Diketoacid HIV-1 Integrase Inhibitors: An Ab Initio Study

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The stable tautomeric forms of two representative arene-substituted diketoacid HIV-1 integrase inhibitors, 5-ClTEP and L-731,988, were investigated by B3LYP with 6-31G*, 6-31G(d,p), and 6-31+G(d,p) basis sets. Optimization with MP2/6-31G* was also performed for 5-ClTEP. The solvation effect was considered using a conductor-like screening model. With the density functional theory method, the trans diketo conformations are more stable than the cis conformers. The difference is 14 kJ mol^{-1} for 5-ClTEP and 33 kJ mol^{-1} for L-731,988. Two trans diketo structures were obtained. The difference between these two trans diketo structures is less than 4 kJ mol^{-1} calculated at the B3LYP/6-311+G(3df,2p) level. Two enol forms prevail over the diketo tautomers and are calculated to have the same free energy. Because there is no barrier observed between these two enol forms, they can interchange easily such that a delocalized transition state is suggested to be the observed form. Contradictory to the results of the MP2 method that predicts a preference for the trans diketo forms, the B3LYP method predicts a preference for the enol tautomers, which is in agreement with the experimental results.

Introduction

HIV-1 integrase inhibitors have become of interest as anti-AIDS drugs since the appearance of drug resistance to HIV-1 reverse transcriptase (RT) and protease (PR) inhibitors. HIV-1 integrase (IN) is involved in 3′-processing and strand transfer reactions[1]. β-Diketoacid (DKA)-containing inhibitors are very promising drug candidates for this target. L-731,988 and L-708,906 (Figure 1) are two of the most active β-diketoacid compounds in the strand transfer assay with recombinant integrase.[2] The inhibitor, 5-ClTEP (1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)-propenone) has been cocrystalized with HIV-1 integrase[3](PDB code 1QS4). Comparisons with the DKA family are possible because the tetrazole ring can be considered as an isostere of the carboxylic acid group. S-1360, which contains a triazole ring, is the only drug used clinically at present. Besides the acid group that has been shown to be critical for the inhibitory activity,[4] there is a common aryl group substituted at the other side of the diketo unit of these inhibitors. Because the two carbonyl oxygen atoms of some inhibitors have been shown to be involved in coordination to magnesium,[5,6] investigation of the arene substituent effect on the conformations of DKAs is necessary.

Diketo compounds can exist either as diketo or keto−enol forms. Substituents connected to the two ends of the diketo groups significantly affect the preference for enol or keto tautomeric forms. When the substituents are H,[7] CH_3,[8] $C(CH_3)_3$,[9] or CF_3,[10] enol tautomers predominate. Keto tautomers are observed when the substituents are F,[11] Cl,[12] OCH_3,[13] or NH_2.[14] Studies were also reported for diketo compounds that have different substituents on both sides of the diketo group.[14] The tautomeric and conformational properties of methyl acetoacetate were studied by electron diffraction and quantum chemistry,[14] indicating that the enol tautomer prevails in the mixture of enol and keto forms and only one enol form, that with the O−H connected to the methyl group, is present. Aqueous solution conformations of various 2,4-diketo carboxylic acids were studied by Brecker et al.[15] Compounds with 4-alkyl, 4-alkenyl, and 4-alicyclic substituents were found to exist in both diketo and enol−keto forms, in addition to their hydrated form. NMR studies on the compounds with aryl substituents such as phenyl or benzyl groups indicated that only the enol forms exist. In the present paper, the structure and stability of two aryl-substituted diketoacid compounds 5-ClTEP and L-731,988 are investigated by quantum chemical calculations.

Methods

The carbonyl oxygen that is connected to the indole or pyrazole ring is denoted as O(P), and the carbonyl oxygen that is connected to the carboxyl or tetrazole is denoted as O(A). Correspondingly, the enol form with the carbonyl group connected to the indole or pyrazole is called enol(P), and the enol form with the carbonyl group connected to the carboxyl or tetrazole is called enol(A). The keto and enol forms of the diketoacid are illustrated in Figure 2.

For the keto tautomeric form, there are two free torsions around the diketo bond. τ 1 stands for rotation about C1−C2, while τ 2 stands for rotation about C2−C3. The potential surface was calculated by optimizing geometries at the HF/STO-3G level followed by a single-point energy calculation with B3LYP/6-31G* for a model diketoacid, with chlorine or fluorine substituted by a hydrogen atom.

Geometry optimization and frequency calculations for the minima were carried out by the B3LYP method using 6-31G*, 6-31G(d,p), and 6-31+G(d,p) basis sets. Single-point energy calculations with a 6-311+G(3df,2p) basis set were then performed based on the 6-31+G(d,p) optimized geometries. Because the frequencies for some of the species are not available

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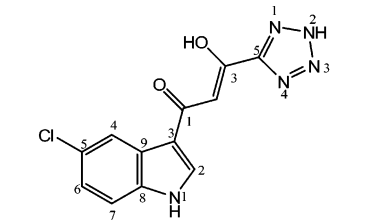
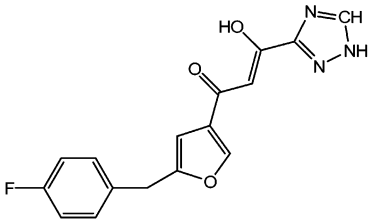
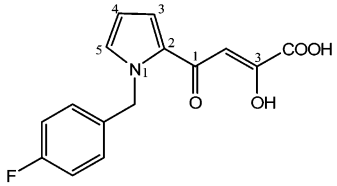


5-ClTEP



S-1360



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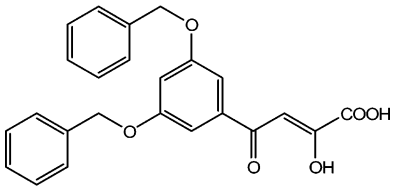
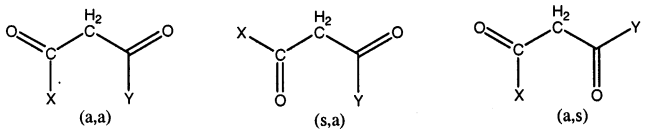


L-708,906

Figure 1. Structures of 5-ClTEP, S-1360, L-731,988, and L-708,906.

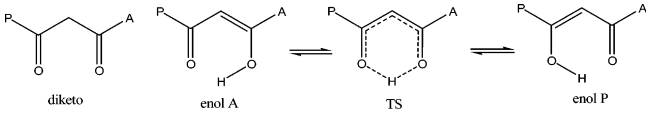
at the B3LYP/6-31+G** level at our present computation source, the frequencies calculated by B3LYP/6-31G** were used and scaled by a factor of 0.9806 when considering the zero-point energy (ZPE). Geometries of the tautomers were also optimized with the MP2/6-31G* method for 5-ClTEP. The polarizable continuum model (PCM) has proven to be reasonably accurate in predicting the solvation energy and acid constants.[16] One polarizable conductor PCM model, a conductor-like screening model (COSMO)[17,18] implemented in Gaussian 03, models a solute immersed in an infinite dielectric constant medium, i.e., conductor. This is a good approximation for solvents of high permittivity such as water. In the present work, the free energies of solvation in water (dielectric constant $\epsilon = 78.39$) were estimated with a B3LYP(COSMO)/6-31+G** single-point calculation based on the B3LYP/6-31+G** optimized geometry, using Klamt's radii and iterative solution. All of the calculations are carried out using Gaussian 98[19] or Gaussian 03[20] software.

The diketo nomenclature follows that used by Belova[13] for methyl acetoacetate. Here "s" stands for synperiplanar (sp) or



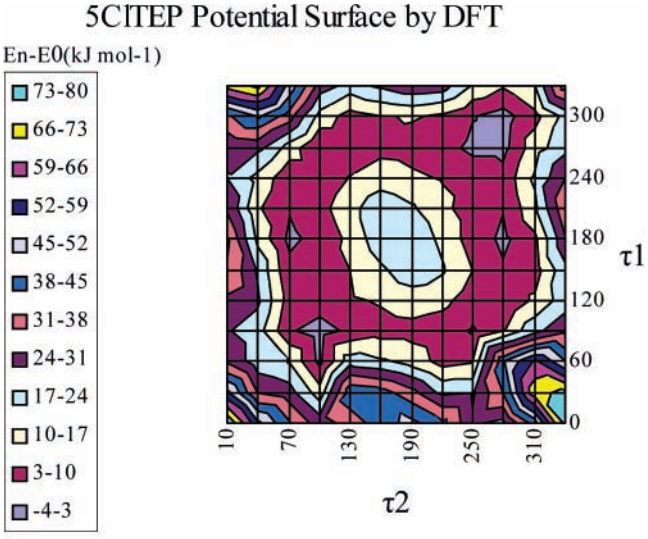
(a,a) (s,a) (a,s)

synclinal (sc), and "a" stands for antiperiplanar (ap) or anticlinal (ac).



diketo enol A TS enol P

Figure 2. Illustration of tautomerism of DKA inhibitors.



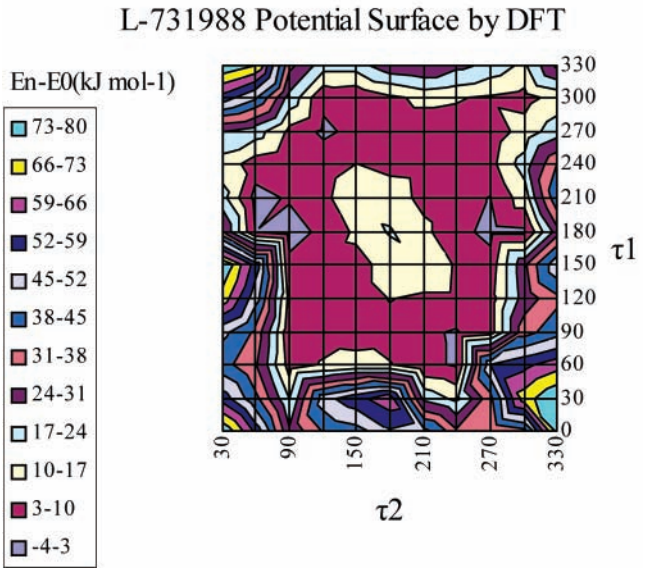


Figure 3. Potential surface of rotation about C1−C2 (τ1), and C2−C3 (τ2) for 5-ClTEP and L-731,988 by B3LYP/6-31G*.

Results and Discussion

5-ClTEP. In the crystal structure of the HIV-1 integrase complex, the carbonyl adjacent to pyrazole is trans with respect to the pyrazole double bond. Scanning the dihedral angles τ1 and τ2 of the trans diketo tautomeric form gives four stationary points (Figure 3). Full geometry optimization with the B3LYP method was carried on these minimum energy structures. The optimized structures at B3LYP/6-31G* are shown in Figure 4. A pair of enantiomers (**1**-*trans*-diketo(a,s)) have τ1 and τ2 values of 73° and 93° and −73° and −93°, respectively, in which the O(P) is facing H(C4′) and O(A) is in close proximity to the H(C2′) atom of the indole to form two intramolecular hydrogen bonds. The other pair of enantiomers was found with τ1 = 178°, τ2 = −74°(**1**-*trans*-diketo(s,a)) and −178°, 74°, respectively, conserving the intramolecular hydrogen bond involving O(P).

The **1**-*trans*-diketo(a,s) is more stable than **1**-*trans*-diketo-(s,a), lower in energy by 5 kJ mol^{-1}. We also considered the

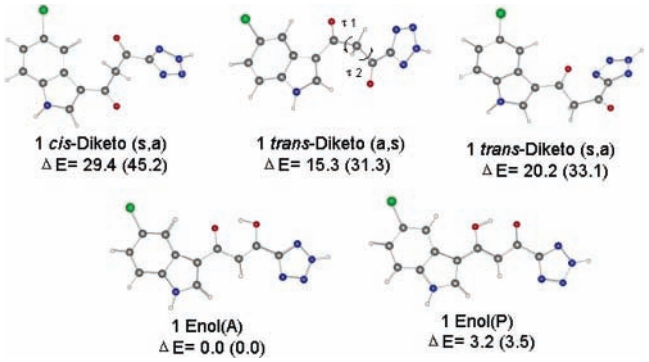


Figure 4. Minimum conformation and relative energies of tautomers of 5-ClTEP calculated with B3LYP/6-31G* (energies in brackets are calculated with BL3YP/6-311+G(3df,2p)//6-31+G**).

cis diketo tautomer in which the carbonyl adjacent to the pyrazole is cis to the pyrazole double bond. Even although O(A) is close to H(C4′) of the indole such that an eight-membered ring containing an intramolecular hydrogen bond can form, its energy is 14 kJ mol^{-1} higher than **1**-*trans*-diketo(a,s). The results are given in Table 1.

For the two enol forms, enol(A) and enol(P), planar conformations are observed. That is, the indole ring, the six-membered ring formed by intramolecular hydrogen bond of the diketo part, and the tetrazole ring are in the same plane. The difference between the two enol tautomers is not distinct, with the enol(A) slightly more stable than enol(P) by only 3 kJ mol^{-1}. However, the enol(A) is obviously more stable than the most stable diketo form **1**-*trans*-diketo(a,s) by about 15 kJ mol^{-1}.

The geometries of the two stable trans diketo forms and the two enol forms optimized with the 6-31G(d,p) or 6-31+G(d,p) basis sets remain the same compared with those optimized with the 6-31G* basis set (Table 2). The enol forms are further stabilized with larger basis sets. The energy difference between **1**-enol(A) and **1**-*trans*-diketo(a,s) increases from 15 kJ mol^{-1} at the B3LYP/6-31G* level to 28 kJ mol^{-1} at the BL3YP/6-31G(d,p) level and 31 kJ mol^{-1} at the B3LYP/6-31+G (d,p) level. However, the difference between the two enol tautomers remains almost the same. Single-point energy calculations at 6-311+G(3df,2p) based on the 6-31+G(d,p) optimized geometry gave the same relative energies as were found with 6-31+G-(d,p).

Free energy calculations (ΔG in Table 1; the electronic energy, zero-point energy, $H_{298}° - H_0°$, and entropy at 298 K are listed in Table 3) show that the enol tautomers are still the dominant form for 5-ClTEP, i.e., enol(A) does not have a notable preference over enol(P). The two trans diketo forms are very close in energy. The conformations of the enol tautomers are consistent with that observed in the cocrystalized complex structure except that the hydrogen bond formed by O(P) and H(C4′), disappears on interacting with the protein because of the deviation about C1−C3′.[3] After considering solvation effects estimated by COSMO at the B3LYP/6-31+G** level, the order of the tautomers remains unchanged. That is, the two enol tautomers still prevail, while the difference between the two trans diketo forms increases and the difference between the enols and **1**-*trans*-diketo(s,a) deceases due to the distinct stabilization of **1**-*trans*-diketo(s,a) in water (see the last column in Table 1).

We calculated the transition state for the interconversion of the two enol tautomers at the B3LYP/6-31+G(d,p) level. The delocalized six-membered ring transition state (which is characterized by an imaginary frequency at −1017 cm^{-1}) has the same free energy as enol(A); hence there is no barrier between the two enol tautomeric forms. This result is in agreement with the experimental observation that only enol forms exist for aryl-substituted 2,4-diketoacid analogues, but no separate isomer could be obtained,[15] which is the result of a fast interconversion between the enol forms. The fact that the two enol forms and the transition state are identical in free energy indicates that the delocalized transition state may exist while interacting with the target enzyme.

Optimization at the MP2/6-31G* level was also performed for the sake of comparison. The key hydrogen bonds found by the density functional theory method are present, but the τ1 and τ2 values of the diketo tautomers are different from the B3LYP optimized ones (Table 2). In contrast to the B3LYP results, the trans diketo tautomers predicted by the MP2 method have lower energies than the enol forms. The two enol tautomers are higher in energy than **1**-*trans*-diketo(s,a) by 10 and 17 kJ mol^{-1}, respectively. **1**-*Trans*-diketo(a,s) is higher in energy than **1**-*trans*-diketo(s,a) by about 3 kJ mol^{-1}. Hence, the B3LYP method predicts that the dominant tautomeric forms are the enol ones, while the trans tautomers are more stable in energy as predicted by the MP2 method for 5-ClTEP. In Belova's study on methyl acetoacetate, it was also indicated that the MP2 method could not distinguish the dominant form of the enol tautomers, but the B3LYP method can reproduce the experimental tautomers very closely.[14]

L-731,988. Potential surface scanning τ1 and τ2 values of the trans tautomers of L-731,988 gave a similar surface to 5-ClTEP. There are four minima that fall into two pairs of enantiomers. Further optimization of the minima was carried

TABLE 1: Relative Energies and Gibbs Free Energy of Tautomers (kJ/mol)

		ΔE						
		MP2	B3LYP					
compound	tautomeric form	6-31G*	6-31G*	6-31G**	6-31+G**	6-311+G(3df,2p)[a]	ΔG_{gas}[b]	ΔG_{sol}[c]
5-ClTEP	**1**-*cis*-diketo(s,a)	7.1	29.4	41.9	44.7	45.2	41.3	30.0
	1-*trans*-diketo(a,s)	−7.0	15.3	27.7	30.7	31.3	27.9	18.5
	1-*trans*-diketo(s,a)	−10.3	20.2	32.7	33.2	33.1	26.2	9.0
	1-enol(P)	7.3	3.2	2.9	2.8	3.5	2.4	0.1
	1-enol(A)	0	0	0	0	0	0	0
L-731,988	**2**-*cis*-diketo(s,s)		45.9	58.1	60.8	60.2	53.5	44.4
	2-*trans*-diketo(s,a)		12.5	24.8	28.3	27.4	20.8	18.3
	2-*trans*-diketo(a,s)		15.5	27.5	32.1	31.1	27.2	25.6
	2-enol(A)		2.9	1.0	2.5	2.3	1.0	2.6
	2-enol(P)		0	0	0	0	0	0

[a] Single-point results based on the B3LYP/6-31+G** optimized geometry. [b] Energies are obtained by single-point calculation with B3LYP/6-311+G(3df,2p), using frequencies calculated with B3LYP/6-31G**. [c] Solvation energies are estimated with the COSMO model with B3LYP/6-31+G**.

TABLE 2: Optimized Dihedral Angles $\tau 1$ and $\tau 2$ of Tautomers (deg)

				B3LYP					
compound	tautomeric form	MP2/6-31G*		6-31G*		6-31G**		6-31+G**	
5-ClTEP	**1**-*cis*-diketo(s,a)	102.8	79.7	98.8	85.9	98.5	85.9	101.6	92.0
	1-*trans*-diketo(a,s)	72.3	80.4	73.4	92.8	73.1	93.5	75.9	94.2
	1-*trans*-diketo(s,a)	170.4	−67.6	178.2	−74.0	178.7	−74.1	177.2	−74.0
	1-enol(P)	180	180	180	180	180	180	180	180
	1-enol(A)	180	180	180	180	180	180	180	180
L-731,988	**2**-*cis*-diketo(s,s)			−92.6	−99.8	−92.5	−100.7	−98.9	−116.0
	2-*trans*-diketo(s,a)			160.6	−64.0	160.6	−63.8	158.2	−62.7
	2-*trans*-diketo(a,s)			77.4	−122.5	77.4	−122.5	78.6	−121.1
	2-enol(A)			179.4	180	180	180	179.6	179.9
	2-enol(P)			178.8	180	178.9	180	178.8	−179.6

TABLE 3: S_{298}°, $H_{298}^\circ - H_0^\circ$, and Electronic Energies Calculated in the Gas Phase

	B3LYP *E*(hartree)			ZPE	$H_{298}^\circ - H_0^\circ$	S_{298}°
species	6-31G*	6-31G**	6-31+G**	(kJ mol^{-1})[a]	(kJ mol^{-1})[a]	(J K^{-1} mol^{-1})[a]
1-*cis*-diketo(s,a)[b]	−1346.440768	−1346.456889	−1346.490767	515.9	44.9	536. 2
1-*trans*-diketo(a,s)	−1346.446145	−1346.462314	−1346.496095	516.4	44.7	535.5
1-*trans*-diketo(s,a)	−1346.444281	−1346.460411	−1346.495144	514.6	45.3	543.3
1-enol(P)	−1346.450726	−1346.471735	−1346.506709	516.3	43.8	524.6
1-enol(A)	−1346.451956	−1346.472851	−1346.50779	516.8	43.8	522.4
1-TS(**1**-enol(A) → **1**-enol(P))	−1346.447316	−1346.469637	−1346.504479	506.7	42.9	516.3
2-*cis*-diketo(s,s)[c]	−1034.286802	−1034.309378	−1034.355258	649.5	50.2	587.3
2-*trans*-diketo(s,a)	−1034.299512	−1034.322028	−1034.36764	648.9	50.5	585.7
2-*trans*-diketo(a,s)	−1034.298364	−1034.321001	−1034.366197	650.5	50.0	580.0
2-enol(A)	−1034.30319	−1034.331109	−1034.37748	650.6	49.0	568.6
2-enol(P)	−1034.304276	−1034.33149	−1034.378434	651.5	48.7	566.1

[a] Calculated at the B3LYP/6-31G** level. [b] **1** denotes 5-ClTEP. [c] **2** denotes L-731,988.

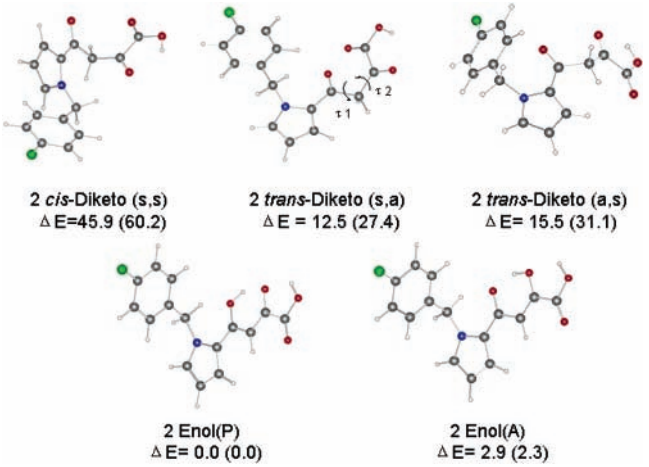


Figure 5. Minimum conformation and relative energies of tautomers of L-731,988 calculated with B3LYP/6-31G*(energies in brackets are calculated with BL3YP/6-311+G(3df,2p)//6-31+G**).

out at the B3LYP/6-31G* level. The most stable diketo conformation (**2**-*trans*-diketo(s,a)) has a six-membered ring with an intramolecular hydrogen bond formed by the hydrogen of the methylene and O(P). At the same time, the carbonyl oxygen of the carboxylic acid is in the region of the H(C) of the benzene ring (d_{O-H} = 2.78 Å), indicating a modest interaction (Figure 5). The other enantiomers (**2**-*trans*-diketo(a,s)) also have the aforementioned six-membered intramolecular hydrogen bond and a new hydrogen bond interaction formed by the carbonyl oxygen of the carboxylic acid group and one of the pyrazole hydrogens (d_{O-H} = 2.5 Å). The **2**-*trans*-diketo(a,s) is higher in energy than **2**-*trans*-diketo(s,a) by 3 kJ mol^{-1}. The cis tautomers are significantly less stable than the trans ones by about 33 kJ/mol. For the two enol tautomers, the dihedral angles about C1−C2′are close to 180°. They share almost same abundance with a difference of 3 kJ mol^{-1}. Similar to the results of 5-ClTEP, with larger basis sets, 6-31G(d,p) or 6-31+G(d,p), the geometries of the trans diketo and enol tautomers are same as that obtained at the 6-31 G* level, while the energy difference between the keto and enol forms becomes larger. The relative energies with 6-31+G(d,p) are as accurate as those obtained with 6-311+G(3df,2p). The difference between **2**-enol(P) and **2**-*trans*-diketo(s,a) is 12 kJ mol^{-1} at the 6-31G* level and increases to 28 kJ mol^{-1} at 6-31+G(d,p), but there is also no distinct difference between the two enol tautomers. The free energy calculation shows no observable difference between the two enol tautomers. The enol tautomers are less stable than **2**-*trans*-diketo(s,a) by 21 kJ mol^{-1}. After inclusion of the solvation effect, the enol forms are still close to each other and more stable than the trans diketo forms. This agrees with the experimental results for its analogues, phenyl- or benzyl-substituted 2,4-diketoacids, for which only the enol signal was observed.[15]

A dicarbonyl compound, methyl acetoacetate, with one substituent CH_3 favoring the enol form and the other OCH_3 favoring the keto form, appears as a tautomeric mixture with the enol form prevailing.[14] With an acidic group such as carboxyl or tetrazole substituent on the O(A) side and an arene substituent on the side of O(P) in the case of DKAs, only the enol forms exist. For methyl acetoacetate, there is only one enol form found by quantum chemical calculations and three stable diketo forms predicted by B3LYP with smaller basis set (6-31G**), but for the DKA system, two enol forms coexist, and only two trans diketones are found by a potential energy scan around the two rotatable bonds of the backbone carbon atoms, regardless of the structural difference between 5-ClTEP and L-731,988. In the gas phase, the difference in free energy between the diketones for methyl acetoacetate is at most 1 kJ mol^{-1} (B3LYP/6-31G**); similarly, the free energy difference between two trans diketones for the DKA system is also small, with 2 kJ mol^{-1} for 5-ClTEP and 6 kJ mol^{-1} for L-731,988. For methyl acetoacetate, optimization with the B3LYP method at different basis sets (6-31G** or 6-31++G**) has very little

influence on geometry or the relative energy. In our calculation for the DKA system, the relative energies of the trans diketo and enol forms are very sensitive to the basis set. The difference increases with the introduction of polarization and diffuse functions, while the relative energy between the two diketo tautomers remains almost the same.

Conclusion

The stable conformation of two representative diketoacid HIV-1 integrase inhibitors was investigated by ab inito methods. The B3LYP method appears to yield results that are closer to the experimental ones compared with the MP2 method. The calculations also show that at least a 6-31+G(d,p) basis set should be used to estimate the energies of the tautomers correctly. For aryl-substituted diketoacid HIV-1 integrase inhibitors, in which the aryl ring can conjugate with the diketo part, the enol forms are the dominant conformations. Two enol tautomeric forms are close in energy and can interchange very easily because there is no barrier to overcome. Two higher-energy trans diketo tautomers are found for these diketoacid HIV-1 inhibitors. The result of this study is expected to facilitate further investigation of the interaction between the diketo inhibitors and HIV-1 integrase.

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Supporting Information Available: Cartesian coordinates of the species discussed in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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