The Study of One Carbon Unit Transfer from Imidazolidine to dUMP Analogue with ONIOM Theory

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Abstract: ONIOM quantum mechanics method is used in this paper to study one-carbon unit transfer from imidazolidine to 6-aminouracil derivates. The computation results show that this reaction can be completed *via* three paths owing to the three different proton transfer modes.   By calculation and analysis, we can find the direct proton transfer is the preferable pathway.

Keywords: H4folate, one-carbon unit transfer, dUMP.

Enzymatic one-carbon unit transfer is an important biosynthetic process[1]. The thymidylate synthase catalyzed conversion of dUMP to dTMP involves the overall transfer of a "CH_3" group from the folate cofactor 5, 10-methylenetetrahydrafolate (5,10-CH2-H4folate) to the uridine derivative [2].   Various steps of the mechanism from dUMP to dTMP have received support from model studies of mechanism-based inhibitors[3, 4, 5].

In this paper, quantum chemical method is employed to study the one-carbon unit transfer from **Rea.2** (R3, 4, 5=Me) to **Rea.1** (R1=H, R2=Me).   The reaction process is characterized hypothetically (see **Figure 2**) by a C-N cleft of **Rea.2** to **Rea.2'**, formation of the tetrahedral intermediate **M0** by **Rea.2'** attacking **Rea.1** nucleophilicly, transfer of proton H-9 by three different paths to N-8, and cleavage of C-7-N-8 until the formation of the final product **E** of the reaction.   Because proton migrating is the rate-limiting step[6,2a], we mainly focus on the process of proton transfer.

Computational method

In this paper, the ONIOM calculation method in the Gaussian98 program is used. With this method the system is divided into two layers, three methyls of **Rea.1** are treated in outer layer, and the other atoms are treated in the inner layer.   In reaction **Rea.2'**, only the $^7CH_2$ and the C-8 atoms are treated in inner layer.   It is the same with the corresponding atoms in the all intermediates and transition states.   We have used the B3LYP hybrid functional and the 6-31G(d) basis set to treat the inner atoms and the remainder is treated with PM3 semi-empirical method.   All stationary points on the reaction path were optimized without constraints and vibrational analysis is performed on

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them.

Results and Discussion

The structures of **Rea.1**, **Rea.2** and **Rea.2'**, and all the optimized structures of the intermediates and transition states generated in the reaction are listed in **Figure 1**.   The

Figure 1   Structures of reactants and optimized structures of intermediates and transition states
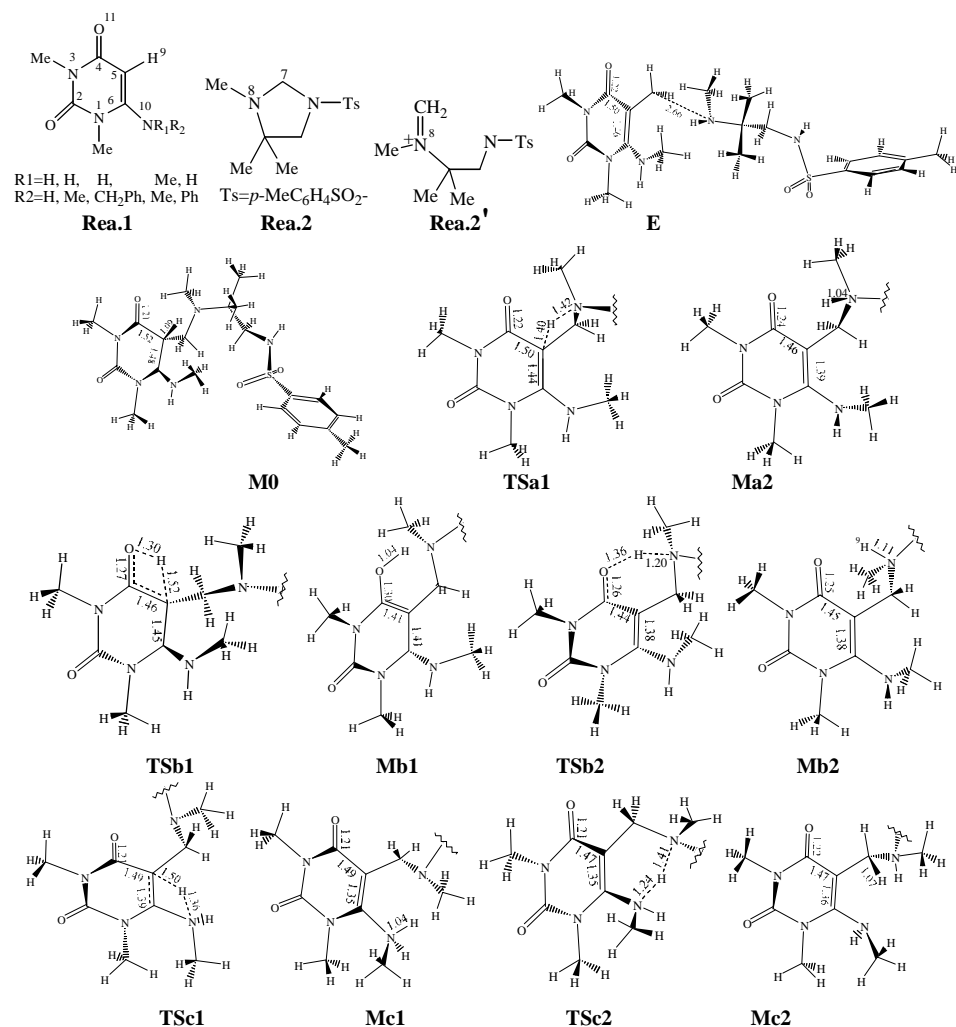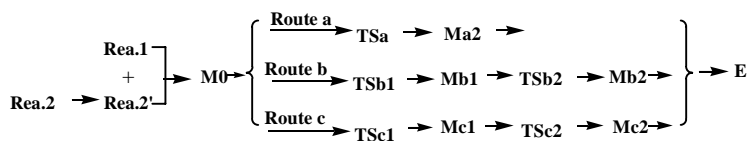

Figure 2   Reaction mechanism of the one-carbon unit transfer of the article

NBO charges of reactants **Rea.1** and **Rea.2'** with B3LYP/6-31G* single-point calculation are –0.430(N-1), 0.857(C-2), -0.497(N-3), 0.614(C-4), -0.132(C-5), 0.456(C-6), N-8(–0.271), O-11(-0.467), N-10(–0.532) respectively. The C-5 atom of **Rea.1** has more negative charge under the influence of the conjugate of the N-10 donor. When the two reactants closing to each other, the nucleophilic attack of **Rea.2'** gives rise to a tetrahedral intermediate **M0** by forming a new covalent bond between C-5 atom of **Rea.1** and C-7 atom of **Rea.2'.** An important feature of this intermediate **M0** is that the N-8, O-11, N-10 atoms separated from C-5 only by one carbon atom have larger negativity, which allows the H9 proton linking to C-5 atom to be readily transferred to one of them. So there exist three pathways for the transfer of H-9. Followed this are the detailed process of the proton migration.

    Route a: In intermediate **M0**, the distance between C-5 and N-8 is very short, so the proton H-9 can migrate from C-5 atom to N-8 directly without any medium. The proton passes through transition state **TSa1** to complete its transfer. The energy variation of the whole process of route **a** can be seen from **Figure 3**. In this route, proton H-9 transfers from C-5 to N-8, passing through 129.49 kJ/mol energy barrier, to form intermediate **M2**, which relative energy is only 3.47 kJ/mol.

    Route b: Proton H-9 transfers from C-5 to O-11 *via* transition state **TSb1** to generate intermediate **Mb1**. After this the H-9 continues to transfer from O-11 to N-8 atom through the six-member cycle formed by the H-9, O-11, C-4, C-5, C-7, N-8 atoms of transition state **TSb2**. The energy variation curve of **Figure 3** shows the proton transfer of this route passes through 227.95 kJ/mol and 5.59 kJ/mol energy barriers respectively. The relative energies of intermediate **Mb1** and the transition state **TSb2** are lower. It is to say, the **Mb1** can exist and it takes up the next reaction step to form intermediate **Mb2** rapidly.

    Route c: Proton H-9 transfers to N-10 atom *via* transition state **TSc1** to reach intermediate **Mc1**. An important feature of **Mc1** is that there are four atoms linking to N-10 atom, which allows one of them transferred. By analyzing the structure data of intermediate **Mc1** we can find H-9 is the best migrating group and N-8 atom is an optimum for the H-9 transferring to. The proton transfer process takes place through a six-member cycle formed by the H-9 and C-5, C-6, N-10, N-8, C-7 atoms. Passing through transition state **TSc2**, the proton H9 reaches to N8 atom to form intermediate **Mc2**. The relative energies of **Figure 3** shows in this route the proton transfer passes through 366.94kJ/mol and 4.18kJ/mol energy barriers respectively.

    Proton transfer can be complete by three different paths. From **Figure 3** we can find that the direct proton transfer (**route a**) is the preferable one. Because the second proton transfer steps of **routes b**, **c** are *via* a six-member cycle mode, both these processes have lower energy barriers. It denotes that despite it is difficult for the first step of proton transfer for **route b**, **c**, once the intermediate **M1** generates, it will reacts rapidly to complete the proton transfer to N8 atom to form intermediate **M2**.

    The proton transfer can complete by three different routes mentioned above. For the need of the proton transfer, intermediate **M2** gained by different proton transfer modes are the bond-rotating isomers. The energy difference of isomers **Ma2**, **Mb2**, **Mc2** is small. The C-7-N-8 cleft can complete the methylene ($^7CH_2$) transfer and lead

to compound **E** which relative energy is only 5.07 kJ/mol.

Figure 3   Relative energy of the proton transfer of the three reaction paths
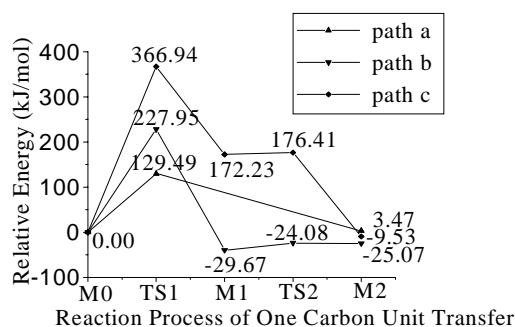
Conclusion

The study shows that the three pathways of the proton transfer are all possible and the direct proton transfer (**route a**) is the preferable one.   For participation of enzyme and other medium, proton migrations in actual reaction may be much easier than that calculated in this paper.   Substitution of N-10 group on C-6 atom makes the **Rea.1** much closing to the dUMP-enzyme duality compound and the whole reaction system models the ternary compound of dUMP-enzyme-substrate.   It can provide valuable reference for further study of such a system.

Acknowledgments

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