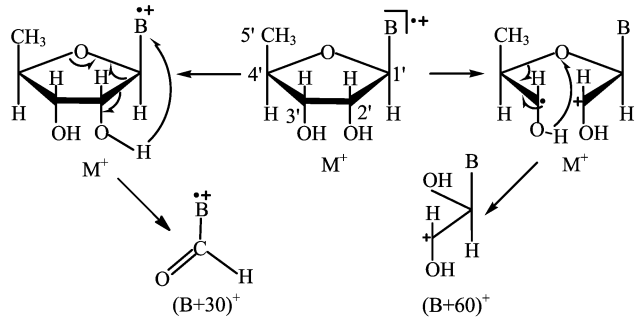
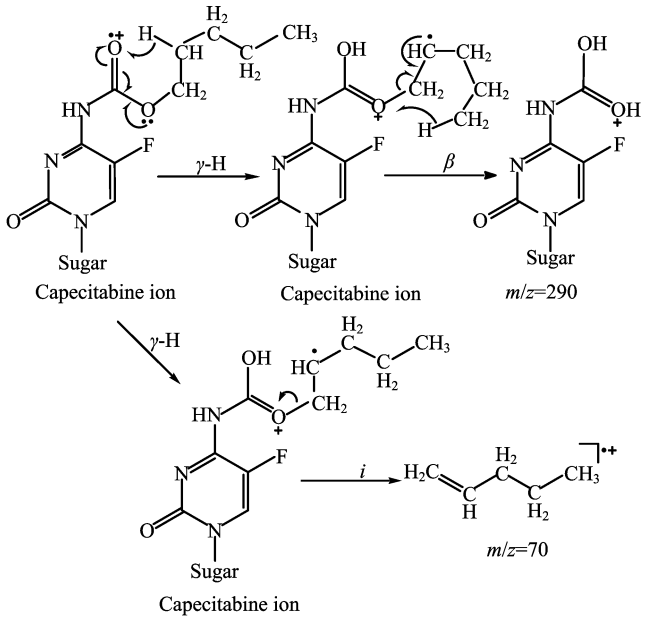


FIG. 4 Photoionization mass spectra of 5′-DFCR at the photon energies of (a) 9.0 eV and (b) 11.5 eV.



Scheme 1 Proposed formation pathways of $(B+30)^+$ and $(B+60)^+$.



Scheme 2 Proposed formation pathways of m/z=290 and 70 ions.

also be observed at m/z=302 with extremely low abundance.

The fragment at m/z=272 for capecitabine (Fig.3(b)) and 158 for 5′-DFCR (Fig.4(b)) are assigned to the $(B+30)^+$ ions. $(B+30)^+$ is a characteristic and abundant fragment in the EI and CI spectra of pentasides. Eggers *et al.* have proposed that hydrogen abstraction from C3′ is a key step to produce $(B+30)^+$ [26]. However, optimized structures at B3LYP/6-311G(d,p) level in this work demonstrate that hydrogen bonding is formed between hydroxy group on C2′ and base moiety for capecitabine and 5′-DFCR ions, with bond lengths of 2.353 and 2.342 Å, respectively (Fig.2). Thus, rearrangement involving hydrogen abstraction from hydroxy group on C2′ is more reasonable. This is also supported by the facts that the $(B+30)^+$ ions nearly can't be found on the spectra of other nucleosides that don't have hydroxy groups on C2′ atom [25]. Formation pathway for this ion is depicted in Scheme 1, where a proton is transferred from the hydroxy group on C2′ atom to the charged oxygen atom on base moiety, followed by radical induced cleavage of C1′−C2′ and C4′−O bonds.

Capecitabine has a carboxylic acid ester substituent on the base skeleton. Thus, it has some specific decomposition products that 5′-DFCR does not have. For example, the fragment at m/z=70 is 1-pentene, arising from γ-hydrogen shift to the charge-bearing heteroatom followed by charge-induced fragmentation (Scheme 2). A competitive pathway to this alkene formation process is the McLafferty rearrangement with double hydrogen transfer, giving rise to the fragment at m/z=290. As demonstrated in Scheme 2, after the γ-hydrogen atom migrates to the charge site, another hydrogen atom is transferred to the new formed charge center. Subsequently, β-cleavage results in the loss of alkenyl group. Another fragment ion at m/z=316 is assigned to $(M\text{-}C_3H_8)^+$ after direct α-cleavage at the alkane group. Moreover, α-cleavage at the carbonyl group can yield the characteristic fragment $(M\text{-}C_5H_{11}O)^+$ at m/z=272, but this peak can not be distinguished from the $(B+30)^+$ of capecitabine due to the limitation of mass resolution.

Loss of H_2O will produce the fragments at m/z=341

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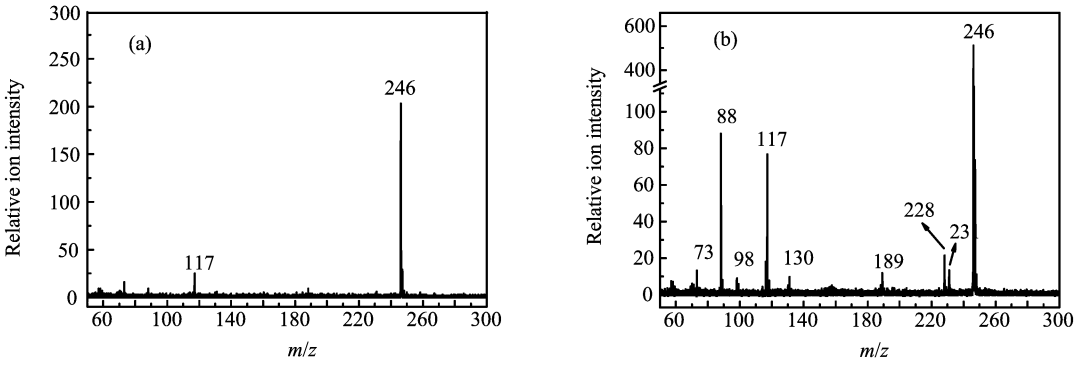

FIG. 5 Photoionization mass spectra of 5′-DFUR at the photon energies of (a) 9.0 eV and (b) 10.0 eV.

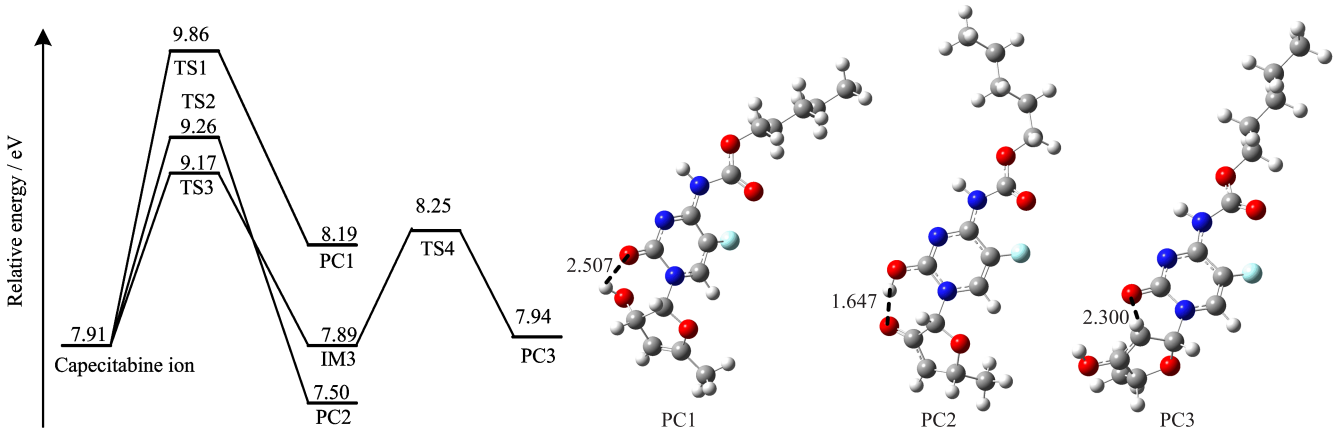

FIG. 6 Reaction minimum energy paths for three possible dehydration channels of capecitabine ion. Bond lengths are in Å.

for capecitabine and at m/z=227 for 5′-DFCR, which will be discussed afterwards.

B. 5′-DFUR

5′-DFUR could be enzymatically converted from 5′-DFCR by cytidine deaminase both in liver and tumour [23]. For the LC mass spectrometric determination of 5′-DFUR in biological matrices, some typical product ions are chosen, such as the m/z=129 ion in negative ionization mode [23, 24], and the losses of a sugar moiety and a fluorine atom product at m/z=108 in negative ionization mode [7].

In our photoionization experiments, in addition to the intense molecular ion at m/z=246 at the photon energy of 9.0 eV, more fragment ions could be formed with the increase of photon energy. As shown in Fig.5, the fragment at m/z=228 is assigned to the dehydration product after intramolecular proton transfer. Loss of a methyl group on C5′ will produce the ion at m/z=231. The $(B+60)^+$ of 5′-DFUR located at m/z=189 is observed with low abundance. The fragment at m/z=130 is assigned to $(B+H)^+$, originating from the elimination of sugar moiety from molecular ion. The B^+ is also observed at m/z=129 with much weaker abundance. α-cleavage and subsequent loss of the 5-FU part will give rise to the sugar moiety at m/z=117. The base moiety of 5′-DFUR has a lower electron density than that of capecitabine, resulting in a higher sugar fragment compared with that of capecitabine. The fragment ion at m/z=88 with relatively high abundance is believed to be the dissociation product from m/z=130 with the loss of CON radical [27]. Loss of the base moiety from the m/z=228 ion results in the fragment ion at m/z=98. The major dissociation pathways are described in Scheme 1.

C. Dehydration mechanisms of capecitabine, 5′-DFCR, and 5′-DFUR

Loss of H_2O for nucleosides has been found in many cases [16, 21, 22]. The fragments at m/z= 341 (Fig.3(b)), m/z=227 (Fig.4(b)), and m/z=228 (Fig.5(b)) are attributed to the dehydration product ions $(M\text{-}H_2O)^+$ of capecitabine, 5′-DFCR, and 5′-DFUR respectively. Since the 5′-deoxypentaose backbone has two hydroxyl groups (on C2′ and C3′), each group can be eliminated from their sugar moieties. To

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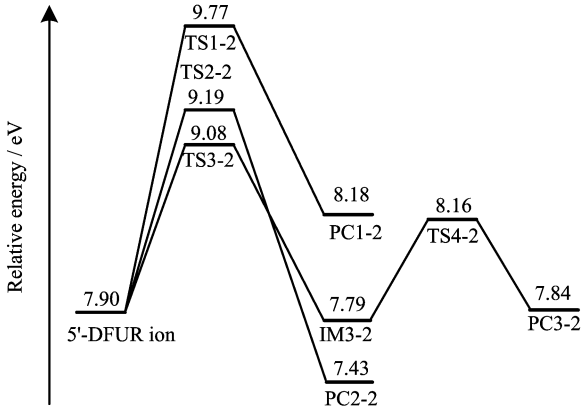

FIG. 7 Reaction minimum energy paths for three possible dehydration channels of 5′-DFCR ion.

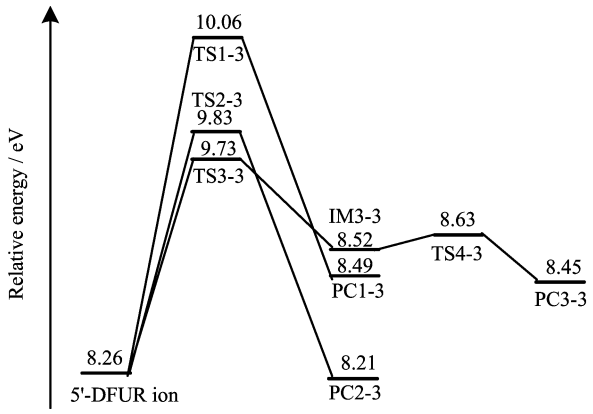

FIG. 8 Reaction minimum energy paths for three possible dehydration channels of 5′-DFUR ion.

learn more about the dehydration processes, *ab initio* calculation at the B3LYP/6-311G(d,p) level was introduced for these fluoro-nucleosides.

Figure 6 is the potential energy surfaces for three possible dehydration channels of capecitabine ion and the geometries of $(M\text{-}H_2O)^+$ product complex (PC). The geometries of transition states (TS) and intermediates (IM) are listed in the supplementary material. Theoretical calculations show that the hydroxy group on C2′ of capecitabine ion prefers to form a hydrogen bond with the oxygen atom on base moiety, as depicted in Fig.2. Three dehydration pathways are proposed according to the calculated results. (i) Capecitabine ion can undergo a hydrogen transfer from C4′ to OH on C3′ via TS1 to form PC1, which is followed by elimination of water on C3′. The barrier for this step is calculated to be 1.95 eV, and corresponding AE for this $(M\text{-}H_2O)^+$ ion is determined to be 9.86 eV. (ii) After intramolecular proton transfer from C2′ to OH on C3′ via TS2, C3′−O bond cleavage leads to the formation of PC2. The barrier for this step and the AE value are calculated to be 1.35 and 9.26 eV, respectively. (iii) Capecitabine ion undergoes C2′−H bond elongation and subsequent proton transfer to OH on C2′ with a barrier of 1.26 eV at TS3, generating an intermediate IM3. The C2′−O bond in this intermediate then breaks with an energy barrier of 0.36 eV to produce PC3. The AE value for the $(M\text{-}H_2O)^+$ of this channel is determined to be 9.17 eV.

Due to structural similarity, 5′-DFCR and 5′-DFUR ions undergo the same dehydration pathway. Their proposed dehydration channels, potential energy surfaces and corresponding energies are shown in Fig.7 and Fig.8. The lowest AE values for the $(M\text{-}H_2O)^+$ ions of 5′-DFCR and 5′-DFUR are determined to be 9.08 and 9.73 eV, respectively. Geometries for the transition states, intermediates and product complex of three nucleoside drugs are also listed in the supplrmentary material. All the theoretical results show that the three nucleosides tend to loss the water via C2′−O bond fission with the lowest appearance energies.

IV. CONCLUSION

The VUV photoionization and dissociative photoionization of capecitabine, 5′-DFCR and 5′-DFUR have been investigated with IR LD/VUV PIMS method at two photon energies. Structural information of the fragments was verified by a commercial EI-TOF-MS. Due to the similar nucleoside structures, the mass spectra of these compounds demonstrate some typical ions, such as $(M\text{-}H_2O)^+$, $(B+H)^+$, $(B+2H)^+$, $(B+30)^+$, $(B+60)^+$, and sugar moiety. Corresponding dissociation channels of these major fragments were discussed. Moreover, McLafferty rearrangement was found to involve in the fragmentation of capecitabine bearing a carboxylic acid ester group. DFT calculations demonstrate the hydrogen-bonding formation on the nucleoside skeletons. C2′−H bond elongation and subsequent proton transfer to OH on C2′, followed by C2′−O bond fission to produce $(M\text{-}H_2O)^+$ was proposed to be the possible dehydration pathway with the lowest appearance energy.

V. ACKNOWLEDGEMENTS

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Supplementary material: Structures of transition states, intermediates and products were shown.

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