Origin of Increased Reactivity in Rhenium-Mediated Cycloadditions of Tetrazines

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ABSTRACT: Pyridyl tetrazines coordinated to metals like rhenium have been shown to be more reactive in [4+2] cycloadditions than their uncomplexed counterparts. Using density functional theory (DFT) calculations, and in particular distortion/interaction and energy decomposition analysis, we analyze the factors that contribute to this increase in reactivity. The reaction of the complexed tetrazine has a more favorable interaction energy compared to the uncomplexed tetrazine, and the main contributor to this favorable interaction energy is an increase in orbital interactions through a lowering of the LUMO in the complexed tetrazine. Additionally, the high regioselectivity of the reaction is shown to be due to a greater degree of charge stabilization in the transition state leading to the major product. This study suggests that the energy of the FMOs is a good indicator of reactivity in these reactions, and the enhanced [4+2] reactivity upon complexation adds another tool to the toolbox of click reactions.

Introduction

The reaction between 1,2,4,5-tetrazines and alkenes was first described in 1959 by Carboni and Lindsey. These azines react with olefins in an inverse electron-demand Diels–Alder cycloaddition followed by a cycloreversion with loss of dinitrogen (Figure 1a). The formed dihydropyridazines can then further tautomerize or be oxidized to the corresponding pyridazine. In 1990, Sauer showed that tetrazines react very rapidly with strained alkenes, such as cyclopropanes and trans-cyclooctenes. This observation led to the introduction of tetrazine ligation as a biocompatible click reaction in 2008. This ligation has since been employed broadly in applications ranging from radiolabeling to material science. A targeted drug delivery system based on this cycloaddition is currently in phase 1 clinical studies.

Commonly used tetrazines include mono- and di-substituted derivatives with alkyl or aryl groups. Due to the inverse electron-demand nature of the initial, rate limiting [4+2] cycloaddition, electron withdrawing substituents increase reactivity. 2-Pyridyl and 2-pyrimidyl substituted tetrazine derivatives are among the most reactive dienes that can be used for these reactions.

2-Pyridyl-tetrazines can be used as ligands for metals, as analogs to 2,2′-bipyridine ligands. Recently, Ringenberg and coworkers investigated the click reactivity of a 2-pyridyl-tetrazine ligand in a rhenium complex towards vinylferrocene, styrene, and trans-cyclooctene (Figure 1b). This reaction allows for efficient labeling with the metal complex, opening possible applications in the fields of chemical biology and medicine.Interestingly, Ringenberg et al. describe an increase in rate constants of two orders of magnitude compared to the tetrazine itself. The same behavior was observed by the same group for a ruthenium complex. This acceleration makes this system particularly interesting since it would allow for labeling at ultra-low concentrations. However, the origin of this increased reactivity could not be determined.

Here we perform a computational analysis using distortion/interaction and energy decomposition analysis to unravel the underlying mechanisms that lead to the observed increase in reactivity.

Figure 1. [4+2] cycloadditions of tetrazine 1 (Tz) and rhenium-coordinated tetrazine 5 (Re-Tz) with styrene (2, St) leads to the formation of intermediates 3 and 6, respectively. This is followed by a retro-[4+2] cycloaddition to form dihydropyridazines 4 and 7. The regioisomers in which the Ph group is ortho to the pyridyl substituent are shown. Subsequent oxidation of products 4 and 7 can form pyridazines.

Computational methods

Density functional theory (DFT) calculations were performed with Gaussian 16 RevA.03. For each structure, all possible conformers were considered. Geometry optimizations were performed with the B3LYP functional, which was shown to closely match experimental values, augmented with Grimme’s D3 empirical dispersion term, and the SDD basis set for Re and 6-311+G(d,p) for all other atoms. Dichloromethane solvation was modeled using
the SMD solvation model. Frequency calculations confirmed the optimized structures as minima (zero imaginary frequencies) or transition state structures (one imaginary frequency) on the potential energy surface. A quasi-harmonic correction was applied using the GoodVibes program. Orbital energies were calculated at the same level of theory in the gas phase.

Distortion/interaction\textsuperscript{38} and energy decomposition analysis\textsuperscript{39} were performed in ADF (2019.304)\textsuperscript{40} with PyFrag 2019\textsuperscript{41} using B3LYP-D3/TZ2P in the gas phase on structures that were optimized with the B3LYP-D3/6-311G(d,p)/SDD-SMD(DCM) level of theory.

**Results and Discussion**

The reactivity of Tz (1) was first compared to that of Re-Tz (5) in the reaction with St (2). In these reactions, the formation of two regioisomers is possible: one in which the phenyl group ends up ortho to the pyridyl ring in the final dihydropyridazine (Figure 2a), and one in which these groups are meta to each other (Figure 2b). Experimentally, the ortho products are the major products observed. The energy barrier for the reaction leading to ortho intermediate 3 was calculated to be 23.5 kcal/mol (Figure 2a, TS-1a). Coordination of Re reduced this barrier to 21.4 kcal/mol (TS-2a) for the formation of meta intermediate 6. This calculated activation barrier is in excellent agreement with experimental results by Ringenberg and coworkers, whose kinetic experiments correspond to a $\Delta G^2$ value of 22.0 kcal/mol.

**Figure 2.** Transition states for the [4+2] cycloaddition to form the ortho products (a) and the meta products (b).

In order to determine what causes the lowering of the energy barrier upon coordination of Re, we performed distortion/interaction and energy decomposition analysis (Figure 3). For this analysis, we compared the geometries along the intrinsic reaction coordinate (IRC) in which the shorter bond-forming distance was 1.95 Å. In addition, we performed an analogous analysis of the two structures at the transition states, which can be found in the Supporting Information. At the so-called consistent geometries of 1.95 Å, the electronic energy was lower for the reaction of Re-Tz + St by 6.1 kcal/mol compared to that of Tz + St. This difference in electronic energy was decomposed into the interaction ($\Delta E_{int}$) and distortion ($\Delta E_{dist}$) components, both of which were decomposed further (Figure 3). Interaction energy was overall favorable for the Re-Tz reaction, by 10.0 kcal/mol compared to the Tz reaction. The greatest contribution to this was orbital interactions ($\Delta E_{orbi}$), which were stronger in the Re-Tz reaction by 7.2 kcal/mol. Electrostatic interactions ($\Delta V_{elstat}$) were also stronger in the reaction with Re-Tz, by 2.2 kcal/mol, as were dispersive interactions ($\Delta E_{disp}$), by 1.9 kcal/mol. Conversely, Pauli repulsive interactions were less unfavorable in the reaction of Tz than Re-Tz, by 1.3 kcal/mol.

**Figure 3.** Distortion/interaction and energy decomposition analysis of TS-1a and TS-2a. Values are shown for TS-2a relative to TS-1a.

Distortion was more unfavorable in the case of the Re-Tz reaction, by 3.9 kcal/mol. Distortion energies were calculated for the tetrazine/Re-tetrazine and styrene components, and the Re-Tz component was shown to be the main contributor to the difference in distortion energies between the two reactions, by 3.6 kcal/mol more than the Tz component. Distortion in the St component was similar in both reactions, with a difference of only 0.2 kcal/mol.

As suggested by energy decomposition of the interaction energies, we performed further analysis of the orbital interactions at play in this reaction. The energies and visualizations of the relevant filled and empty molecular orbitals of the styrene and tetrazine components for the Tz and Re-Tz structures at the consistent geometry are depicted in Figure 4. The analogous data for the starting materials and transition states is included in the Supporting Information. Since the reaction of tetrazines with dienophiles is an inverse electron-demand Diels–Alder reaction, the orbitals that contribute most to the reactivity are the LUMO of the diene and the HOMO of the dienophile. This can be seen by the HOMO/LUMO gap between the styrene and Tz/Re-Tz: 2.8 and 1.9 eV, respectively. In contrast, the normal-electron demand orbital gaps are 5.9 and 7.0 eV for Tz and Re-Tz, respectively. We thus conclude that the enhanced orbital interactions in the Re-Tz reaction, which is ultimately the major contributor to enhanced reactivity compared to the Tz reaction, originates from a lowered LUMO in the coordinated tetrazine.
We subsequently analyzed the regioselectivity of this reaction. The reaction is highly regioselective, with formation of the ortho product favored over formation of the meta product. Transition states for formation of the meta products are shown in Figure 2b. For the reaction of Tz with styrene, the ortho TS is lower in energy than the meta TS by 4.6 kcal/mol. For the reaction of Re-Tz with styrene, this difference is 4.7 kcal/mol. Thus, both systems are highly regioselective. This is unusual for bioorthogonal tetrazine ligation, where poor regioselectivity is often observed. However, the high degree of regioselectivity in this reaction is not surprising, given the asymmetric electronic and steric nature of both the dienes and dienophile; furthermore, this selectivity has been reported before.

The transition states leading to the favored ortho products are more asynchronous than the transition states leading to the meta products. In the ortho transition state, the more nucleophilic terminal carbon of styrene can interact strongly with the less sterically hindered tetrazine carbon, leading to a short carbon-carbon distance of about 1.96 Å. In addition, charges built up during this interaction can be well-stabilized by the aryl substituent at the ipso carbon, leading to the highly asynchronous, and energetically favored approach. In the transition state leading to the meta product, the terminal carbon of the styrene also participates in the initially-forming bond. In this case, charge buildup on the unsubstituted tetrazine carbon could not be stabilized in a potential asynchronous transition state. This thus leads to a more synchronous transition state that is higher in energy.

For the reaction of Re-Tz with St, we also studied the reaction profiles for the entire reaction sequence for formation of the ortho and meta products; these energy profiles are shown in Figure 5. It should be noted that in both of these pathways, the preferred addition of styrene is from the face that contains the Cl ligand—this mode of addition is favored over addition to the CO face by 0.8 kcal/mol. Both pathways shown in Figure 5 correspond to addition to the Cl face. As discussed previously, the difference in energy barriers between TS-2a and TS-2b is 4.7 kcal/mol. These transition states lead to the formation of [4+2] cycloaddition products Int-1a and Int-1b, which are both at 6.2 kcal/mol. Subsequent retro-[4+2] cycloadditions have low energy barriers, at 9.2 and 10.6 kcal/mol relative to starting materials SM, and are highly exergonic, leading to low-energy products at -52.9 and -53.1 kcal/mol for Int-2a and Int-2b. Int-2a then preferentially undergoes isomerization to form a double bond between the two distal carbons of the dihydropyridazine (Int-3a). Int-3a, in which the Ph group is on the same face as the Cl ligand, is 0.2 kcal/mol lower in energy than the epimer in which the Ph group is on the same face as the CO ligand. It is likely that these two structures can reach a thermodynamic equilibrium at this stage through alkene isomerization.

We also studied the possibility of a retro-[4+2] cycloaddition occurring with the N₂ that is coordinated to the Re center. For the ortho pathway, the transition state for this reaction was at 23.1 kcal/mol, which is significantly higher than the 9.2 kcal/mol that is observed for recycloaddition to expel the distal N₂. This is reflected in the greater stability and entropy of product Int-2a compared to that which would be formed in the case of the other retro-[4+2] pathway.

Figure 4. Reacting orbitals and orbital energies of fragments at the consistent geometry where the shorter forming bond is 1.95 Å. Reactions of Re-Tz (left) and Tz (right) with St are shown.
**Figure 5.** Free energy diagram for formation of ortho and meta dihydropyridazine products in the reaction of Re-Tz with St.

**Conclusion**

With the considerable increase in Diels–Alder reactivity, the rhenium-mediated tetrazine ligation adds another interesting tool to the click chemistry toolbox. Using energy decomposition methods, we show that this higher reactivity is mainly due to a lowered LUMO of the tetrazine and therefore increased orbital interactions. These results also suggest that FMO interactions are a good indicator of cycloaddition reactivity of such tetrazine ligands in metal complexes. The high regioselectivity of the reaction with styrene is explained through a better stabilization of charges at the ipso-carbon of the mono-substituted tetrazine.

**ASSOCIATED CONTENT**

Supporting Information

Energies and geometries of computed structures, orbital energies at transition state geometry and for starting materials, EDA at transition states (PDF)

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**Notes**

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