Tetrazine metallation boosts rate and regioselectivity of inverse electron demand Diels–Alder (iEDDA) addition of dienophiles†

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Reported herein is the coordination of rhenium complexes to tetrazine ligand in [ReCl(CO)₃(TzPy)] [1] (TzPy = 3-(2-pyridyl)-1,2,4,5-tetrazine) and the rates of addition of different dienophiles to the tetrazine. Tetrazine coordination lowers the ΔS‡ contribution to ΔGo for iEDDA addition.

The inverse electron demand Diels–Alder (iEDDA) reaction between 1,2,4,5-tetrazines and olefins is a substrate controlled click-reaction, and does not require the addition of a catalyst (CuAAC) or light (thiol–ene). The modification of the 1,4-positions on the tetrazine can be synthetically arduous but rational design of the tetrazine diene and dienophile has resulted in very fast iEDDA reaction rates, where rates > 10⁶ M s⁻¹ have been reported. These factors have made the iEDDA addition a useful reaction in several applications, chiefly among them in biological labelling experiments. The iEDDA addition is, however, not regioselective and it produces a mixture of regioisomers, e.g., 1,4- and 1,5-isomers (Scheme 1).

Transition metal(s) are known to coordinate tetrazines and the tetrazine moiety is more electrophilic than the aquo ligand. Additionally, tricarbonylrhenium(I) complexes with pyridine donor ligands have found uses as electrocatalysts to CO₂ reduction.

Herein we report the synthesis and rate of addition of three dienophiles to the metallotetrazine [ReCl(CO)₃(TzPy)] [1], see ESI, for synthetic and kinetic details. The ReCl(CO)₃ moiety was chosen to coordinate TzPy because tricarbonylrhenium(i) complexes with pyridine donor ligands have found uses as imaging reagents in cells and they have also shown cytotoxic activity for cancer treatment.

The tetrazine moiety in [1] can add dienophiles and the rate of addition of vinylferrocene (ViFc), styrene (Ci), and trans-cyclooctene (TCO) to [1] were measured and are reported in Table 1. Different dienophiles were also tested for their ability to add to the tetrazine.

† Electronic supplementary information (ESI) available: Containing experimental section, DFT calculations, crystallographic tables, and molecule coordinates determined by DFT description. CCDC 1991846–1991849 and 2008845. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d0cc03805a

Scheme 1 Addition of dienophile to [1].
to add to [1], such as vinyl and allyl functionality (e.g. vinyl-trimethoxysilane and allyltrimethylsilane), a bulky olefin (e.g. quinine) and phenylacetylene to [1] was also demonstrated (see ESI†) indicating coordination of TzPy does not inhibit addition of electron rich olefins. The addition of Ci and TCO to [1], respectively, showed enhanced rates compared to the reported rates between the symmetric 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (Py2Tz) and the self-similar dieneophiles. 7,17,18 The work herein was performed in 1,2-dichloroethane (DCE) due to the poor solubility of [1] in H2O, although [1] does show moderate solubility in MeOH, the comparison is used to demonstrate the enhanced rate compared to the reported k2 values.

The rate of the addition of ViFc, Ci, or TCO to [1] was measured using time-resolved variable-temperature UV vis spectroscopy in C2H4Cl2, respectively and the rates and thermodynamic values are reported in Table 1. The rate for [1] + ViFc k2 = 2.80 ± 0.1 M⁻¹ s⁻¹ at 22 °C was 160 times faster than the control reaction TzPy + ViFc k2 = 0.0180 M⁻¹ s⁻¹ at 22 °C. The Eyring analysis19 of [1] + ViFc found ΔH‡ = 22.6 kJ mol⁻¹ and ΔS‡ = −150 J mol⁻¹ K⁻¹, with ΔG‡(25 °C) = 68 kJ mol⁻¹. The Eyring analysis of TzPy + ViFc showed a small increase in the ΔH‡ = 27 kJ mol⁻¹, however the ΔS‡ = −192 J mol⁻¹ K⁻¹ contributed more to the ΔG‡(25 °C) = 84 kJ mol⁻¹. Coordination of TzPy lowers the ΔΔG‡ = 16 kJ, and the ΔΔG‡(calc.) = 14 kJ was in good agreement with experimental value (see ESI†). The discrepancy between DFT and experimental values can be attributed to solvent effects.

TzPy shows resonance stabilization of δ⁺ at the C4 of the tetrazine, and coordination of TzPy enhances this resonance structure due to back-bonding of the δ⁻ on the ReCl(CO)3 moiety (Fig. 2). The addition of ViFc to TzPy shows a larger contribution of ΔS‡ to the transition state ΔG‡. One rational is that the molecular structure of TzPy (see Fig. S27, ESI†) is planar while the TzPy in [1] (Fig. 1) tilts towards the Cl-ligand. This distortion may approximate the dien-dieneophile transition state (Scheme 2), another contribution could be that back-bonding affords a weakening of the double bonds in the tetrazine. Additionally, coordination of TzPy restricts its motion, which may also contribute to a lower transition state energy. The effect as to why coordination of the tetrazine lowers the ΔG‡ is currently under investigation.

ViFc is an electron rich dienophile (δ⁻ on C-carbon, fulvene resonance with δ⁺ on Fe atom),20 therefore the addition of the unactivated styrene (Ci) to [1] was also studied. The rate of addition of Ci to [1], k2 = 6.03 ± 0.02 × 10⁻² M⁻¹ s⁻¹ was nearly 20 times faster than the addition of Ci to Py2Tz (k2 = 3.0 × 10⁻³ M⁻¹ s⁻¹).18 The Eyring analysis of the addition of Ci to [1] found a larger ΔH‡ = 55 kJ mol⁻¹ was more than double the ΔH‡ for the addition of ViFc to [1]. The lower contribution of ΔS‡ = −125 J mol⁻¹ K⁻¹ to the transition state (Table 1) can be attributed to the size of the Ph versus Fe. The rate of the addition of TCO to [1] was k2 = 4.06 ± 0.52 × 10⁻⁵ M⁻¹ s⁻¹ which is 200 time faster than the addition of TCO to Py2Tz.7 The ΔH‡ = 26 kJ mol⁻¹ is on the order of the addition of ViFc to [1], however there is a significantly lower contribution of the ΔS‡ = −50 J mol⁻¹ K⁻¹ to ΔG‡ = 41 kJ mol⁻¹, as should be expected with the strained TCO dieneophile. The difference in rates between the addition of ViFc and TCO respectively to [1], shows

### Table 1 2nd order rate constants of iEDDA and thermodynamic values

<table>
<thead>
<tr>
<th>Reaction</th>
<th>k2/M⁻¹ s⁻¹</th>
<th>ΔH‡/kJ mol⁻¹</th>
<th>ΔS‡/J mol⁻¹ K⁻¹</th>
<th>ΔG‡/DFT/kJ mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1] + ViFc</td>
<td>2.80 ± 0.1</td>
<td>23</td>
<td>−150</td>
<td>68 (40)</td>
</tr>
<tr>
<td>TzPy + ViFc</td>
<td>1.80 × 10⁻²</td>
<td>27</td>
<td>−192</td>
<td>84 (54)</td>
</tr>
<tr>
<td>[1] + Ci</td>
<td>6.03 ± 0.02×10⁻²</td>
<td>55</td>
<td>−125</td>
<td>92 (62)</td>
</tr>
<tr>
<td>Py2Tz + Ci</td>
<td>3.0 ± 0.3×10⁻³</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[1] + TCO</td>
<td>4.06 ± 0.52×10⁻⁷</td>
<td>26</td>
<td>−50</td>
<td>41 (28)</td>
</tr>
<tr>
<td>Py2Tz + TCO</td>
<td>2.0 × 10⁻⁴</td>
<td>—</td>
<td>—</td>
<td>(37°)</td>
</tr>
</tbody>
</table>

a Rate at 25 °C in C2H4Cl2. b At 25 °C. c Gas phase reaction TPSS(def2-TZVP/J level of theory (see ESI, for full details). d MeOH ref. 18. e 9:1 MeOH/H2O ref. 7 and C2H4Cl2 see ESI figure S. f DFT M06L/6(311)+G(d,p) level ref. 7.

Fig. 1 Molecular structure of [1] determined crystallographically, co-crystallized CH2Cl2 was omitted for clarity.

Scheme 2 Reaction mechanism of olefin with tetrazine.

Fig. 2 Lowest unoccupied molecular orbital (LUMO, yellow/blue) and highest occupied MO (HOMO, red/green) of [1], isosurface at 0.02, TPSS basis set at def2-TZVP/J level of theory (see ESI† for full details).
that coordination lowers the entropic barrier ($\Delta S^\ddagger$) as the enthalpic barriers ($\Delta H^\ddagger$) are nearly isoenergetic (Table 1).

The addition of dienophiles to the tetrazine diene is generally unselective (Scheme 2), and the 1,3-protoprototropic isomerization is rapid which prevents the 4,5-dihydropyridazine (Scheme 2, intermediate 2) from being isolated.15 The Alder–Stein principle (the relative stereochemistry of dienes and dienophiles is conserved), and Alder’s endo-rule (the endo-adduct is the kinetically preferred product), apply in the iEDDA addition, but stereochemical information is often lost due to rapid isomerization and rearomatization of the 1,4-dhp to the 1,2-pyrazine, especially under aerobic conditions (Scheme 2).

The addition of ViFc, TCO, or Ci to [1] at room temperature generated the species [1Fc], [1TCO], and [1Ci] and crystals were obtained directly from the reaction mixtures where the molecular structures were determined crystallographically. The molecular structure of [1Fc] showed the 4,5-dhp isomer, while [1TCO] and [1Ci] were found as the 1,4-dhp isomer (Fig. 3). DFT analysis for the 1,3-protoprotropic isomerization for all three complexes showed the 1,4-dhp was energetically favorable, however, the 1,4-dhp isomer of [1Fc] was only observed as a minor product in the $^1$H-NMR (see ESI†). DFT analysis found rearomatisation for both [1Fc] and [1Ci] were both endergonic (see ESI†).

The 4,5-dhp isomer of [1Fc] is, to the best of our knowledge, the first molecular structure of this intermediate. Based on the structure of [1Fc] the endo approach of the dienophile to [1] at CO-face of the molecule (Chart 1, Fig. 1) is favorable. The $^1$H NMR21 of the reaction product [1Fc] showed a mixture of two major 4,5-dhp products in a ratio of 80:12, and a minor product (~8%) that appears to be the 1,4-dhp isomer due to slow 1,3-protoprotropic isomerization. The major 4,5-dhp product is the same found in the crystal state, however, based purely on these data the dienophile’s exo approach at the Cl-face cannot be differentiated from the endo approach. However, DFT calculations found that exo approach of any dienophile to [1] at either Cl- or CO-face produced unrealistic activation energy (>50 kcal mol$^{-1}$) and was therefore not considered. The endo approach of ViFc to [1] was found to be favorable at the CO-face versus the Cl-face of [1] based on DFT calculations (Fig. S5, ESI†).

The crystal structure of [1TCO] and [1Ci] revealed that the 1,3-protoprotropic isomerization had occurred. The $^1$H NMR of the reaction mixture for [1Ci] confirmed the 1,4-dhp isomer in a ratio of 46:46 with 8% other products (Fig. S22, ESI†). According to DFT analysis the endo approach of Ci to [1] is nearly isoenergetic at both the faces, however, only the product of the endo CO-face addition was found in the crystal state (Fig. 3). Analysis of the reaction mixture from [1] + TCO also showed two similar products in a ratio of 71:29, and the 1,4-dhp isomer was assigned based on $^1$H NMR analysis, the molecular structure, and DFT calculations (Fig. S25, ESI†). These data show that the endo approach of dienophiles is favored to occur and only the 1,4-additon and not the 1,5-addition are observed.

We report the increased rate of the iEDDA addition of three dienophiles to the metallotetrazine [1]. The combination of the strong endo effect and the back-bonding from the tetrazine to the metal is thought to increase the rate of this reaction. Coordination of the rhenium(s) moiety to the tetrazine lowers the $\Delta S^\ddagger$, while the nature of the dienophile shows the larger influence on the contribution of $\Delta H^\ddagger$ to the transition state $\Delta G^\ddagger$. The metallotetrazine [1] also allows for the facial approach of the dienophile to be prejudiced, albeit the Cl- and CO-face of [1] only imparts a small influence. Currently we are exploring this
complex for its biological activity, and for immobilization of the complex onto solid supports to generate new electrocatalysts.

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Conflicts of interest

There are no conflicts to declare.

Notes and references