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A computational analysis of the interaction between flavin and thiol(ate) groups. Implications for flavoenzyme catalysis

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A computational analysis of the interaction between flavin and thiol(ate) groups. Implications for flavoenzyme catalysis

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A computational analysis of the interaction between flavin and thiol(ate) groups has been performed using three density functional theory methods (B3LYP, MPW1PW91, and MPWB1K). Oxidized flavin forms a charge-transfer complex with the thiolate anion prior to the generation of a covalent-adduct at the C4a position of the isoalloxazine ring. The computed reactions require a concerted proton transfer to the N5 position of the flavin and show relatively small barriers (<13 kcal/mol). Using different model systems, it was demonstrated that this proton can be delivered to the flavin via a protonated cysteine, or from the carboxyl moiety of glutamic and aspartic acids by utilizing a series of intervening water molecules.

Keywords: charge-transfer; flavin; thiolate; DFT; C4a-adduct

1. Introduction

The interaction between a sulfhydryl group and an electron-deficient flavin ring is a crucial aspect of the mechanism of action of a number of flavoenzymes. Examples include members of the pyridine nucleotide disulfide oxidoreductase family (*1, 2*), a range of sulfhydryl oxidases (*3–5*), phosphopantothenoylcysteine decarboxylase (*6*), and a series of blue light photoreceptors (*7–9*). In many enzymatic cases, these interactions involve transitory charge-transfer (CT) complexes that may subsequently react via the attack of a cysteinyl-sulfur atom on the C4a position of the isoalloxazine ring (*1–5*). This flavin-adduct frequently represents a key step in the redox catalysis, and is often resolved by the attack from a second cysteine to generate a disulfide bond with the transfer of 2-reducing equivalents to the flavin ring. Such is the case in the flavin-linked sulfhydryl oxidases (*3–5*). Here, adduct formation bridges the oxidation of dithiol substrates with the reduction of molecular oxygen to give hydrogen peroxide: $2\,RSH+O_2 \rightarrow RS–SR+H_2O_2$ (*3–5*). Herein we describe a computational analysis of the interaction between flavin and thiol(ate) groups to provide insight to guide further mechanistic studies of this important class of flavin-linked catalysts.

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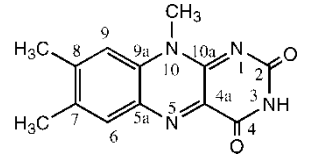
2. Computational details

Quantum chemistry calculations were carried out using the Gaussian98 and Gaussian03 program system (*10a,b*) utilizing gradient geometry optimization (*11*). All geometries were optimized using the B3LYP functional (*12a–d*), hybrid meta MPWB1K (*12e*), and MPW1PW91 (*12f*) with 6-31G(d) and 6-311+G(d,p) basis sets (*13*), denoted here as smaller basis set (SB) and larger basis set (LB). Vibrational frequency calculations were performed to characterize the stationary points as either minima or transition structures (first-order saddle point). Vertical energies of the singlet excited state transitions have been calculated using the time-dependent DFT method (TD DFT) (*14a–c*). CT interactions were estimated based upon the relative energy of the complex *vs.* isolated partners. The partial charges were calculated using full Natural Bond Orbital analysis (NBO method implemented in Gaussian03) (*15a*). Corrections for solvation and optimizations in dielectric medium with the toluene dielectric constant ($\varepsilon = 2.379$) were made using the CPCM model (*15b,c*) implemented in Gaussian03. For solvent calculations, TD calculations, and NBO analysis, we employed the same method as it was used in the optimization. The model systems in this study are designed using a fragment of the active site of the X-ray structure for the sulfhydryl oxidase Erv2p (*16*) (accession code 1JR8 in the Protein Data Bank). To model FAD, we used a tricyclic oxidized flavin molecule (Fl_{ox}).

$Fl_{ox}=$ 

Most of calculations were performed using GridChem computational resources and services, Computational Chemistry Grid (*17*) (www.gridchem.org).

3. Results and discussion

In an earlier computational study on the role of oxidized flavin, Fl_{ox}, in the catalytic activity of acyl-CoA dehydrogenase (*18a*), we demonstrated that the developing enolate can be subjected to a strong CT interaction with the flavin. This type of complex, thioenolate/Fl_{ox}, was characterized by an intense CT absorption band assigned to $\pi \rightarrow \pi^*$ transfer from the highest occupied molecular orbital of thioenolate to the lowest virtual orbital of flavin. We also noted that the energy of this CT vertical (Franck–Condon) excitation depends strongly on the contact distance, but appears less sensitive to the polarity of the microenvironment. In another study (*18b*), it was found that the flavin N(5) H-bond to a SER– or THR–OH function appears to have a strong influence on the pK (αC–H ionisation) of the CoA ligand since it induces an increase by $\approx$1 pK unit and on the pK of the active center base in the uncomplexed state. This generally indicates that H-bonding to the FAD modulates its electron-accepting ability, and thus, may also strongly influence the energy, contact distance, and absorption characteristics of CT complexes. In this study, we consider another biochemically-important CT system, thiolate/Fl_{ox}: a key intermediate in the transfer of reducing equivalents in flavoproteins of the pyridine nucleotide disulfide oxidoreductase and the sulfhydryl oxidase families [see Introduction].

First, the simplest model CH_3S^-/Fl_{ox} (Figure 1) was optimized without any constraints. Two minima (Figures 1A and 1B) were found for this complex differing by the orientation of the thiolate and the relative stability. Complex A is 2.9 kcal/mol lower in energy than complex B, which is, probably, due to a stronger H-bonding between hydrogen of methyl group in thiolate

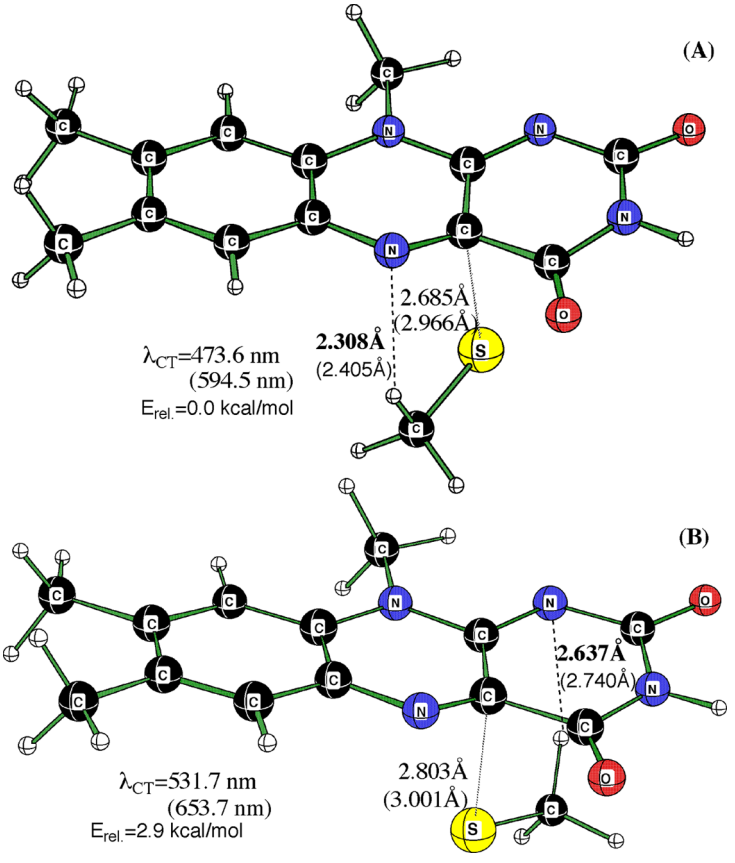


Figure 1. CH_3S^-/Fl_{ox} complexes with different orientation of thiolate (A, B) optimized at the B3LYP/LB [LB = 6 − 311 + G(d,p)] level of theory. Wavelength, λ_{CT}, corresponds to the lowest vertical strongly allowed singlet–singlet transition. Numbers in parentheses correspond optimizations in dielectric medium with the toluene dielectric constant ($\varepsilon = 2.379$).

and N atom of flavin. Geometry A is more similar to the presumed orientation in the sulfhydryl oxidase Erv2p (*16*). Here, the sulfur atom is 2.685 Å away from the C4a atom of the flavin. Unfortunately, we cannot compare the results directly with the published Erv2p X-ray structure (*16*), because it was determined using the oxidized protein containing a disulfide bond (comprising a loop between CYS54 and CYS57) and adjacent to the flavin. The methylthiolate approaches the flavin in Complex B with a different orientation, leading to a longer S···C4a distance of 2.803 Å. Coincidently, Complex B in gas phase has a CT absorption maximum that is much closer to the typical experimentally-observed transitions in the 500–600 nm region (*19a,b*). B3LYP optimizations in solvent (toluene) result in the elongation of S···C4a distances in both complexes to 2.966 Å (A) and 3.001 Å (B). In this case, the Complex A has a CT absorption, 594.5 nm, which satisfies the experimental observation.

In this study, in addition to B3LYP calculations[1], CH_3S^-/Fl_{ox}Complex (A) also has been re-optimized using MPW1PW91 and MPWB1K methods (Table 1). Both methods are recommended for investigation of non-covalent interactions (*12e–g*). For current models, these mPW exchange functional-based methods produce tighter complexes with shorter S···C4a contact distances and, thus, shorter absorption wavelengths.

The use of LB does not affect the results significantly in contrast to previously studied thioenolate/Fl_{ox} complexes (*18a*). We suggest that this is caused by larger CT interaction in case of

Table 1. Equilibrium S···C4a contact distances (d, Å), lowest vertical strongly allowed singlet-singlet transitions (λ_{CT}, nm), and charge transferred to flavin (Q_{Fl},e) calculated for the Fl_{ox}/SMe^- complexes.

CT complex	Method	d, Å	λ_{CT}, nm	f_{osc}	Q_{Fl}, e
Fl_{ox}/SMe^- (Figure 1A)	B3LYP/SB	2.694	451.11		–0.52
Fl_{ox}/SMe^-(Figure 1A)	B3LYP/LB	2.685	473.64	0.24	–0.55
Fl_{ox}/SMe^- (similar to geometry in Figure 1A)	MPW1PW91/LB	2.494	423.92	0.26	–0.63
Fl_{ox}/SMe^-(similar to geometry in Figure 1A)	MPWB1K/SB	2.464	377.42	0.35	–0.57
Fl_{ox}/SMe^- (Figure 1B)	B3LYP/SB	2.826			
Fl_{ox}/SMe^- (Figure 1B)	B3LYP/LB	2.803	531.72	0.21	

thiolate anion, which also is indicated by shorter contact distances. In this study, for CH_3S^-/Fl_{ox}, it is 23.3 kcal/mol [B3LYP/6-311+G(d,p)], whereas in $CH_3CH2CH(C{=}O)SCH_3^-/Fl_{ox}$, it is 16.3 kcal/mol [the same level of theory, ref. (*18a*)].

In spite of the larger energy of the CT interaction in CH_3S^-/Fl_{ox} than in the $CH_3CH_2CH(C{=}O)SCH_3^-$/Flox complex, the charge transferred to Fl_{ox} from thioenolate (Table 1) is slightly larger than from thiolate (*18a*) (0.66 e *vs.* 0.55 e). This difference is due to the different HOMO orbitals responsible for the CT complex formation and its absorption characteristics. In the case of CH_3S^-, it is a lone pair of sulfur atom (HOMO-1), which donates electrons to the lowest virtual π^* orbital of flavin [lp(S)→ π^*, LUMO].

Interestingly, in both cases for CT complexes of oxidized flavin with thioenolate and thiolate, we observed an increase of the CT absorption wavelength with increasing contact distance. This can be understood in terms of work required for the electron to be excited onto the flavin π^* orbital, which is already partially populated due to CT transfer, and thus has a partial negative charge. The larger the distance, the less charge is transferred to the flavin, and the less energy is required to excite an electron to its π^* orbital. For CH_3S^-/Fl_{ox}, the dependence is almost linear for the CT complex absorption wavelength and charge transferred to flavin (Table 2).

In the next models, explicit water molecules have been placed around flavin H-bond-accepting sites, and the complexes were re-optimized. No constraints were imposed again. We found (Table S1, Supporting Information) that there is no significant effect of the H-bonded water molecules on CT complex geometry and absorption.

Marked increase of S···C4a distance is observed when thiol molecule CH_3SH is H-bonded to thiolate, $CH_3SH{\cdots}CH_3S^-/Fl_{ox}$, or when additional water molecule is bonded to thiolate in $W{\cdots}CH_3SH{\cdots}CH_3S^-/Fl_{ox}{\cdots}W(N5)$ (Table S1, Supporting Information). We also note that when $CH_3NH_2$is H-bonded to oxygen of flavin C2=O, the increase of the S···C4a distance and the

Table 2. CH_3S^-/Fl_{ox} CT complex absorption wavelength (λ_{CT}, nm) and charge transferred to flavin (Q_{Fl}, e) *vs.* S···C4a distance (d, Å) calculated at the B3LYP/6-31G(d) level of theory.

d, Å	λ_{CT}, nm	Q_{Fl}, e
2.694[a]	451.11	–0.52
2.8[b]	475.13	–0.49
2.85[b]	487.95	–0.47
2.9[b]	501.68	–0.46
2.95[b]	516.29	–0.45

[a]S···C4a distance in the B3LYP/6-31G(d)-optimized CT complex without constraints; [b]S···C4a distance was increased, no re-optimization was performed.

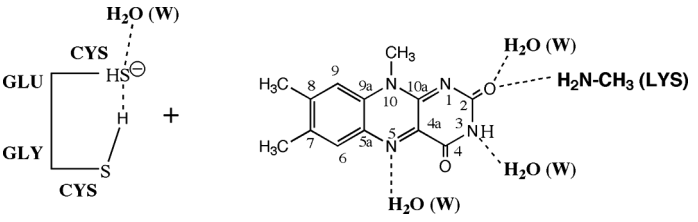


Figure 2. Schematic representation of the CT complex, its components, and H-bonding interactions based upon Erv2p active site structural information.

wavelengths of CT absorption are larger than when water molecules are used. None of these relatively small models give satisfactory wavelengths for the CT absorption (>500 nm), except the model B in Figure 1 with the 'wrong' orientation of thiolate methyl group (in this sense, B3LYP results are better than the other two DFT methods). We next proceeded to larger, more realistic, models of the Erv2p active site including a representation of the CYS–GLY–GLU–CYS active site loop (Figure S1, Supporting Information). The S–S bond was substituted by the S–H···S$^-$ unit as indicated in the schematic representation of the CT complex designed from the active site structure (Figure 2). The number of H-bonded water molecules (W) was varied, while the total charge of the systems studied was always '–1'. The best agreement ($\lambda_{CT} = 586.12$ nm) with the experimental CT absorption was found for the B3LYP/6-31G(d)-optimized aggregate with two water molecules H-bonded to N3 and N5 atoms of flavin and NH_2CH_3 (mimicking LYS11 of the active site) H-bonded to oxygen of O=C2. Again, the gas-phase MPWB1K-optimizations and TD MPWB1K calculations on these systems produce tighter complexes and shorter CT absorption wavelengths.

The above studies clearly indicate the stability CT complexes between thiolate and the isoalloxazine ring of FAD. Nevertheless, a range of experimental observations suggests that sulfur adducts at the C4a position of the isoalloxazine ring of the FAD can be formed (*1–5*). Using several reaction models, we show here that adduct formation requires a proton relay to N5 of the flavin. All our models are based upon an oxidized flavin molecule (FAD = Fl_{ox}), a thiol/thiolate system (–S–H···S–), a donor (R–H), which will transfer a proton through a chain of n ($n = 0,1,2$) water molecules (W), and, in the biggest model, a CH_3NH_2 molecule H-bonded to the C2=O and N3-H of the flavin. It is described by a general schematic representation in Figure 3.

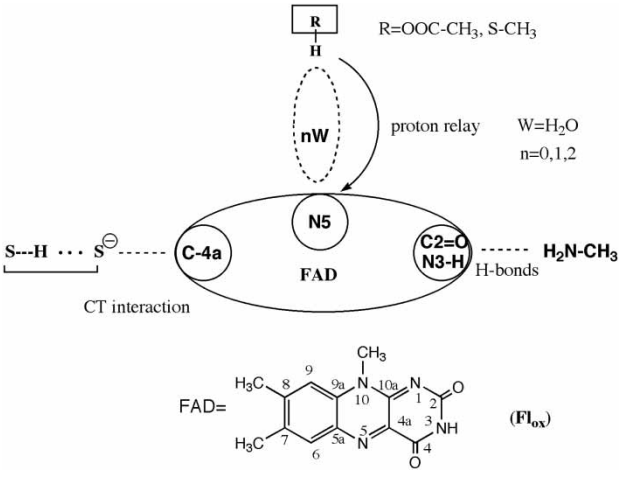


Figure 3. Schematic representation of the model, which includes CT interaction, proton relay, and H-bonding interactions at the active site of the Erv2p.

Table 3. R-1 [CH_3S^-+Fl_{ox}(N5)···H–SCH_3]→P-1 [CH_3S^-···H–(N5)-adduct] Reaction energetics (kcal/mol) calculated at different levels of theory (see Figure 4).

	B3LYP/SB[a]	B3LYP/LB[b]	MPW1PW91/SB[a]	MPWB1K/LB[b]
ΔE	5.9	6.2	1.6	–0.8
$\Delta E^{\neq}$	10.0	9.6	5.4	5.1
Δ(E+ZPVE)[c]	7.9	7.9	3.7	1.3
Δ(E+ZPVE)$^{\neq}$	8.5	8.0	3.8	
Imaginary frequency	1082.2i cm^{-1}	910.5i cm^{-1}	927.4i cm^{-1}	

[a]SB=6-31G(d); [b]LB=6-311+G(d,p); [c]ZPVE is a zero-point vibrational energy correction.

In the first reaction model, we assume that the H–SCH_3 group forms a hydrogen bond with the N5 of flavin in concert with the CT interaction. The reactant complex (R-1), transition structure (TS-1), and product complex (P-1, see Figure S2 in Supporting information) were fully optimized at different level of theory (Table 3 and Figure 4). We find significant differences in C4a···S distances for R-1 and TS-1, when B3LYP and MPWB1K are applied. The barriers and endothermicity are smaller for mPW-based methods (Table 4).

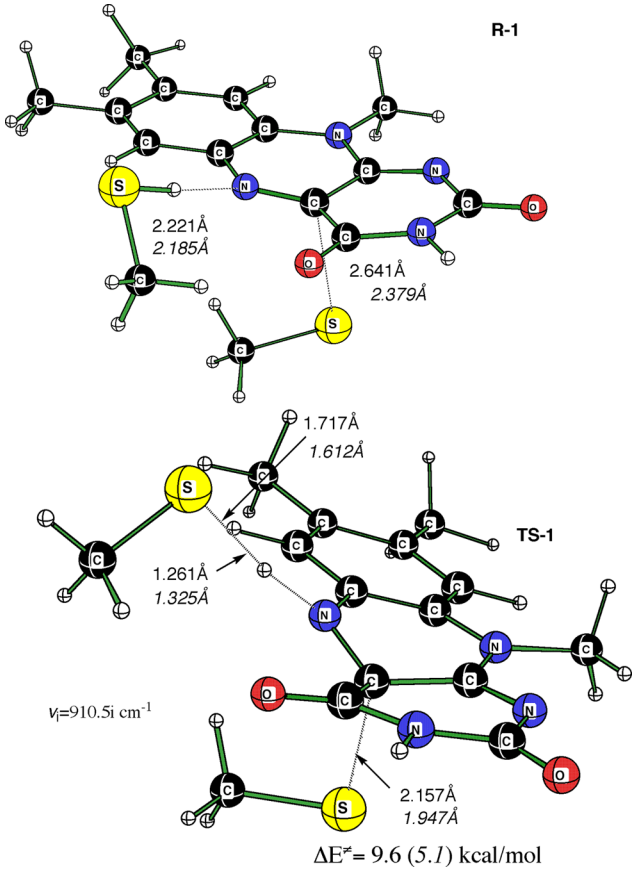


Figure 4. Reactant CT complex R-1 and transition structure TS-1 for the reaction [CH_3S^-+Fl_{ox}(N5)···H–SCH_3]→[CH_3S^-···W···H–(N5)-adduct] optimized at the B3LYP/6-311+G(d,p) [plain numbers] and MPWB1K/6-311+G(d,p) [*italic numbers*] levels of theory.

Table 4. R $[CH_3S^- + Fl_{ox}(N5)\cdots nW\cdots H\text{–}SCH_3] \rightarrow$ P $[CH_3S^{-}\cdots nW\cdots H\text{-(N5)-adduct}]$ reaction energetics (kcal/mol) calculated at the B3LYP/SB and B3LYP/LB level of theory.

	B3LYP/SB[a] ($n = 1$)	B3LYP/BB[b] ($n = 1$)	B3LYP/SB[a] ($n = 2$)	B3LYP/BB[b] ($n = 2$)
ΔE	3.5	4.5	5.8	6.8
$\Delta E^{\neq}$	11.5	13.1		
Δ(E+ZPVE)[c]	5.3	6.3	7.2	
Δ(E+ZPVE)$^{\neq}$	8.3	9.3		
Imaginary frequency	1168.3i cm^{-1}	1313.6i cm^{-1}		
S···C4a in reactant (CT)	2.600 Å	2.603 Å	2.589 Å	2.570 Å
S–C4a in product (adduct)	1.978 Å	1.974 Å	1.965 Å	1.963 Å

The number of water molecules $n = 1$ and 2.
[a]SB = 6 − 31G(d); [b]LB = 6 − 311 + G(d,p); [c]ZPVE is zero-point vibrational energy correction.

The animations of an imaginary frequency in all transition structures at the different levels of theory show vibrations that are characteristic of a concerted proton transfer and an S-C4a bond formation/cleavage. The large involvement of the proton transfer in this reaction is represented by the characteristic value of an imaginary frequency (Table 3).

Since it is not realistic in the case of Erv2p that the H-bond at the N5 position is directly contributed by a –SH group, we introduced one and two molecules of water between them in order to form a proton relay chain. These results are given in Table 4. The energetics for one and two water molecules in such a relay show only minor effects. Optimized transition structures represent concerted S–C bond formation and a proton relay. Animation of the imaginary frequency shows three concerted vibrations involved: two bouncing hydrogens (S···H···O and O···H···N5) and a S–C stretching vibration. Interestingly, in this animation, the S–C bond is formed when the proton is delivered. Thus, we would characterize this reaction as non-synchronous concerted. In order to further characterize this concerted TS, we performed intrinsic reaction coordinate (IRC) calculations for the transition structure optimized at the B3LYP/6-31G(d) level of theory. These calculations confirmed that the optimized transition structure is directly connected to the reactant CT complex $CH_3S^- + Fl_{ox}(N5)\cdots W\cdots H\text{-}SCH_3$ and product complex, $CH_3S^{-}\cdots W\cdots H\text{–(N5)-adduct}$.

Other potential residues in the enzyme might also donate a proton (like the thiol in the case above), and initiate the formation of the S–C4a bond. Modeling a GLU or ASP residue was achieved using CH_3COO^-H. The results for the reaction with CH_3COO^-H as a proton donor and one water molecule in a proton relay chain are reported in Table 5 and in Figure 5. In comparison with the case of CH_3SH (Table 5), this reaction has a smaller barrier and is less

Table 5. R-2 $[CH_3S^- + Fl_{ox}(N5)\cdots W\cdots H\text{–}OOCCH_3] \rightarrow$ P-2 $[CH_3COO^{-}\cdots W\cdots H\text{–(N5)-adduct}]$ reaction characteristics calculated at the B3LYP/SB, B3LYP/LB and MPWB1K/LB levels of theory.

	B3LYP/SB[a]	B3LYP/LB[b]	MPWB1K/LB[a]
ΔE, kcal/mol	–0.8	1.0	–4.4
$\Delta E^{\neq}$, kcal/mol	8.0	7.8	5.2
Δ(E+ZPVE)[c], kcal/mol	0.7		
Δ(E+ZPVE)$^{\neq}$, kcal/mol	3.9		
Imaginary frequency	1191.8i cm^{-1}	1165.6i cm^{-1}	
S···C4a in R-2 (CT)	2.548 Å	2.561 Å	2.031 Å
S–C4a in P-2 (adduct)	1.972 Å	1.966 Å	1.882 Å
Charge on Fl^{d}_{ox}	–0.60	–0.66	–0.06 (on adduct[e])

[a]SB = 6 − 31G(d); [b]LB = 6 − 311 + G(d,p); [c]ZPVE is zero-point vibrational energy correction; [d]NBO charges are calculated at the B3LYP/LB level of theory; [e]$C_{14}H_{16}N_4O_2S$.

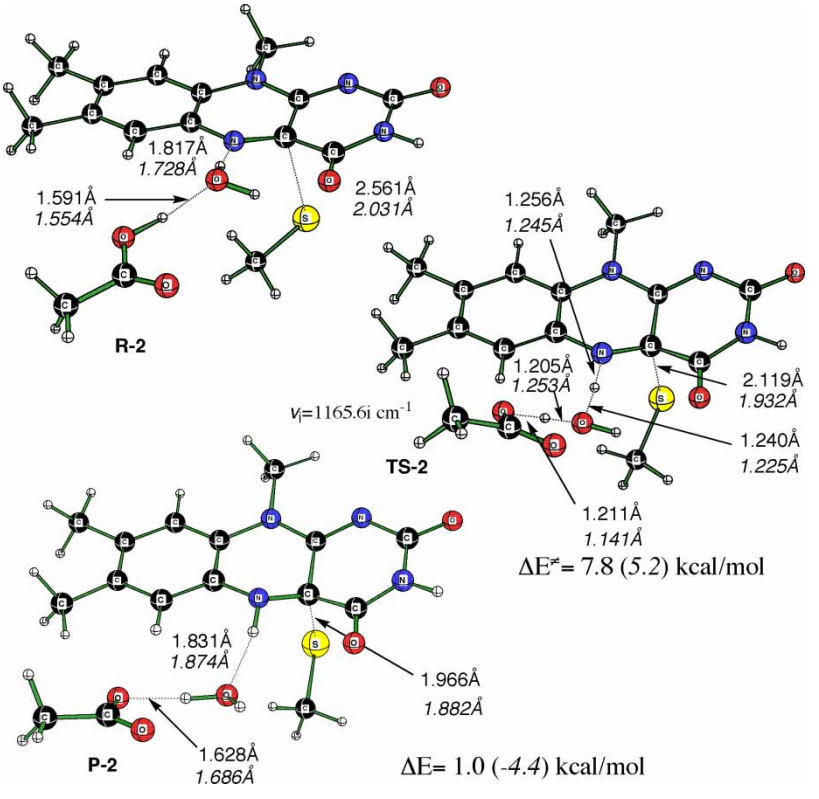


Figure 5. Reactant CT complex R-2, transition structure TS-2, and product complex P-2 for the reaction [CH_3S^- + Fl_{ox}(N5)···W···H–$OOCCH_3$]→[CH_3COO^-···W···H–(N5)-adduct] optimized at the B3LYP/6-311+G(d,p) [plain numbers] and MPWB1K/6-311+G(d,p) [italic numbers] levels of theory.

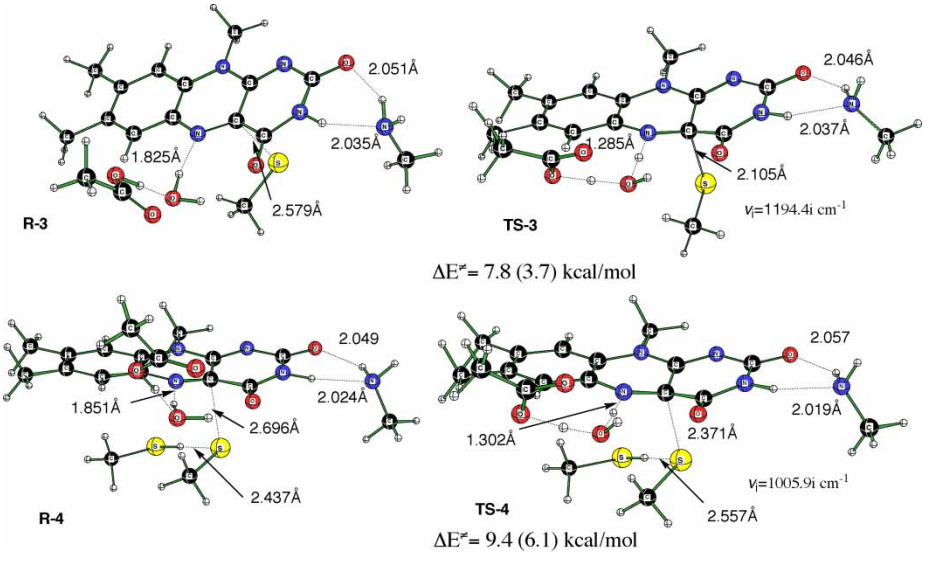


Figure 6. Reactant CT complexes R-3,4 and transition structures TS-3,4 for the reaction [CH_3S^- + CH_3NH_2···Fl_{ox}(N5)···W···H–$OOCCH_3$]→[CH_3COO^-···W···H–(N5)-adduct···CH_3NH_2] and [CH_3S–H···CH_3S^- + CH_3NH_2···Fl_{ox}(N5)···W···H–$OOCCH_3$]→[CH_3COO^-···W···H–(N5)-adduct···CH_3NH_2] optimized at the B3LYP/6-31G(d). ZPVE-corrected barriers are given in parentheses.

endothermic. This means that this type of functional group will donate a proton more efficiently. The slight reduction of S···C4a distance in CT complexes (from 2.603 to 2.561 Å) indicates that CH_3COO^-H-through-water effect on the CT interaction between Fl_{ox} and thiolate is stronger.

H-bonding of the NH_2CH_3 molecule to the flavin (TS-3, Figure 6) that mimics LYS11 almost does not affect the reaction barrier. Without NH_2CH_3 (TS-2, Figure 5), at the B3LYP/SB+ZPVE level, the barrier is 3.9 kcal/mol (Table 5). It slightly lowered to 3.7 kcal/mol when NH_2CH_3 was attached.

Anything that forms a hydrogen bond with thiolate stabilizes its anionic state, thereby increasing the barrier of the reaction. As an example of this statement, we used the model with thiol H-bonded to a thiolate (TS-4, Figure 6). Indeed, this addition of thiol molecule increases the barrier to 6.1 kcal/mol.

4. Conclusions

This computational study demonstrates that CT complexes of thiolate with FAD are quite a stable molecular unit (minimum that does exist independently on chosen method/model/medium) and can be a precursor for the sulfur-adduct at the C4a position of the isoalloxazine ring of the FAD. Calculations on various model systems indicate that the CT complex geometry, absorption, and C4a-adduct formation energetics depend on solvation and on the presence of the H-bonding species in the active site. We also found that the reactions studied require a concerted proton transfer to the N5 position of the flavin, and that they show relatively small barriers (<13 kcal/mol). It was demonstrated that this proton can be delivered to the flavin via a protonated cysteine, or from the carboxyl moiety of glutamic and aspartic acids by utilizing a series of intervening water molecules.

Acknowledgements

This work was supported by NIH GM26643. GridChem is also acknowledged for the computational resources (www.gridchem.org) (see ref. (*17*).

Note

1. B3LYP method was successfully applied by Houk and co-authors in their theoretical enzymes (theozymes (*20a*)) studies (*20b*).

Supporting information available

Total energies, TD DFT and NBO analyses, and Cartesian coordinates. Is available free of charge via the internet at www.informaworld.com

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A computational analysis of the interaction between Flavin and Thiol(ate) groups. Implications for flavoenzyme catalysis

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Supporting Information

Full reference 10a,b:

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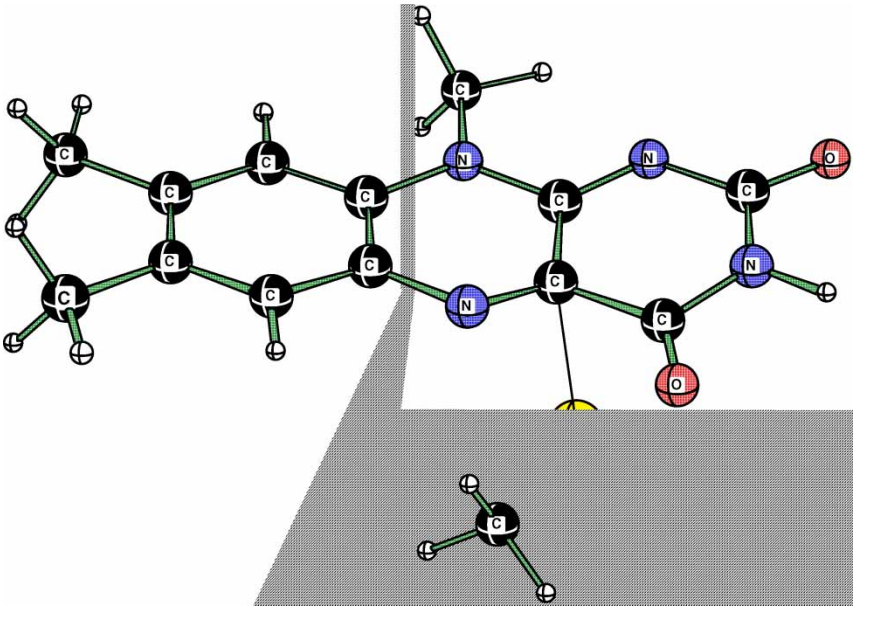
Figure 1A

TD(Nstates = 12,50-50) rb3lyp/6-311+g(d,p) // rb3lyp/6-311+g(d,p)
E = −1310.5529483



Zero-point correction = 0.275378 (Hartree/Particle)
Thermal correction to Energy = 0.295893
Thermal correction to Enthalpy = 0.296837
Thermal correction to Gibbs Free Energy = 0.226164
Sum of electronic and zero-point Energies = −1310.277571
Sum of electronic and thermal Energies = −1310.257056
Sum of electronic and thermal Enthalpies = −1310.256112
Sum of electronic and thermal Free Energies = −1310.326785
TD(Nstates = 12,50-50) rb3lyp/6-311+g(d,p) pop = nbo
Excited State 4: Singlet-A 2.6177 eV 473.64 nm f = 0.2398
Charge unit 2 (C13H12N4O2) −0.54945
Charge = −1 Multiplicity = 1

C	0.7471200612	−2.1695945276	2.3225058529
S	−0.947478488	−1.5189779782	2.1820200926
N	−2.7714598839	−0.0993100575	−0.4592226391
C	−3.3665910262	−1.3199524598	−0.662086457
O	−4.5783433714	−1.4725884769	−0.7435454037
N	−2.5322499995	−2.4374643978	−0.825747236
C	−1.1521817887	−2.4869998506	−0.6973103356
O	−0.5356656157	−3.523246977	−0.8643023221
C	−0.5347866135	−1.1497529145	−0.4453349861
N	0.7600518303	−1.0205398684	−0.6488338093
C	1.2903094761	0.2342658113	−0.4815745761

C	0.4922128247	1.3743075112	−0.2203158107
N	−0.8975901181	1.2240698828	−0.2224222552
C	−1.4665842121	−0.0213769736	−0.3563574599
C	−1.754513382	2.3841523493	−0.0015514244
H	1.2485026776	−2.0675259259	1.3490921509
H	1.3246849706	−1.6077714563	3.0631064243
H	−3.0025592074	−3.3246270666	−0.9412887565
H	−1.5672498847	3.1453531167	−0.7642670339
H	−2.7851002336	2.0480239833	−0.0619351609
H	−1.5612325778	2.8079358607	0.9884571855
C	2.6825584529	0.4094869945	−0.5649338822
H	0.7466937436	−3.2288698315	2.5927051103
C	1.109596864	2.6162812259	−0.0286955773
C	2.4952391177	2.7668603016	−0.0986073555
C	3.2988197442	1.6404286509	−0.3791782609
H	0.5066421113	3.4902809989	0.1819110061
H	3.2709861096	−0.4789524149	−0.7681093893
C	3.1128376928	4.1259587403	0.1290709558
H	3.7929349779	4.1248283552	0.9891078005
H	3.6993574878	4.4590663765	−0.7353557705
H	2.3436308846	4.8782623847	0.3175333526
C	4.8023941256	1.7597287844	−0.4658826545
H	5.2546075313	0.7914280259	−0.6894396082
H	5.1124323728	2.4615240522	−1.24906659
H	5.2375459203	2.1213240499	0.4731030104

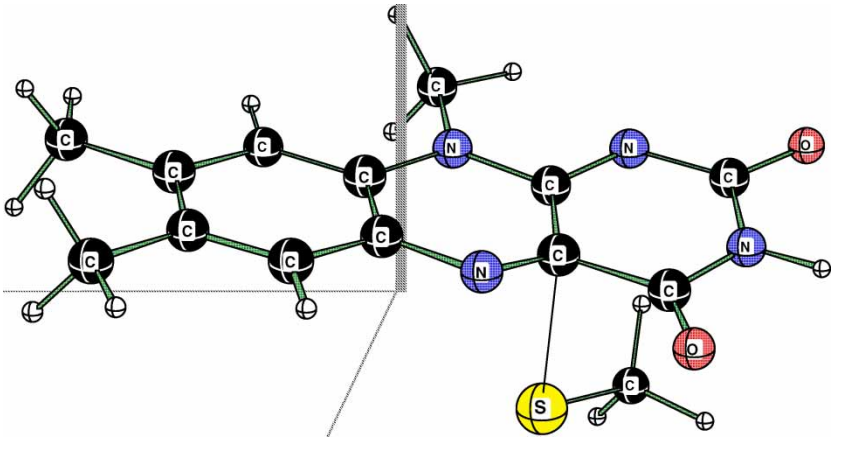
SOLVENT (Toluene) EFFECT CALCULATION USING C-PCM model with B3LYP/6-311+G(d,p)
Total free energy in solution:
with all non electrostatic terms (a.u.) = −1310.592315
Excited State 4: Singlet-A 2.0856 eV 594.48 nm f = 0.2982
Charge = −1 Multiplicity = 1

C	0.7944205041	−2.1331321436	2.3895138348
S	−0.9681988972	−1.6334784325	2.3257596441
N	−2.790175166	−0.111992311	−0.4165690558
C	−3.3660543345	−1.3262727846	−0.6257448548
O	−4.5881966361	−1.4948337043	−0.6468449281
N	−2.5429025731	−2.437538917	−0.855984145
C	−1.1675658848	−2.4639458842	−0.8008932834
O	−0.5340909243	−3.4911620357	−1.0035037719
C	−0.5535020675	−1.1281113875	−0.5674035375
N	0.7396870608	−1.0065553221	−0.7107851133
C	1.2811409159	0.2382524047	−0.5151765997
C	0.4798417129	1.3720783748	−0.2454769973
N	−0.9073560558	1.2228377097	−0.239367818
C	−1.4733226418	−0.0123058739	−0.3829294902
C	−1.7562096642	2.4013251734	−0.0231051831
H	1.2223194333	−2.0936805177	1.3794306203
H	1.3760862768	−1.4617111354	3.0265216654

H	−3.0153280707	−3.3236361233	−0.9902283255
H	−1.5351227353	3.1569560003	−0.7778235434
H	−2.7916414453	2.0926866326	−0.1039562422
H	−1.5717736494	2.8117205687	0.9717887639
C	2.6787073128	0.3970258672	−0.5794333441
H	0.9066409544	−3.1543643137	2.7628117912
C	1.0998384895	2.6126144508	−0.0351653384
C	2.4835327065	2.7507048367	−0.0915109923
C	3.2947330979	1.619607577	−0.374584946
H	0.5022978886	3.4885402717	0.1771531236
H	3.2673026611	−0.4893243054	−0.7880241995
C	3.1074482935	4.101778027	0.1490383701
H	3.8021818904	4.0729586405	0.9948500725
H	3.6827951324	4.434832279	−0.7211173876
H	2.3467604669	4.854671277	0.3607516069
C	4.79681653	1.7411575786	−0.4442170674
H	5.253265088	0.7802562679	−0.6868817325
H	5.1055951077	2.4646643718	−1.2056009876
H	5.2156017968	2.0833931615	0.5079575785

Figure 1B

TD(NSTATES = 12,50-50) b3lyp/6-311+G(d,p)// b3lyp/6-311+G(d,p)
E = −1310.5483434 a.u.
Excited State 4: Singlet-?Sym 2.3317 eV 531.72 nm f = 0.2140



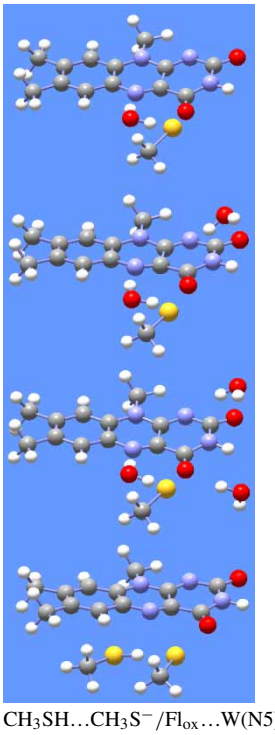
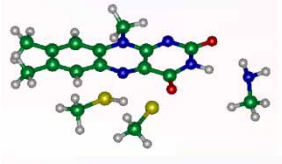
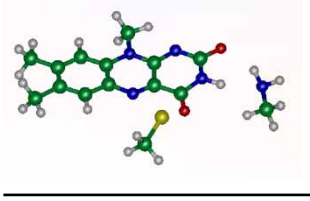
N	2.1564582824	−1.2110594439	−0.8677961648
C	3.333185279	−0.5264170525	−1.0203294745
O	4.3935819113	−1.0585376347	−1.3256397095
N	3.3099147519	0.868097091	−0.8374040502
C	2.219325871	1.6681018253	−0.530793962
O	2.3169965341	2.8778670723	−0.4667958633
C	0.9453057212	0.8935426442	−0.3997340172
N	−0.1833818953	1.5516202586	−0.4051522435

C	−1.3370706698	0.814507427	−0.3101649404
C	−2.5674493374	1.4805270961	−0.187203018
C	−3.7745537629	0.8065100799	−0.0509481162
C	−3.7645781168	−0.6050390837	−0.0376860021
C	−2.5526698791	−1.2842519484	−0.1745965164
C	−1.3390187036	−0.6016273967	−0.3188136844
N	−0.127737865	−1.2626112438	−0.5219610095
C	1.0574278796	−0.5568471861	−0.5602994507
C	−0.0946153359	−2.7199583166	−0.5655262371
S	1.5077302318	0.2504807814	2.2703048139
H	−2.5331030754	2.5646355031	−0.1848288139
H	−2.5637743126	−2.3663860226	−0.1611051944
H	−0.7617805511	−3.0892430336	−1.3490562198
H	0.9263747973	−3.0204711135	−0.7799112621
H	4.1975583232	1.3409633294	−0.9405436161
C	−5.0449098896	−1.3880535958	0.1288548581
H	−5.7687177123	−1.159816128	−0.6623048282
H	−4.8528465817	−2.4629650189	0.1036964053
H	−5.5379952635	−1.161428717	1.0814404584
C	−5.0655565304	1.5777980419	0.0915802204
H	−4.8794941399	2.6527644169	0.0493852598
H	−5.7794199199	1.3309832778	−0.7030850291
H	−5.5653762353	1.3644573756	1.0436956429
C	2.9594303911	−0.8527944922	2.4484058523
H	2.8600872908	−1.4571532048	3.3564241488
H	3.030157753	−1.5395495091	1.5981018204
H	3.8934271162	−0.2868828342	2.5120335777
H	−0.4056991766	−3.1305512118	0.4003864166

SOLVENT (Toluene) EFFECT CALCULATION USING C-PCM model with B3LYP/6-311+G(d,p)
Total free energy in solution:
with all non electrostatic terms (a.u.) = −1310.589197
Excited State 4: Singlet-A 1.8968 eV 653.65 nm f = 0.2655
Charge = −1 Multiplicity = 1

N	2.1495479502	−1.223727373	−0.8583945061
C	3.3135189149	−0.5331471544	−0.9945533758
O	4.3903919399	−1.0750558388	−1.2560021938
N	3.2950034215	0.8624916965	−0.8514698162
C	2.1992944018	1.6474325253	−0.5747484505
O	2.2824531418	2.8651972025	−0.5026299957
C	0.9267238297	0.877596413	−0.4648969228
N	−0.1957904001	1.5357973137	−0.4200631143
C	−1.353425669	0.8093765669	−0.3216583851
C	−2.5809594958	1.4854308823	−0.1862287864
C	−3.7838803142	0.8133157857	−0.0483978357
C	−3.7726651658	−0.6069590594	−0.0514253392
C	−2.5689447332	−1.2899613844	−0.2018929053
C	−1.3533412724	−0.60544522	−0.3401276423

Table S1. Equilibrium S…C4a Contact Distances (d, Å), Lowest Vertical Strongly Allowed Singlet-Singlet Transitions[a] (λ_{CT}, nm) and Charge Transferred to Flavin (Q_{Fl}) Calculated for the CH_3S^{ψ}/Fl_{ox} Complexes. For TD Calculations and NBO Analysis, the Same Method as for the Optimization was Used. SB = 6-31G(d); LB =6 − 311+G(d,p)

CT complex	Method	d, Å	λ_{CT}, nm	f_{osc}	Q_{Fl}, e
	B3LYP/SB	2.603			
	B3LYP/LB	2.616	456.95	0.24	−0.58
	MPW1PW91/LB	2.395	413.68	0.24	−0.68
	B3LYP/LB	2.615	462.01	0.22	
	B3LYP/LB	2.637	462.84	0.24	
	B3LYP/LB	2.773	496.68	0.22	−0.46
	MPW1PW91/LB	2.608			
$CH_3SH…CH_3S^-/Fl_{ox}…W(N5)$	B3LYP/LB	2.702	475.68	0.23	−0.49
	MPW1PW91/LB	2.522			
$CH_3SH\&W…CH_3S^-/Fl_{ox}…W(N5)$	MPWB1K/SB	2.583	398.34	0.41	−0.41
	B3LYP/SB	2.765	482.31	0.30	−0.39
	B3LYP/LB	2.803	507.67	0.21	−0.44
	B3LYP/LB	2.697	478.77	0.24	−0.54

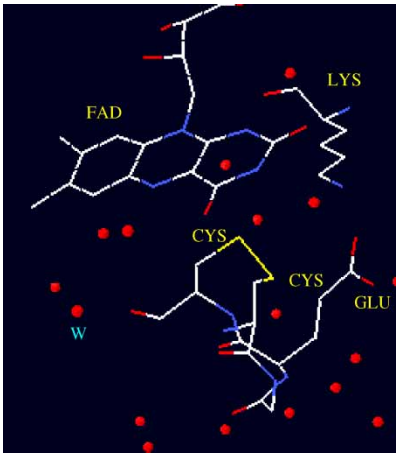


Figure S1. Fragment of Erv2p active site structure. Red balls are water molecules (W).

N	−0.143958716	−1.2691899972	−0.5346452263
C	1.0303350677	−0.5711341369	−0.5961844218
C	−0.1341746659	−2.734671212	−0.6161652842
S	1.621353071	0.3059686972	2.3987770934
H	−2.5496300596	2.569144321	−0.1795674246
H	−2.5856529092	−2.3712073851	−0.2072851409
H	−0.7805167538	−3.0634018869	−1.4313290717
H	0.883954101	−3.0553001217	−0.8020485527
H	4.1856953906	1.3350699227	−0.9489502175
C	−5.0550579213	−1.3833135946	0.1095561898
H	−5.7681516045	−1.1429305296	−0.6860386953
H	−4.8705163325	−2.4583046858	0.0865854145
H	−5.5473000173	−1.1433269808	1.0578222535
C	−5.0755200218	1.5771662285	0.1106576164
H	−4.8960605636	2.6531998331	0.0923706772
H	−5.7837238417	1.3382789005	−0.6896720973
H	−5.5715428269	1.334361408	1.0562409556
C	3.0698764423	−0.8229813517	2.5416875293
H	3.013948875	−1.4150546286	3.4596991505
H	3.0926424398	−1.5181726131	1.6967306156
H	4.0109770905	−0.2664811312	2.5509915468
H	−0.4876896136	−3.158849379	0.3258864103

Figure S2. R-1 at B3LYP/6-311+G(d,p)

E = −1749.3076647
Zero-point correction = 0.322759 (Hartree/Particle)
Thermal correction to Energy = 0.348562
Thermal correction to Enthalpy = 0.349506
Thermal correction to Gibbs Free Energy = 0.264137
Sum of electronic and zero-point Energies = −1748.984906

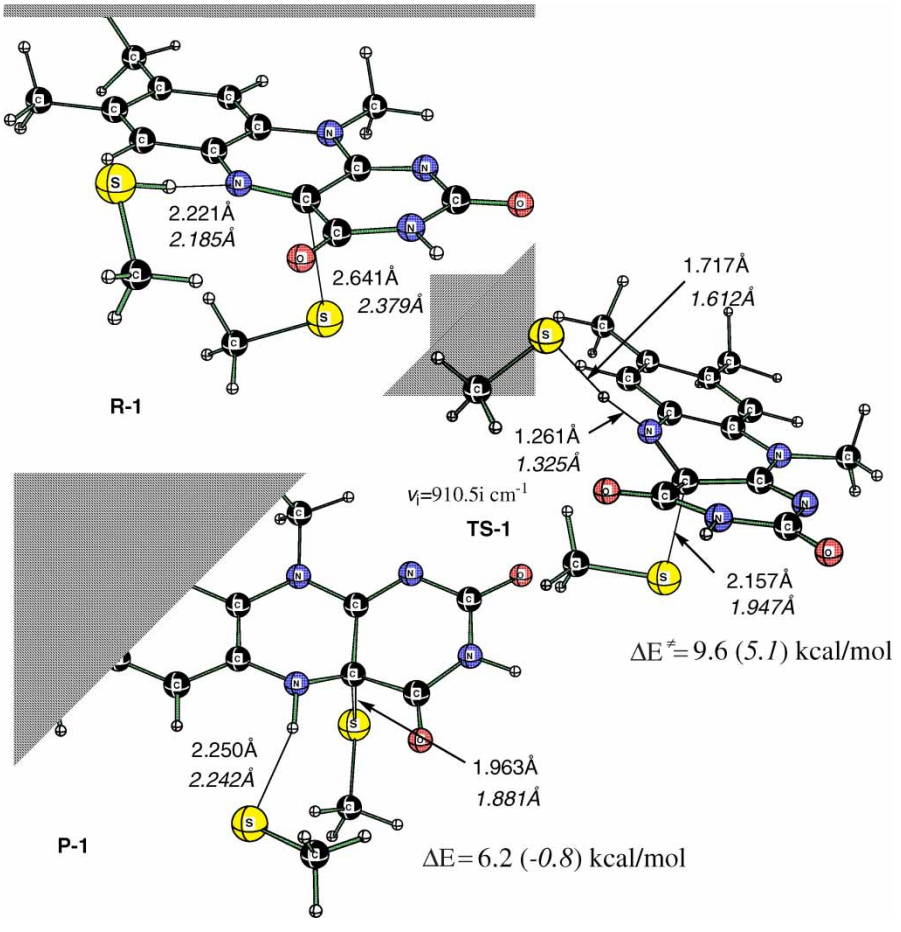


Figure S2. Reactant charge-transfer complex R-1, transition structure TS-1 and product complex P-1 for the reaction $[CH_3S^- + Fl_{ox}$ (N5)…H-$SCH_3] \rightarrow [CH_3S-…W…H$-(N5)-adduct] optimized at the B3LYP/6-311+G(d,p) [plain numbers] and MPWB1K/6-311+G(d,p) [italic numbers] levels of theory.

Sum of electronic and thermal Energies = −1748.959103
Sum of electronic and thermal Enthalpies = −1748.958159
Sum of electronic and thermal Free Energies = −1749.043528
Charge = −1 Multiplicity = 1

C	1.4145861339	4.754940856	−0.0690294925
S	0.0409293566	4.1277754115	−1.1147725936
C	0.7928924691	0.8385157849	2.8509841807
S	1.6109390068	−0.7060038982	2.3472539693
N	2.0428590032	−2.3369879637	−0.7248620663
C	3.3038176123	−1.7973612255	−0.7802274872
O	4.3102422276	−2.4560493504	−1.0012834518
N	3.4305615471	−0.4063611069	−0.617256694
C	2.4345237608	0.491429424	−0.2794248417
O	2.6621467127	1.6845226557	−0.1665392302
C	1.081998737	−0.1300099059	−0.1753667892
N	0.0333295847	0.6717230788	−0.2155687625
C	−1.2052774407	0.0759042563	−0.1929050625

C	−1.3744448848	−1.3267263468	−0.2767406272
N	−0.2403689764	−2.1226704213	−0.475632422
C	1.0165290451	−1.569038831	−0.44976987
C	−0.3854638636	−3.5658799917	−0.6399393035
H	1.1194511962	4.7939954921	0.9797804967
H	2.2783383512	4.1033078334	−0.1805345076
H	0.3374486463	1.3005867531	1.9630390812
H	−0.0017831787	0.6436044469	3.5769099884
H	4.3739660118	−0.0441696432	−0.6407240772
H	0.1724733136	2.8180341947	−0.7680334131
H	−1.0372400684	−3.7852212943	−1.4899445168
H	0.6032270343	−3.9783943656	−0.8156189008
H	−0.812530988	−4.0056106939	0.2663180045
H	1.5064976069	1.5487868856	3.2762167568
H	1.6529159208	5.7618558209	−0.4149881364
C	−2.6640711346	−1.8682012465	−0.2178646455
C	−3.7942910426	−1.0604782553	−0.0900523315
C	−2.3561393076	0.8751178451	−0.081321619
C	−3.6372622256	0.3413079952	−0.0280776717
H	−2.7993656842	−2.9409289582	−0.2663188708
H	−2.2055329886	1.9476054365	−0.0218568046
C	−5.1633840578	−1.6931132829	−0.0163616606
H	−5.6739099613	−1.4471662705	0.9220472824
H	−5.8155965732	−1.3506392183	−0.8282686669
H	−5.0961343555	−2.7814184118	−0.0811712206
C	−4.8341353674	1.2531009662	0.1055029949
H	−4.5218958646	2.2984119512	0.1383163341
H	−5.529556305	1.1387848829	−0.734269014
H	−5.4037161609	1.0454711422	1.0187846021

Figure S2, TS-1 rb3lyp/6-311+g(d,p)

E = −1749.2923784 Frequencies - - −910.4777
Zero-point correction = 0.320287 (Hartree/Particle)
Thermal correction to Energy = 0.345252
Thermal correction to Enthalpy = 0.346196
Thermal correction to Gibbs Free Energy = 0.264180
Sum of electronic and zero-point Energies = −1748.972091
Sum of electronic and thermal Energies = −1748.947127
Sum of electronic and thermal Enthalpies = −1748.946182
Sum of electronic and thermal Free Energies = −1749.028199
Charge = −1 Multiplicity = 1

C	0.8790996253	4.4240181541	−0.7847019015
S	−0.1173623657	3.229555858	−1.7716428279
C	0.9961655579	1.5009879251	2.5774174622
S	1.4245120526	−0.2322986669	2.2233308583
N	2.0952407448	−2.335026852	−0.4842147177
C	3.3602350582	−1.8110315422	−0.5906795801

O	4.3580357926	−2.4915980082	−0.7657782074
N	3.4971419667	−0.4113962517	−0.5397011675
C	2.5223017169	0.5150501799	−0.2318462299
O	2.7543911494	1.7053452742	−0.2051730808
C	1.173999099	−0.0932522802	0.0856728014
N	0.1014675267	0.688836002	−0.2635481215
C	−1.152989651	0.1304949784	−0.1317613636
C	−1.3138115528	−1.2661457718	−0.0330688207
N	−0.1766182511	−2.0838136524	−0.2139970482
C	1.0764008441	−1.5545865347	−0.2293918796
C	−0.3522256429	−3.5219605569	−0.4084586566
H	0.991736566	5.3515556544	−1.352555238
H	0.3910008473	4.665318446	0.1648930781
H	0.3779737966	1.8788140204	1.7574517806
H	0.4140279896	1.5339267575	3.5004386021
H	4.4353492177	−0.053885346	−0.6600623834
H	0.1421347293	1.8034586077	−0.8511511439
H	−1.0676420182	−3.7009193499	−1.2141738443
H	0.6140613419	−3.9457947855	−0.6628576999
H	−0.7218203343	−3.9856968767	0.510791462
H	1.8868811101	2.1225315634	2.6695819167
H	1.8668680488	4.0126233857	−0.5752539577
C	−2.5902142491	−1.8061739126	0.1506970554
C	−3.7261127897	−0.9988206908	0.2011868484
C	−2.3051528678	0.9336367018	−0.1092028123
C	−3.577356902	0.3958182276	0.0494043193
H	−2.7122065595	−2.8771261746	0.2535340134
H	−2.1680102503	2.0017392519	−0.2364208545
C	−5.0857465741	−1.6221715521	0.4091870882
H	−5.5719995262	−1.247349046	1.3175671978
H	−5.7662105628	−1.406433559	−0.4229739044
H	−5.0086559358	−2.7083101264	0.5004365455
C	−4.7789941304	1.3114008537	0.0681207733
H	−4.4757659186	2.3483768461	−0.0840005889
H	−5.4957906834	1.0535638665	−0.7199659102
H	−5.3199515436	1.2537338801	1.0200995083

Figure S2, P-1 at B3LYP/6-311+G(d,p)

E = −1749.2982565
Zero-point correction = 0.326015 (Hartree/Particle)
Thermal correction to Energy = 0.351012
Thermal correction to Enthalpy = 0.351956
Thermal correction to Gibbs Free Energy = 0.269951
Sum of electronic and zero-point Energies = −1748.972241
Sum of electronic and thermal Energies = −1748.947245
Sum of electronic and thermal Enthalpies = −1748.946300
Sum of electronic and thermal Free Energies = −1749.028305

Charge = −1 Multiplicity = 1

C	0.62953	4.7387	−1.07667
S	−0.68164	3.71918	−0.27473
C	1.35981	1.8037	2.26065
S	1.35663	−0.00801	1.99417
N	2.26378	−2.31667	−0.4015
C	3.50073	−1.73295	−0.51061
O	4.54168	−2.36349	−0.5835
N	3.55089	−0.3287	−0.60639
C	2.50402	0.55133	−0.44782
O	2.62478	1.74202	−0.6355
C	1.20834	−0.09311	0.03837
N	0.07607	0.55283	−0.5093
C	−1.15817	−0.04676	−0.3166
C	−1.22463	−1.4364	−0.12464
N	−0.024	−2.18713	−0.21193
C	1.19381	−1.58795	−0.22314
C	−0.10816	−3.64162	−0.35151
H	0.72807	5.7074	−0.57503
H	1.59561	4.22899	−1.034
H	0.64057	2.31152	1.60713
H	1.0591	1.94568	3.30085
H	4.4588	0.07368	−0.79774
H	0.05765	1.60828	−0.52322
H	−0.77727	−3.89231	−1.17729
H	0.88957	−4.02085	−0.54685
H	−0.4924	−4.08727	0.57017
H	2.35506	2.21783	2.10204
H	0.39084	4.92889	−2.12856
C	−2.46555	−2.04984	0.06709
C	−3.65203	−1.31706	0.05258
C	−2.35157	0.68569	−0.35084
C	−3.58839	0.07351	−0.17186
H	−2.51856	−3.11865	0.23059
H	−2.27953	1.75518	−0.52493
C	−4.97314	−2.01435	0.27516
H	−5.49178	−1.6298	1.16105
H	−5.65504	−1.87728	−0.57208
H	−4.83111	−3.08876	0.41584
C	−4.84258	0.91464	−0.2066
H	−4.59675	1.9636	−0.37624
H	−5.52388	0.59248	−1.00305
H	−5.39987	0.8469	0.73476

Figure S3

R−2 E = −1616.21944464 RB3LYP/6-311+G(d,p)
Charge = −1 Multiplicity = 1
O 2.3479512784, −1.4241380556, −1.1322936116

C	0.1972508443	−1.1728183695	2.6278192593
S	−1.5658670785	−1.425017498	2.2642051025
N	−3.6091594129	−1.1460435374	−0.5237372462
C	−3.5178751522	−2.5092328723	−0.6584516725
O	−4.4865832075	−3.2401102265	−0.7983098562
N	−2.2318713482	−3.0819211318	−0.6834967678
C	−1.0313972908	−2.4447393235	−0.4480708445
O	0.0306432493	−3.0474711107	−0.5029638308
C	−1.1651448715	−0.9743030461	−0.2246684545
N	−0.0771073103	−0.2293392523	−0.377608896
C	−0.2295729642	1.1345065303	−0.277708791
C	−1.5017540876	1.7452715154	−0.1893521249
N	−2.6402446528	0.9329939603	−0.2872021924
C	−2.5257415469	−0.4324385465	−0.3395734399
C	−3.9674299363	1.5420429805	−0.2780560576
H	0.6896245999	−0.7872133402	1.7243311807
H	0.3268321985	−0.4347436989	3.4238024721
H	−2.198444047	−4.0885885149	−0.7706886012
H	1.5183738394	−0.9526664407	−0.8593971443
H	−4.0580225776	2.2604788855	−1.097039013
H	−4.6969005531	0.7475932621	−0.4006560758
H	−4.1367248395	2.0546007465	0.6734283741
C	0.9050218628	1.963258601	−0.2693390286
H	0.6862691647	−2.1071377295	2.9116628198
H	3.7645186435	−0.8976412801	−0.6335764877
O	4.6008863839	−0.4076321888	−0.3347451228
H	2.1025466961	−2.3461517155	−0.9801350554
C	−1.5881830267	3.1369058144	−0.0690219723
C	−0.4509975552	3.9446795662	−0.0464631929
C	0.8223196349	3.3451228238	−0.1549836875
H	−2.5581052723	3.610534442	0.0105216053
H	1.8742877966	1.4819102324	−0.3413043087
C	−0.596613563	5.4409966747	0.0926597049
H	−0.0982531374	5.8158031086	0.9942013505
H	−0.1534353045	5.9740936733	−0.7564450741
H	−1.6487170936	5.7283779204	0.1522121209
C	2.0808954144	4.179913014	−0.1397030594
H	2.9656905038	3.547661134	−0.230861913
H	2.0990967533	4.9026771502	−0.9635672424
H	2.1749793072	4.7549592684	0.7886342041
C	5.6473718651	−1.2172454281	−0.2198801798
O	5.6343793824	−2.4078847596	−0.4553119421
C	6.8820811374	−0.4717686271	0.2489642846
H	7.1130524808	0.3396255323	−0.445822185
H	6.690773573	−0.0199421608	1.2256496251
H	7.7255083145	−1.1567978932	0.3160538721

Figure S3, TS-2

E = −1616.2070605 RB3LYP/6-311+G(d,p)
Zero-point correction = 0.356479 (Hartree/Particle)
Thermal correction to Energy = 0.385079
Thermal correction to Enthalpy = 0.386024
Thermal correction to Gibbs Free Energy = 0.292878
Sum of electronic and zero-point Energies = −1615.850582
Sum of electronic and thermal Energies = −1615.821981
Sum of electronic and thermal Enthalpies = −1615.821037
Sum of electronic and thermal Free Energies = −1615.914183

O	1.8569508716	−1.8225015191	0.5873565939
C	−0.7865765166	−1.2356881358	2.9999275922
S	−2.2124690311	−0.5373453775	2.1028013456
N	−3.5382719365	−0.244190031	−1.0604352979
C	−3.869260622	−1.5756872099	−1.032639671
O	−4.9501196825	−2.0111898126	−1.3920800707
N	−2.8779245821	−2.489333402	−0.6185326332
C	−1.6547559073	−2.1903896244	−0.0649238554
O	−0.8530887906	−3.0559865425	0.2322825038
C	−1.4138850405	−0.7100768984	0.1478268326
N	−0.0867665465	−0.3538340803	0.0598431802
C	0.2136402395	0.9873513865	0.0209208351
C	−0.7717189417	1.9405315426	−0.3079606147
N	−2.0430848753	1.4712943648	−0.7133872632
C	−2.3883081579	0.1634198661	−0.586915797
C	−2.9912755595	2.4093999456	−1.3134101591
H	0.1434069235	−0.9216715838	2.5239597748
H	−0.81656978	−0.8374138378	4.0161267169
H	−3.1260999734	−3.4682594105	−0.6726309333
H	0.861061092	−1.1416752535	0.2999396694
H	−2.5190189306	2.9272095089	−2.1512093531
H	−3.85053306	1.8437275067	−1.6589713988
H	−3.3127479504	3.1451875366	−0.5709703388
C	1.5187418689	1.4465763957	0.2687043576
H	−0.8267713955	−2.3246748483	3.0249339257
H	2.9547623421	−1.6165373866	0.1354721515
O	4.0506740825	−1.3022298031	−0.2730468604
H	1.6220846131	−2.7472324655	0.4564462898
C	−0.4489913988	3.300065776	−0.31553566
C	0.8418284155	3.7486364216	−0.0359163222
C	1.8457181869	2.7975881902	0.2436542276
H	−1.2110920276	4.0326877425	−0.5492205932
H	2.2874520583	0.7136939788	0.4816443286
C	1.1455172687	5.2277708522	−0.0458078497
H	1.5167184808	5.5746662761	0.9255868719
H	1.9140085922	5.4827523649	−0.784945776
H	0.2515634478	5.8091282399	−0.2842578761
C	3.267113102	3.2255720974	0.5227478498

H	3.9102596111	2.3567332965	0.6695397285
H	3.6812448398	3.8127517945	−0.3046726631
H	3.3327722692	3.8519114259	1.4203016439
C	4.7758600026	−2.3011030541	−0.6751972771
O	4.443197776	−3.4846462369	−0.6598614356
C	6.1482921097	−1.8807120741	−1.2023307271
H	6.6865258309	−1.3214948544	−0.4319435123
H	6.7272392994	−2.7555026057	−1.4987510627
H	6.0230254425	−1.2131208951	−2.0596834426

Figure S3, P-2

E = = −1616.217791 RB3LYP/6-311+G(d,p)
Zero-point correction = 0.364382 (Hartree/Particle)
Thermal correction to Energy = 0.393312
Thermal correction to Enthalpy = 0.394256
Thermal correction to Gibbs Free Energy = 0.301755
Sum of electronic and zero-point Energies = −1615.853409
Sum of electronic and thermal Energies = −1615.824479
Sum of electronic and thermal Enthalpies = −1615.823535
Sum of electronic and thermal Free Energies = −1615.916036
Charge = −1 Multiplicity = 1

O	1.7108013038	−2.3340651185	0.2624332096
C	−0.7991735831	−1.9925139864	2.4897460434
S	−1.9243712929	−0.6352878576	1.9975094416
N	−3.8170316217	0.3783809207	−0.6941838069
C	−4.3960943339	−0.8634549107	−0.7678309479
O	−5.5864958862	−1.0455988745	−0.9498428073
N	−3.5456245072	−1.9879984774	−0.6885707494
C	−2.2051680724	−1.9880238131	−0.390694265
O	−1.5231492612	−2.990562158	−0.4320301269
C	−1.6603322215	−0.6332712537	0.0489867297
N	−0.3246003854	−0.4613676032	−0.3741945974
C	0.2473817593	0.7935307742	−0.1981848474
C	−0.5884054143	1.9205069146	−0.1907181129
N	−1.9758024079	1.7357633529	−0.4344286959
C	−2.5502889542	0.5085502833	−0.4008680783
C	−2.7959768102	2.9020410638	−0.7681354225
H	0.1477966487	−1.9410012033	1.9511763173
H	−0.6167824828	−1.8466456449	3.5561648936
H	−3.977622672	−2.8859871808	−0.8620881622
H	0.3160515791	−1.2673387818	−0.2571699546
H	−2.322332742	3.4599418198	−1.5780232922
H	−3.7763787974	2.5510073364	−1.0731581487
H	−2.8995116112	3.5525304977	0.1045369062
C	1.6279887425	0.9735508198	−0.0728632034
H	−1.2611432843	−2.9648886082	2.3225935882
H	2.6389277048	−1.9376352492	0.2404081509
O	4.0997066448	−1.236908911	0.0780417301

H	1.835533137	−3.1731832462	−0.1943428697
C	−0.0320886737	3.1902111459	−0.0141730382
C	1.3430945184	3.3683959906	0.1358232406
C	2.1858573795	2.2388150844	0.0935478356
H	−0.6741520517	4.0615101961	0.0094287649
H	2.2854429514	0.1132994313	−0.1034868098
C	1.9059101348	4.7550518278	0.3378646216
H	2.4299012814	4.8435575237	1.2963553002
H	2.6310636857	5.0163486125	−0.4409219544
H	1.113548447	5.5075261601	0.3220126564
C	3.6837479682	2.3621986313	0.2378845812
H	4.1510556622	1.375867416	0.2132173113
H	4.1134958578	2.9710756627	−0.5667284528
H	3.9549689457	2.8489581668	1.1819900373
C	4.8507042298	−2.0781229168	−0.5223036152
O	4.5250791174	−3.2167138159	−0.9039660714
C	6.2884371238	−1.5926466915	−0.7883079856
H	6.9006343939	−2.3929149957	−1.2073918907
H	6.2625815073	−0.7527782894	−1.4904608444
H	6.7371768749	−1.2262140085	0.139653647

Figure S4

R-3 RB3LYP/6-31G(d)
E = −1711.6785377
Zero-point correction = 0.433671 (Hartree/Particle)
Thermal correction to Energy = 0.467403
Thermal correction to Enthalpy = 0.468347
Thermal correction to Gibbs Free Energy = 0.361716
Sum of electronic and zero-point Energies = −1711.244866
Sum of electronic and thermal Energies = −1711.211135
Sum of electronic and thermal Enthalpies = −1711.210191
Sum of electronic and thermal Free Energies = −1711.316821
Charge = −1 Multiplicity = 1

O	−1.7505170922	1.0748932609	2.2345097134
C	−0.4697047396	3.0284683807	−0.9620314155
S	−0.0567856992	1.924847957	−2.3499999381
N	0.5372783245	−1.4703794574	−2.7463626591
C	−0.7941458117	−1.5876745897	−3.073955959
O	−1.1633530577	−2.1519127719	−4.1071108282
N	−1.7552465612	−1.0910787061	−2.1827759773
C	−1.5086798334	−0.3280397729	−1.0599686356
O	−2.4233698271	0.0720892601	−0.3459683356
C	−0.0566275156	−0.1152994965	−0.7719722461
N	0.2763454626	0.2731806994	0.4548590305
C	1.6181014024	0.3667899193	0.7414425987
C	2.6162018763	−0.1088490313	−0.1449828358
N	2.2079485414	−0.7558201803	−1.3196107168

C	0.8800610639	−0.8007863523	−1.6721941785
C	3.2048000974	−1.3297637482	−2.2158448847
H	−0.484934878	2.4465629396	−0.0274097384
H	0.2782248448	3.8223822258	−0.8510622165
H	−2.7353711034	−1.1826327532	−2.4808300249
H	−0.9783091726	0.7793005845	1.6802968445
H	3.8067444393	−2.0756913212	−1.6847000465
H	2.6715232747	−1.7974548012	−3.0406355781
H	3.8652404499	−0.5428909912	−2.5986789357
C	2.0306622634	0.9383821238	1.9589381751
H	−1.4584961592	3.4823743396	−1.0892606088
H	−1.9564014843	0.117061467	3.484401625
O	−1.9918921854	−0.4839725051	4.3138676481
H	−2.4714223989	0.9029203714	1.6008912926
C	3.9663527909	0.02400654	0.2042088273
C	4.3590999967	0.6080122335	1.4107239297
C	3.3699689185	1.0694923852	2.3094823731
H	4.7365224424	−0.3291702281	−0.4732598282
H	1.2518392515	1.2939124593	2.6285495533
C	5.8277998282	0.7368260481	1.7395548815
H	6.126479621	1.7847594838	1.8823160872
H	6.0897598808	0.2097691716	2.6677817187
H	6.4496087098	0.323525863	0.9384220793
C	3.7512799901	1.7022489114	3.6277630522
H	2.8603753819	1.9943705768	4.191873952
H	4.3348828531	1.0172638236	4.2585174835
H	4.3670144137	2.6016103568	3.4882319298
C	−3.2519325308	−0.7590987078	4.6352848835
O	−4.2353303608	−0.3552162086	4.040070348
C	−3.3289227731	−1.6587239199	5.8596178058
H	−2.8246037681	−1.1837752297	6.7084526738
H	−4.3727053263	−1.8561006576	6.1101902227
H	−2.8096950179	−2.6031773818	5.662241414
H	−3.1750185312	−1.8522129851	−4.3702795692
N	−4.0446732913	−1.4428230766	−4.0166525532
H	−4.7928193363	−2.1163281052	−4.1688169876
C	−4.330747861	−0.1833851491	−4.7055421142
H	−3.4944722623	0.5033793137	−4.5405324368
H	−4.4868625881	−0.2709303709	−5.7956598179
H	−5.2258024714	0.2793666546	−4.273335857

Figure S4 TS-3 rb3lyp/6-31g(d)

E = −1711.66604175 Frequencies--−1194.4005
Zero-point correction = .427089 (Hartree/Particle)
Thermal correction to Energy = .459859
Thermal correction to Enthalpy = .460803
Thermal correction to Gibbs Free Energy = .358360
Sum of electronic and zero-point Energies = −1711.238952

Sum of electronic and thermal Energies = −1711.206183
Sum of electronic and thermal Enthalpies = −1711.205239
Sum of electronic and thermal Free Energies = −1711.307682
Charge = −1 Multiplicity = 1

O	−1.0799602625	2.4265649093	0.8517192028
C	0.9405803025	0.8008804218	3.0292036224
S	1.4790821361	−0.7444809048	2.2108203252
N	2.5582175891	−1.8504125376	−0.8622178085
C	3.5552187101	−0.9043189096	−0.841415094
O	4.7147659177	−1.1715888747	−1.1646788787
N	3.2250240763	0.4154981611	−0.5027006995
C	2.0071837614	0.8533839605	−0.0324897198
O	1.7829788929	2.0368996847	0.1732344449
C	0.9902879124	−0.246320641	0.225316769
N	−0.3132056077	0.1806550438	0.0517515117
C	−1.2960011938	−0.7761708032	−0.0130251356
C	−0.9838394511	−2.1357916155	−0.2475036761
N	0.3598723768	−2.4651910425	−0.5471446096
C	1.3558283658	−1.5440304386	−0.4399500714
C	0.6702153946	−3.7998159666	−1.052269182
H	0.0868940496	1.2378858827	2.5038028684
H	0.63911518	0.5433952011	4.0496741736
H	3.9808107997	1.1087924669	−0.5882105882
H	−0.6800784197	1.358953176	0.4093357397
H	0.0550256246	−4.0196421258	−1.9313003863
H	1.7250126851	−3.8187950153	−1.3163669542
H	0.4668936076	−4.5500612892	−0.2797543275
C	−2.6562024776	−0.4284454574	0.1048531668
H	1.7485631896	1.5366324914	3.0605116039
H	−2.2186300264	2.7256238666	0.5466092548
O	−3.3647300436	2.9527133983	0.2242156635
H	−0.5068089408	3.0841182171	0.4246023927
C	−2.0033893288	−3.0934228652	−0.2763896633
C	−3.3450935226	−2.7417262441	−0.1126577602
C	−3.672237357	−1.3780412175	0.0598458913
H	−1.7582230419	−4.1387486086	−0.4342175193
H	−2.9148075064	0.6185506854	0.2240065519
C	−4.4152863917	−3.8076335905	−0.1372644788
H	−4.9879892449	−3.8400647134	0.8005581417
H	−5.1471767874	−3.6425092534	−0.9408544189
H	−3.9789114817	−4.801163488	−0.2891958916
C	−5.1090232446	−0.9308282257	0.2055552278
H	−5.1662969866	0.1582277054	0.2923327551
H	−5.7213287515	−1.2348213408	−0.6550887245
H	−5.5845392314	−1.365657232	1.0962725374
C	−3.3918373343	3.906400017	−0.6667170801
O	−2.4191181808	4.5454872905	−1.0685391808
C	−4.7983741905	4.1906569597	−1.2003509478
H	−5.4752680262	4.425220248	−0.3706065011
H	−4.7776526499	5.0231045088	−1.9078862616

H	−5.1964997545	3.2974934027	−1.6964527136
H	5.8667897707	0.5190212318	−1.1480615213
N	5.9308865849	1.5307568614	−1.0007072374
H	6.1708935359	1.9539881907	−1.8951576666
C	6.9395904593	1.8514011856	0.0078045692
H	6.6704184027	1.3557101943	0.9462687025
H	7.9709492261	1.5483193022	−0.2467403691
H	6.9447523901	2.9313046857	0.1970371842

Figure S4, R-4 RB3LYP/6-31G(d)

E = –2150.3880674
Zero-point correction = 0.480854 (Hartree/Particle)
Thermal correction to Energy = 0.520130
Thermal correction to Enthalpy = 0.521074
Thermal correction to Gibbs Free Energy = 0.397534
Sum of electronic and zero-point Energies = −2149.907214
Sum of electronic and thermal Energies = −2149.867938
Sum of electronic and thermal Enthalpies = −2149.866994
Sum of electronic and thermal Free Energies = −2149.990533

O	−2.3290826556	0.7030475645	2.3636636638
C	−0.7871842322	2.781082472	−0.5757906811
S	−0.4165486823	1.765229461	−2.0468146749
N	0.2150671118	−1.6262077952	−2.6198042009
C	−1.1001771452	−1.7590549573	−2.9984522341
O	−1.4215350133	−2.2756439272	−4.0712001388
N	−2.1054172666	−1.331471519	−2.1172324952
C	−1.9193740469	−0.6620363866	−0.9287651093
O	−2.8678301865	−0.3241762334	−0.2264605451
C	−0.4831853909	−0.4780452998	−0.5527608195
N	−0.2040560911	−0.1614155925	0.6990195706
C	1.124474342	−0.0787746298	1.049531984
C	2.1634756373	−0.4311684345	0.1526794877
N	1.8190120569	−0.9681349425	−1.0909074477
C	0.5027946216	−1.0366557095	−1.4846537539
C	2.8685073734	−1.3758989875	−2.0233508323
H	−0.9991404391	2.1233578081	0.2792005239
H	0.0535167862	3.4303440389	−0.3054603845
H	−3.0736993008	−1.4469174955	−2.4484346869
H	−1.5213048374	0.4121154253	1.8662563005
H	3.5174519835	−2.1234028125	−1.5543608968
H	2.3797209867	−1.8028334192	−2.896088696
H	3.4597496706	−0.5014411838	−2.3178521342
C	1.4730720558	0.3685660155	2.3368614381
H	−1.6741391216	3.4016592238	−0.7436366792
H	−2.3924885975	−0.0800947635	3.7543924917
O	−2.2677176082	−0.535994611	4.6605374754
H	−3.0111805289	0.4213522551	1.7265555554
C	3.4974044158	−0.2835785313	0.5608979453

C	3.8268047038	0.1798815781	1.83606383
C	2.793976203	0.5024559229	2.7483658569
H	4.2997543604	−0.5239459331	−0.1273290089
H	0.6604619012	0.6187592068	3.0137168098
C	5.2786443638	0.326291021	2.2269588318
H	5.5259702711	1.3610079903	2.5009951758
H	5.533086456	−0.2955998192	3.0964261906
H	5.9403216243	0.0349817977	1.4050237402
C	3.1112508795	0.9940945623	4.1412610419
H	2.193666969	1.1850027794	4.7057227912
H	3.70523854	0.2639045702	4.7079971858
H	3.6925167149	1.9262900601	4.1246635548
C	−3.3692160535	−1.1974916581	5.0062049067
O	−4.3983954996	−1.2362763656	4.3562514505
C	−3.1907757786	−1.9163140887	6.3342862249
H	−2.9083486493	−1.2018502116	7.1152711856
H	−4.1175735302	−2.4224842055	6.6098246245
H	−2.3786965194	−2.6477853392	6.2558886202
H	−3.4532931459	−2.1927220887	−4.3193054278
N	−4.3591348524	−1.8940761852	−3.9461271049
H	−4.9910878203	−2.6912075548	−3.987696235
C	−4.8896466055	−0.7705421859	−4.7186355706
H	−4.1691658643	0.0524902526	−4.6797343451
H	−5.0949693823	−0.988868047	−5.7817774137
H	−5.8206637249	−0.4141103401	−4.2624296988
S	3.2029869107	2.6789108815	−2.8094353371
H	1.8901992398	2.3177144536	−2.6041608094
C	3.4740279188	3.3869300087	−1.1367347847
H	2.8211497033	4.2463733342	−0.9636422964
H	3.3041762609	2.6351126567	−0.3627864323
H	4.514709764	3.7195984329	−1.0880194825

Figure S4, TS-4 RB3LYP/6-31G(d)

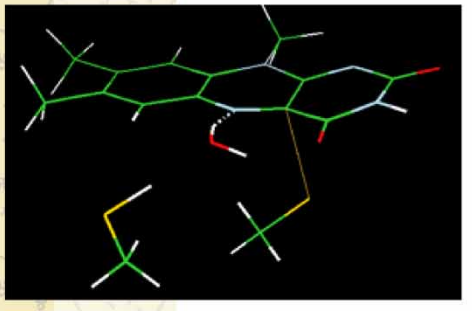
E = −2150.3730254 Frequencies-−1005.9408
Zero-point correction = .475580 (Hartree/Particle)
Thermal correction to Energy = .513876
Thermal correction to Enthalpy = .514820
Thermal correction to Gibbs Free Energy = .397152
Sum of electronic and zero-point Energies = −2149.897446
Sum of electronic and thermal Energies = −2149.859149
Sum of electronic and thermal Enthalpies = −2149.858205
Sum of electronic and thermal Free Energies = −2149.975873

O	−0.9424126486	2.7131225555	1.235656396
C	0.6979411419	0.0407368041	2.9398157469
S	1.6992955632	−0.9099361442	1.7441639478
N	2.5716162503	−1.0889816512	−1.540448094
C	3.594369331	−0.2055198614	−1.2714971316
O	4.7503129805	−0.4211567869	−1.6390519966

N	3.2962287478	0.9956683885	−0.6125855795
C	2.0813738975	1.3410589284	−0.063347652
O	1.8776817556	2.4310963949	0.4493062018
C	1.0236557365	0.2786257509	−0.1928115058
N	−0.2494645954	0.7136646285	−0.1114222277
C	−1.2671001899	−0.1526505274	−0.4611409733
C	−0.9880245781	−1.3903210901	−1.085144853
N	0.3409373722	−1.6781523884	−1.4481953174
C	1.3644773842	−0.8437075877	−1.096314936
C	0.6358623592	−2.8976705965	−2.1985738647
H	0.1498365217	0.8370428156	2.4236116362
H	−0.0263142031	−0.6072693029	3.4437694023
H	4.0828727412	1.6484713591	−0.4846970325
H	−0.5717081678	1.7918697505	0.5423909279
H	0.0579613291	−2.9141863684	−3.1287726096
H	1.7002333022	−2.8966070847	−2.4214704466
H	0.3821068366	−3.7740898661	−1.5924016928
C	−2.6094488958	0.1984780953	−0.23759836
H	1.3447851706	0.5088764856	3.6879240455
H	−2.0278904302	2.9380302856	1.1170062272
O	−3.2992605306	3.2807831048	0.8306816946
H	−0.5348210183	3.5166993951	0.8626086825
C	−2.0458197785	−2.2559960836	−1.3947032538
C	−3.3750226154	−1.9141812332	−1.1335233054
C	−3.6603994156	−0.6537442844	−0.5621262151
H	−1.8384811914	−3.2177757968	−1.8500531003
H	−2.8337412169	1.1748042482	0.1798253432
C	−4.4820030689	−2.8818533408	−1.479578216
H	−5.0739140466	−3.1595740863	−0.5966571884
H	−5.1879183697	−2.4543051349	−2.2049292524
H	−4.0802667682	−3.8044322116	−1.911542927
C	−5.080040738	−0.2104679582	−0.2922701162
H	−5.0923662593	0.8061146214	0.1115877761
H	−5.6914936795	−0.2209207916	−1.2051904244
H	−5.583578171	−0.8686669181	0.4297000053
C	−3.2083514292	4.3889808004	0.1627419296
O	−2.1588160965	4.9999463036	−0.0842270737
C	−4.5517062602	4.9345168123	−0.3333285384
H	−5.2433814807	5.0507448382	0.5090936809
H	−4.4163051836	5.8948512915	−0.8370556352
H	−5.0094884651	4.2194372439	−1.0275949627
H	5.9732568056	1.1430178201	−1.1011523102
N	6.0510332699	2.0640887437	−0.6603155123
H	6.396048156	2.7125176181	−1.3654456183
C	6.9570021289	2.0192116748	0.4871282789
H	6.5736306647	1.292242934	1.2102726763
H	7.9989210229	1.7399415199	0.2501038657
H	6.9755726348	2.9968840258	0.9826215365
S	0.1287784605	−4.498929327	1.7482156472
H	0.753232112	−3.2857103298	1.7138602906

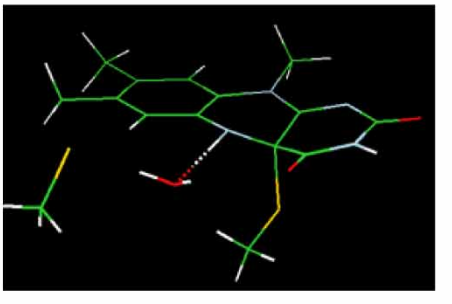
C	−1.5351157212	−3.8556070405	2.1853115614
H	−1.5246253021	−3.3719769406	3.1652752177
H	−1.89298192	−3.1534359228	1.4295710998
H	−2.2107625276	−4.7144301576	2.2232810322

Proton relay.



Reactant complex

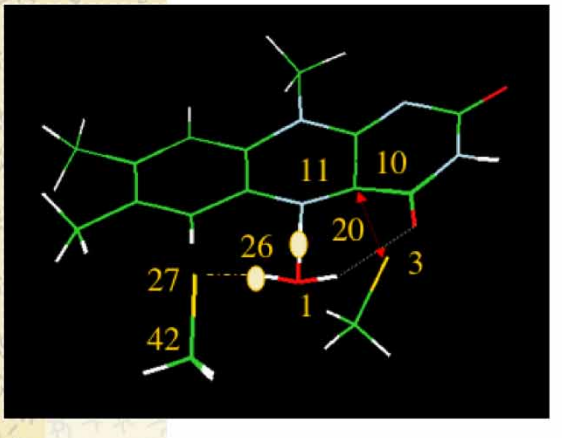
$MeSH + H_2O + CT\{Flox\ldots SMe\}$



Product complex

$MeS^- + H_2O + Adduct\{HFlSMe\}$

Proton relay. TS for simulteneous two-proton transfer and C-S bond formation.



ModRedundant input Section:

1 26 D
1 20 D
11 20 D
26 27 D
27 42 D
3 10 D

B3LYP/6-31G(d) freq=noraman opt=(ts,noeigen,ModRedun)