Mechanistic Studies

DOI: 10.1002/ange.200703681

A Polar Radical Pair Pathway To Assemble the Pyrimidinone Core of the HIV Integrase Inhibitor Raltegravir Potassium**

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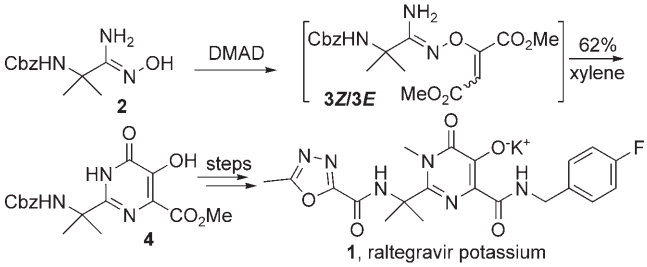
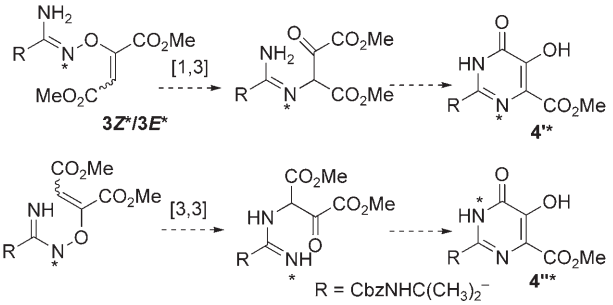
The UN and WHO currently estimate that 40 million people are living with HIV/AIDS worldwide.[1] Combination therapies using reverse transcriptase and protease inhibitors have been highly successful, but the emergence of drug resistance prompts a search for alternative treatments. A rapidly expanding area of HIV/AIDS research targets a third enzyme, integrase, the catalyst responsible for the integration of proviral DNA into the host cell chromosome.[2] Research at Merck has led to the identification of raltegravir potassium (**1**),[3,4] a potent and well-tolerated HIV-1 integrase inhibitor that targets strand transfer, the second of two catalytic cycles mediated by the integrase enzyme.[5]

Raltegravir potassium (**1**) consists of a central hydroxypyrimidinone heterocyclic core that is rapidly assembled by a two-component coupling reaction between amidoxime **2** and dimethyl acetylenedicarboxylate (DMAD) to give **3*Z*/3*E***, followed by a thermal rearrangement to give product **4** (Scheme 1).[6] Amidoxime–DMAD adduct isomers **3*Z*** and **3*E*** are converted to hydroxypyrimidinone **4** at significantly different rates: **3*Z***, the major isomer, reacts at a temperature of 125 °C within 2 h, while **3*E*** only rearranges at an elevated temperature of 135 °C within 5 h. Plausible mechanisms for this reaction involving [1,3] and [3,3] shifts[5a–b] are shown in Scheme 2. ^{15}N-enriched rearrangement precursors **3*Z**/3*E**** (65:35) were synthesized. Thermolysis of **3*Z**/3*E**** resulted in the formation of hydroxypyrimidinone **4′***, in which the ^{15}N label was unexpectedly found to be exclusively at the position *ortho* to the ester substituent. Since this outcome is not consistent with a [3,3]-sigmatropic rearrangement mechanism (**4″***), a formal [1,3] rearrangement must have occurred.



Scheme 1. Synthesis of raltegravir potassium. Cbz = benzyloxycarbonyl.



Scheme 2. Plausible [1,3]- and [3,3]-rearrangement mechanisms.

An in-depth experimental and computational study of the mechanism has now shown that the apparently attractive concerted [3,3]-pericyclic route is eschewed in favor of a direct N–O cleavage to form a polar radical pair (PRP) with a substantial preference for recombination. Quantum mechanical calculations were used to determine how models **6*Z*** and **6*E***, with CO_2Me replacing CBz, undergo transformation to **5**, a model for pyrimidinone **4**. B3LYP density functional theory,[7] as implemented in Gaussian03,[8] was used to compute optimized geometries of intermediates and transition states. Energies were obtained from a calibration involving a smaller computational model where G3-(MP2)B3,[9] a high-accuracy method, and B3LYP energetics were compared (other functionals and methods involving CCSD(T) were also compared, see Supporting Information for details).

As summarized in Scheme 3, the tautomerization of amidoxime adducts **6*Z*/*E*** to **7*Z*/*E*** is endothermic by ≈ 12 kcal mol^{-1}. Acid-base catalysis is required to form **7**, since concerted [1,3]-hydrogen shifts have prohibitively high barriers. Although the activation energies of the allowed [3,3] shifts of **7*Z*/*E*** are 25 kcal mol^{-1} (cf. **TS5**), these rear-

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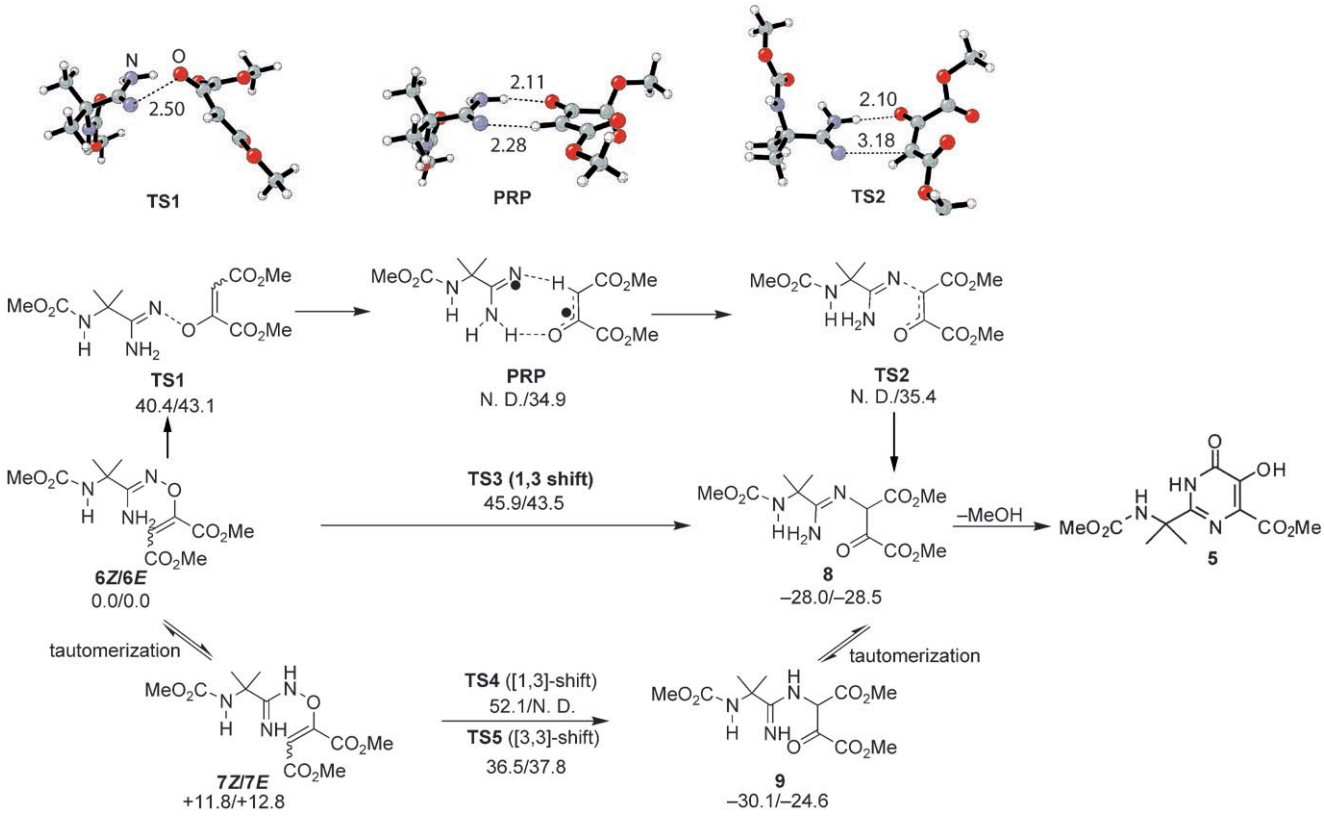
[**] We are grateful to the NSF (CHE-0548209 to K.N.H.) and the NIH (GM36700 to K.N.H.) for financial support. Computational resources from NSF-PACI and the UCLA ATS are appreciated. We thank T. J. Novak for mass spectrometric assistance, and P. Bulger for helpful discussions.

Supporting information for this article (experimental details and B3LYP/6-31+G(d,p) Cartesian coordinates, G3(MP2)B3 results and full reference list) is available on the WWW under http://www.angewandte.org or from the author.



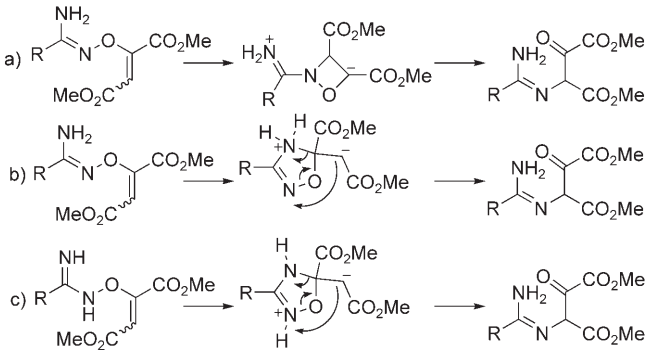
Scheme 3. Corrected B3LYP energies and relevant transition structures and intermediates in the possible rearrangement mechanisms of amidoximes **6*Z*** and **6*E***. All energies [kcal mol^{-1}] are referenced to **6*Z*** and **6*E***. Pictures are for structures in the stepwise rearrangement of **6*E***. (N.D. = not determined.)

rangements do not occur because the tautomeric reactants are not formed in toluene. The activation energies for the [1,3] shifts of the amidino fragments (**TS3, TS4**) of **6*Z*/*E*** and **7*Z*/*E*** are 40–46 kcal mol^{-1}, typical of thermally forbidden [1r,3s]-sigmatropic shifts, and are nearly the same as N–O homolysis activation barriers of **6*Z*/*E***.[10] The bond dissociation energies for the complete separation of the amidino and vinyloxy fragments in **6*Z*/*E*** are $\approx$42 kcal mol^{-1}, several kcal mol^{-1} lower than the barriers for [1,3] shifts.

Transition structures were found for diradical formation (**TS1**) from **6*Z*/*E***. Significantly, **TS1**–**6*Z*** is 3 kcal mol^{-1} lower in energy than **TS1**–**6*E***, in excellent agreement with the experimentally favored reactivity of **3*Z*** in comparison with **3*E***. **PRP** is a tightly hydrogen-bound radical pair due to the polar nature of each radical fragment. It is 8 kcal mol^{-1} more stable than **TS1**–**6*E***, and the transition structure for collapse of the radical pair to product is essentially identical in energy. The geometries of **TS1** and **TS3** are quite similar, being dissociative in nature; these differ most significantly in the orientation of the migrating amidino moiety in relation to the vinoxyl moiety (see Supporting Information for further details). Given the significant error bars associated with the computed barriers, the calculations do not definitively differentiate between reaction via **TS3** and radical pair formation for one of these substrates (**6*E***). However, the relative reactivities computed for **6*Z*/6*E***, which are similar to the experimental results, and the intramolecular trapping of a radical pair (see below) are consistent only with the radical pair mechanism.

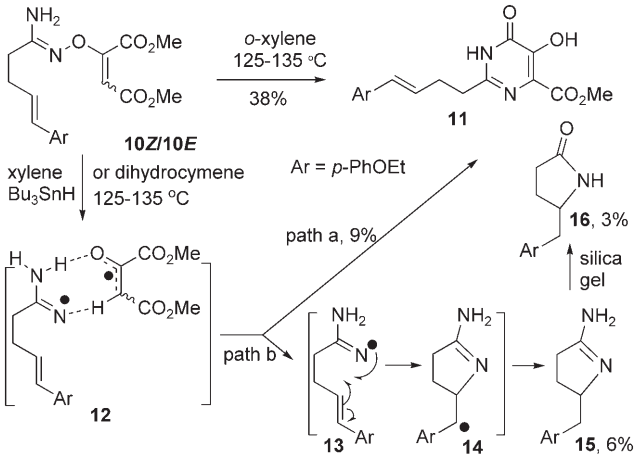
Alternative stepwise mechanisms, involving the formation of 1,2-oxazetidine[11] or 1,2,4-oxadiazole intermediates followed by rearrangement, can potentially lead to [1,3]-rearrangement products as shown in Scheme 4. These nucleophilic mechanisms were found to be significantly disfavored compared to the polar radical pair pathway (see Supporting Information for details).

An experimental probe for the presence of radical intermediates resulting from thermally induced homolytic cleavage of the N–O bond was derived by incorporating an



Scheme 4. a)–c) Plausible stepwise nucleophilic mechanisms.

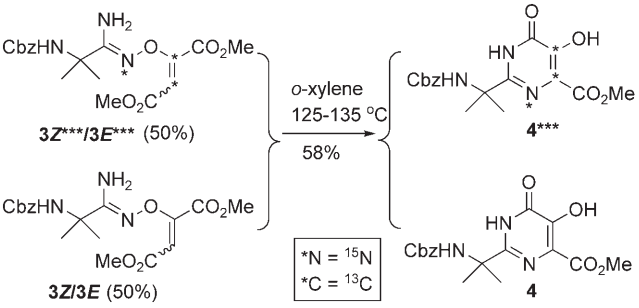
alkene into a model substrate to act as a potential intramolecular radical trap (Scheme 5).[12] When vinyl amidoxime mixture **10*Z*/10*E*** (6:1) was heated in *o*-xylene at 125 °C in the presence of a hydrogen atom source, **11** was formed along



Scheme 5. Trapping of proposed radical intermediate.

with **16**, the product of hydrolysis of **15**. This supports the presence of the radical pair resulting from N–O cleavage in the reaction mixture. Reaction of the corresponding reactant with the styrenyl group reduced gave a reduced product analogous to **11** in 66 % yield.

Evidence consistent with the polar radical pair mechanism was provided by a crossover experiment (Scheme 6).[12] A 1:1 mixture of labeled **3*Z****/**3*E**** and unlabeled **3*Z*/3*E*** was heated in *o*-xylene at 125 °C for 2 h and at 135 °C for 4 h to



Scheme 6. ^{15}N- and ^{13}C-labeled vinyloxyamidine rearrangement.

afford hydroxypyrimidinones **4*** and **4**, which were identified by high-resolution mass spectrometry. No crossover between the labeled and unlabeled fragments was observed. This result reinforces the computational results discussed previously: **PRP** (Scheme 3) recombines to give product within the solvent cage.

Experimental and computational investigations have provided evidence for the intermediacy of a novel polar radical-pair in the assembly of the hydroxypyrimidinone core of the anti-HIV drug raltegravir potassium. It is likely that these intermediates, which are held together by strong electrostatic attractions, rapidly collapse leading to formation of the heterocyclic product.

Received: August 11, 2007
Revised: February 26, 2008
Published online: April 24, 2008

Keywords: density functional calculations · heterocycles · inhibitors · pericyclic reaction · radicals

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