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Substituent Effect on the Efficiency of Desulfurizative Rearrangement of Allylic Disulfides

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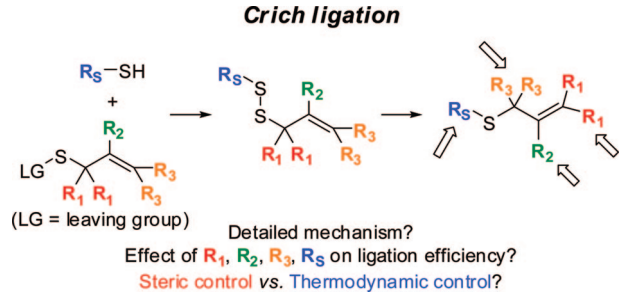

Crich ligation is a new method for the functionalization of peptides and proteins under mild conditions. To more fully understand the mechanism of the ligation and to explore the effect of substitution on its efficiency, a systematic theoretical study is carried out for the first time. It is found that the MP2 method wrongly predicts the substituent effect whereas the ONIOM(CCSD(T):B3LYP) method overestimates the free energy barriers by ca. 4 kcal/mol. Only the ONIOM(G3B3:B3LYP) method is found to be reliable as well as feasible for studying the ligation. The rate-limiting step of the ligation is found to be the [2,3]-sigmatropic rearrangement of the alkyl allyl disulfide, followed by an S_N2 phosphine-mediated desulfurization. The S−S bond is significantly polarized during the rearrangement and, therefore, the reaction proceeds more rapidly in polar solvents. R_S and R_3 substitutions elevate the free energy barrier of the ligation, whereas the R_2 substitution does not exert a useful effect. Only the substitution at R_1 can effectively reduce the free energy barrier of the reaction to less than 20 kcal/mol (a value required to allow the reaction to complete in minutes at 25 °C). Therefore, secondary and tertiary allyl alkyl disulfides can undergo the ligation at the room temperature. Marcus theory analysis indicates that the major factor for the retardation of the reaction by substituents at R_S and R_3 and for the acceleration by substituents at R_1 is the thermodynamic equilibrium between the disulfide and thiosulfoxide. To shift the equilibrium to favor the ligation, placement of substituents at R_1 is obligatory for alkyl allyl disulfides. Nonetheless, alkyl buta-2,3-dienyl disulfides may also undergo the ligation at room temperature without the help of the R_1 substituent.

1. Introduction

The development of increasingly efficient and selective chemical methods for the ligation between polyfunctionalized peptides and proteins, and for the attachment of small molecules and oligosaccharides to proteins remains a challenging frontier in both bioorganic chemistry and chemical biology.[1] Mild reaction conditions, compatibility with water-containing media, and high chemoselectivity are the basic requirements for such chemical methods. Famous recent examples in this field include the Huisgen azide−alkyne cycloaddition,[2] the native chemical ligation,[3] and the Staudinger ligation.[4] The applications of these ligation approaches have greatly expedited the research on various medicinally important biomacromolecules.

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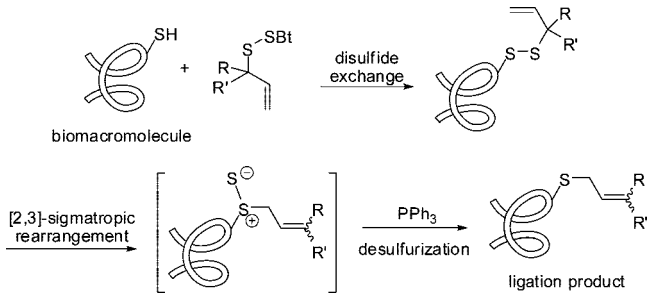
SCHEME 1. The Crich Ligation



In addition to the above popular approaches, the formation of a mixed disulfide is also an established ligation method. The application of the disulfide ligation has been successful in a number of related disciplines,[5] but an often encountered problem of this ligation is that the disulfide bond is not stable in the presence of thiols and other reducing agents.[6] To overcome the impermanence problem of the disulfide ligation method, Crich et al. recently invented an ingenious method of chemical ligation based on the facile formation of mixed allylic disulfides, which are then rendered permanent by an interesting desulfurizative [2,3]-sigmatropic rearrangement producing a stable thioether linkage (Scheme 1).[7–10]

Note that the Crich ligation can make use of both allylic selenosulfides and disulfides.[7,8] However, the seleno version of the ligation could not be well adapted for the preparation of primary allyl sulfides from thiols because of complications in the synthesis of the required tertiary selenosulfides.[10] Accordingly, it is expected that the ligation based on the rearrangement of allylic disulfide[8] would be a more durable method in future applications. Additional studies on the disulfide version of the ligation will possibly be directed to the improvement of the ligation efficiency and the examination of its utility in the functionalization of increasingly more complexed peptides and proteins.

In this context we consider it to be warranted to carry out a systematic theoretical study on the detailed mechanism of the Crich ligation. With a clear mechanism in hand we next examine theoretically the effect of substitution at several potential positions on the efficiency of the ligation. By comparing the theoretical predictions with the available experimental results we can confirm the validity of our theoretical model. The agreement between theory and experiment also indicates that the theoretical model developed in the present study may be helpful in the future improvement of the ligation and study of related [2,3]-sigmatropic rearrangement reactions.

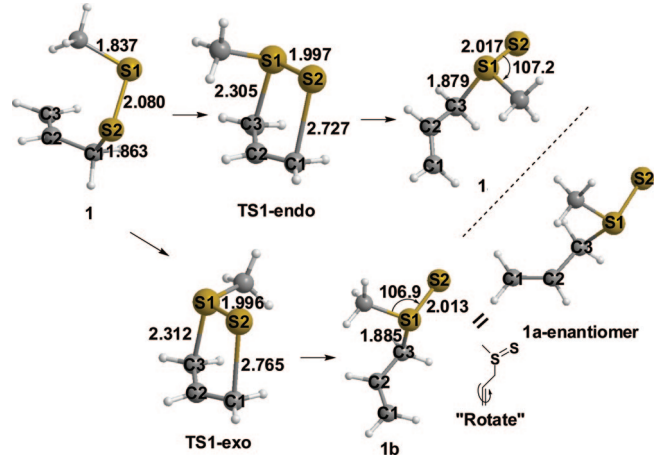


FIGURE 1. [2,3]-Sigmatropic rearrangement of disulfide **1** to thiosulfoxide **1a** and **1b** (bond lengths in Å; bond angles in deg).

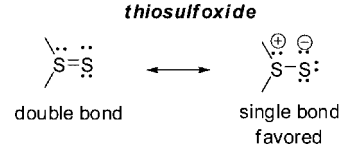


FIGURE 2. Resonance structures of thiosulfoxides.

2. General Mechanism

The mechanism of the Crich ligation was proposed to proceed through a [2,3]-sigmatropic rearrangement of the allylic disulfide intermediate followed by a phosphine-mediated desulfurization reaction.[10] This proposed mechanism relates the ligation to several other famous transformations of organosulfur compounds including the Evans−Mislow rearrangement of allylic sulfoxides,[11] the [2,3]-sigmatropic rearrangement of allyl sulfinyl carbanions,[12] and the [2,3]-Wittig rearrangement of allylic sulfur ylides.[13]

2.1. [2,3]-Sigmatropic Rearrangement. To more fully understand the Crich ligation, we have optimized the ground-state, transition-state, and product structures of the [2,3]-sigmatropic rearrangement of a model allylic disulfide **1** (Figure 1). The density functional theory B3LYP method and the 6-31G* basis set are used for the geometry optimization. Note that the validity of using the B3LYP functional to investigate the sigmatropic rearrangements has been shown in several previous theoretical studies. Recent examples include the studies on the anionic [2,3]-sigmatropic Wittig rearrangements of deprotonated 4-hetera-1-pentenes,[14] on the sigmatropic rearrangement of silyl amides to *N-cis*-propenyl amides,[15] on the Lewis acid-mediated [2,3]-sigmatropic rearrangement of allylic α-amino amides,[16] on the [3,3]-sigmatropic Johnson−Claisen rearrangements,[17] and on the Pd(II)-promoted [3,3]-sigmatropic

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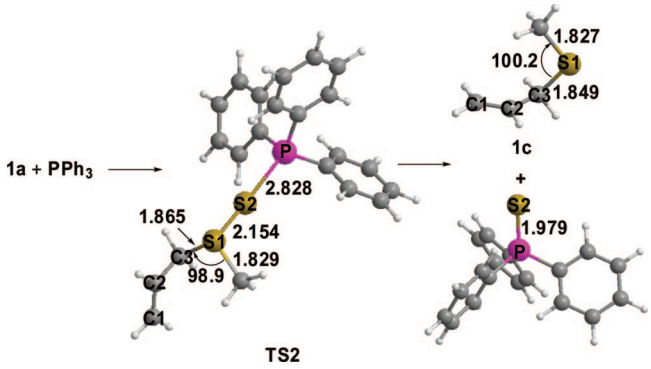

FIGURE 3. Desulfurization mediated by triphenylphosphine (bond lengths in Å, bond angles in deg).

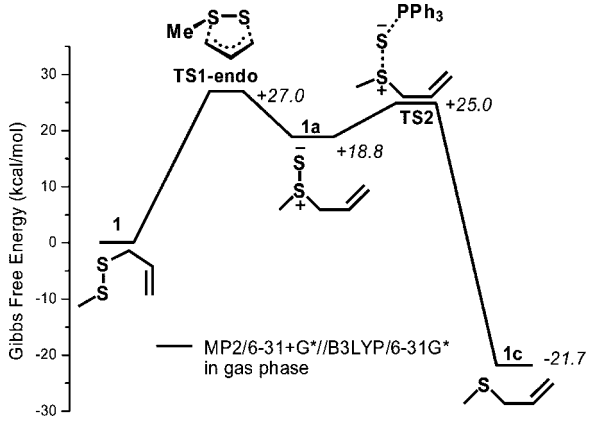

FIGURE 4. Free energy profile for the desulfurizative rearrangement of **1** to **1c** (25 °C).

TABLE 1. Solvation Free Energies (ΔG_{sol}) and Free Energy Barriers ($\Delta G^{\neq}$) for the [2,3]-Sigmatropic Rearrangement of Disulfide 1 in Gas-Phase, Benzene, and Methanol (kcal/mol, 25 °C)[a]

solvent	ΔG_{sol} (**1**)	ΔG_{sol} (**TS1-endo**)	$\Delta G^{\neq}$ (**TS1-endo**)	ΔG_{sol} (**TS1-exo**)	$\Delta G^{\neq}$ (**TS1-exo**)
gas	0.0	0.0	+27.0	0.0	+28.4
benzene	+2.2	+1.0	+25.7	+1.0	+27.2
methanol	−0.7	−3.3	+24.4	−3.4	+25.7

[a] Note: Gas-phase calculations are conducted by using the MP2/6-31+G*//B3LYP/6-31G* method, whereas the solvation free energies are calculated by using the IEF-PCM method with UA0 radii.

Cope rearrangement.[18] Furthermore, the change of 6-31G* basis set to more flexible basis sets (e.g., 6-311++G**) is not found to produce any significant effect on the calculations.

Through theoretical calculations it is found that compound **1** prefers a nonplanar conformation where the C−S−S−C dihedral angle is 91.7°. The cause of this nonplanar conformation has been previously discussed in terms of the "gauche effect".[19] The S−S bond length of **1** is 2.080 Å. From the Natural Bond Orbital (NBO)[20] analysis it is found that the bond order of this S−S bond is 1.06, which indicates that the S−S bond in **1** is a typical single bond. The NBO charge on S_1 is +0.086 and the charge on S_2 is +0.080, which indicates that the S−S bond in **1** is largely a nonpolar bond.

From **1** to the rearranged product we have successfully located two transition state structures. They are named **TS1-endo** and **TS1-exo** respectively to designate the relative orientation of the S1-substituent within the five-membered ring. The gas-phase free energy barrier for **TS1-endo** is calculated to be +27.0 kcal/mol at the MP2/6-31+G*//B3LYP/6-31G* level, whereas the free energy barrier for **TS1-exo** is +28.4 kcal/mol at the same level of theory. Therefore, the endo mode is favored in the rearrangement, which is consistent with some recent studies on related sigmatropic rearrangements.[21] The bond order of the S−S bond in **TS1-endo** is 1.27, a value higher than that of compound **1**. Accordingly the S−S bond in **TS1-endo** is 1.997 Å and shorter than that in **1**. The NBO charge on S_1 is +0.418 and the charge on S_2 is −0.348, indicating that the S−S bond is significantly polarized in the transition state.

The product of the rearrangement is thiosulfoxide **1a** or **1b** as derived from **TS1-endo** or **TS1-exo**, respectively. These two thiosulfoxides are enantiomers to each other and are produced in an equal quantity through the rearrangement (note that **1a** or **1b** can also be produced from the enantiomer of **TS1-exo** or **TS1-endo**, respectively). The bond length of the S−S bond in **1a** (or **1b**) is 2.017 Å, which is shorter than that in **1** (2.080 Å) but longer than that in **TS1-endo** (1.997 Å). In agreement with this observation, the bond order of the S−S bond in **1a** is calculated to be 1.19, a value higher than that in **1** (1.06) but lower than that in **TS1-endo** (1.27). This bond order value shows that the S−S bond in the thiosulfoxide is principally a single bond (Figure 2).[22] The NBO charge on the S_1 atom is +0.669 and the charge on S_2 is −0.536.

2.2. Desulfurization. The free energy change for the rearrangement from **1** to **1a** is +18.8 kcal/mol in the gas phase. Thus, to drive the reaction to completion the thiosulfoxide must be rapidly reduced to form the final thioether product. Here we theoretically examine the phosphine-mediated reduction of the thiosulfoxide intermediate. Note that the mechanisms of disulfide bond reductions by phosphines and by thiols were theoretically studied very recently by Bach and co-workers.[23] It was shown that gas-phase S_N2 attack of a tertiary phosphine on a neutral disulfide most likely proceeded through a phosphonium cation−thiolate anion salt intermediate, whereas in protic solvents a typical uncomplicated S_N2 pathway was anticipated due to the availability of H-bonding to stabilize the departing thiolate anion.[23]

In comparison to Bach's study, we propose that PPh_3-mediated reduction of thiosulfoxide should proceed through a direct S_N2 pathway without any explicit involvement of the solvent molecules. This argument is supported by two reasons: (1) the reduction can take place smoothly in benzene where the H-bonding is not available and (2) a thiosulfoxide is already a highly polarized molecule so that its reduction by PPh_3 does not cause a significant charge separation. Indeed, through geometry optimization we readily locate the transition state for the reduction step. We also find that the reduction is highly exothermic in the gas phase.

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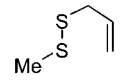
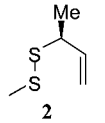
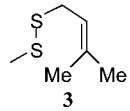
TABLE 2. Comparison of the Estimated Experimental and Theoretical Free Energy Barriers (60 °C, in Benzene, in kcal/mol)[a]

Compound	Est. Exp.	MP2/ 6-31+G*	MP2/ 6-311++G**	ONIOM (CCSD(T):B3LYP)	ONIOM (G3B3:B3LYP)
 **1**	24.2	25.9	26.7	28.7	23.7
 **2**	22.4	25.6	26.5	26.0	20.8
 **3**	25.9	23.5	23.8	29.9	25.9

[a] Gibbs free energy corrections and solvation free energies are added by using the theoretical values at the B3LYP/6-31G* level calculated for 60 °C.

Figure 3 shows the transition state (**TS2**) for PPh_3-mediated reduction of thiosulfoxide **1a** optimized by the B3LYP/6-31G* method. In **TS2** the S−S bond elongates from 2.017 Å (as in **1a**) to 2.154 Å. The free energy of **TS2** is +25.0 kcal/mol as calculated relative to the starting material **1**. This value is lower than the free energy of **TS1-endo** (+27.0 kcal/mol) indicating that the rate-determining step in the overall transformation is the [2,3]-sigmatropic rearrangement of the disulfide to the thiosulfoxide intermediate (Figure 4). This supposition is consistent with the experimental observation that the reaction is of pseudo-first order depending only on the concentration of the disulfide.[24]

The overall reaction free energy for the transformation from disulfide **1** to thioether **1c** is calculated to be −21.7 kcal/mol. As a result the phosphine-mediated desulfurizative rearrangement of allylic disulfides is strongly favored in thermodynamics, which constitutes the main driving force for the success of the ligation.

2.3. Solvent Effect on the Efficiency of Crich Ligation. The above calculations indicate that the rate-determining step in the ligation is the [2,3]-sigmatropic rearrangement of the disulfide. Consequently in the following studies we focus only on the [2,3]-sigmatropic rearrangement step. Note that the above calculations are conducted in the gas phase, whereas a useful ligation should take place in the solution. As a result, it is important to know the effect of solvation on the efficiency of the [2,3]-sigmatropic rearrangement of an allyl alkyl disulfide.

To this end we calculate the solvation free energies for compound **1** and **TS-endo** in benzene (to represent a nonpolar solvent) and methanol (to represent a polar solvent). The IEF-PCM solvation model with the UA0 radii[25] is used in our calculation at the B3LYP/6-31G* level, whose validity in handling the solvation of organic compounds in various organic solvents has been recently confirmed.[26] Through the calculation it is found that the solvation free energy of **1** in benzene is +2.2 kcal/mol, whereas the solvation free energy of **TS1-endo** in benzene is +1.0 kcal/mol (Table 1). The more favorable solvation of the transition state is consistent with the observation that the S−S bond in **TS1-endo** is more polarized than that in disulfide **1** (Section 2.1). As a result, the free energy barrier in benzene is +25.7 kcal/mol, which is 1.3 kcal/mol lower than that in the gas phase.

In comparison to benzene (dielectronic constant = 2.25), the solvation free energies of **1** and **TS1-endo** in methanol (dielectronic constant = 32.63) are −0.7 and −3.3 kcal/mol, respectively. These solvation free energies cause a further reduction of the free energy barrier to +24.4 kcal/mol in methanol, which is 1.3 kcal/mol lower than that in benzene. This magnitude of barrier reduction is consistent with the experimental observation that the rate of desulfurizative rearrangement of 1,3-dimethylbut-2-enyl *tert*-butyl disulfide with PPh_3 increases by 6−10-fold when the solvent is changed from benzene to ethanol/benzene (volume ratio 9/2).[27] Thus, polar solvents such as methanol stabilize the transition state more than the disulfide and consequently accelerate the ligation.

Note that in Table 1, the free energy barrier for **TS1-exo** is always higher than that for **TS1-endo**. Therefore, the solvent effect does not seem to be able to switch the endo/exo selectivity in the rearrangement reaction. Furthermore, the positive solvation free energies in benzene are presumably caused by the nonelectrostatic contributions in the solvation process.[26]

2.4. Improve the Accuracy of Energy Calculation. In the above energy calculations we have temporarily used the MP2/6-31+G*//B3LYP/6-31G* method.[28] Here we seek to improve the accuracy of the energy calculation by comparing the performances of different levels of theoretical approaches.

According to the experiment, the pseudo-first-order rate constants for the phosphine-mediated rearrangement of compounds **1**−**3** (Table 2) are 8.9×10^{-4}, 140×10^{-4}, and 0.7×10^{-4} s^{-1} at 60 °C in benzene, respectively.[24] These rate constants are translated to free energy barriers of 24.2, 22.4, and 25.9 kcal/mol (at 60 °C) by using transition-state theory with the transmission coefficient equated to unity. Our calculation shows that the MP2/6-31+G* method slightly overestimates the free energy barrier of **1** by +1.7 kcal/mol. Further examinations, however, reveal that the MP2 method surprisingly fails to predict the trend of the substituent effects, where **3** is wrongly predicted

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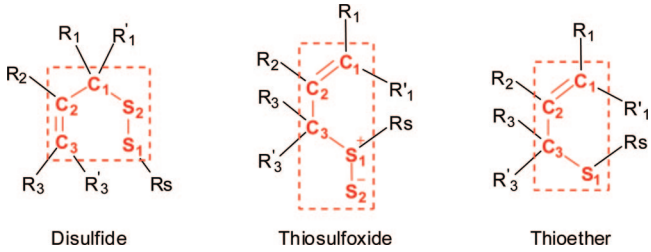


FIGURE 5. ONIOM layers and atom numbering scheme (the atoms in the rectangular dashed lines belong to the core layer, while the remaining atoms belong to the low layer).

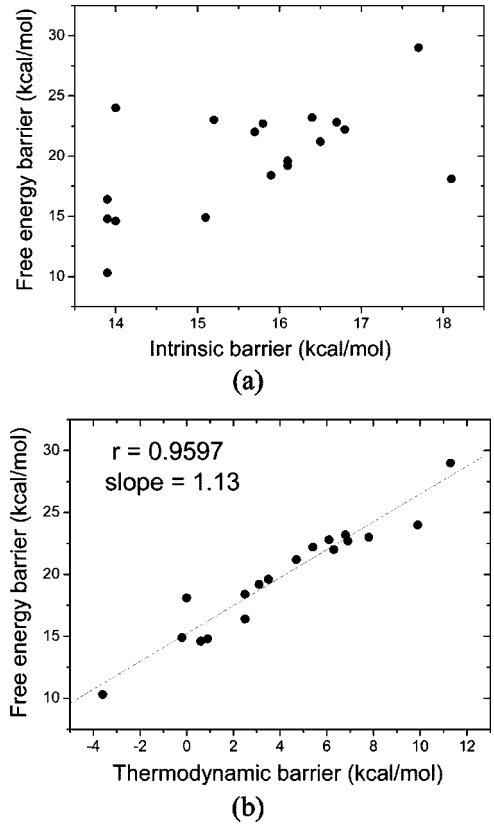
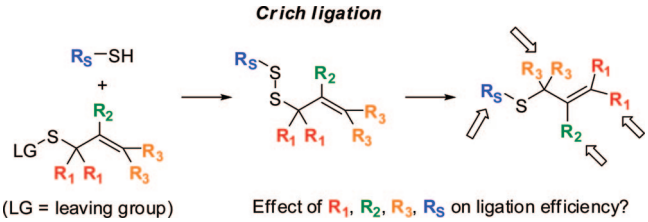


FIGURE 6. Dependence of the reaction free energy barriers on the intrinsic and thermodynamic barriers.

to have a lower barrier than **1**.[29] The use of a more flexible basis set (i.e., 6-311++G**) does not correct this error.

At this point we decided to use higher level ab initio methods (e.g., CCSD(T) and G3B3). To compromise the accuracy and cost we implement the ONIOM strategy (Figure 5).[30] The atoms directly involved in the sigmatropic rearrangement are placed in the core layer and treated by the CCSD(T)/6-311++G** or G3B3 method, whereas the remaining atoms are placed in the low layer and handled at the B3LYP/6-31G* level. Using this approach the free energy barriers of compounds **1**−**3** are calculated to be 28.7, 26.0, and 29.9 kcal/mol at the ONIOM-(CCSD(T):B3LYP) level. These values are about 4 kcal/mol higher than the estimated experimental results. By comparison, the ONIOM(G3B3:B3LYP) method provides predictions of 23.7, 20.8, and 25.9 kcal/mol, which are in fairly good agreement with the estimated experimental barriers with an error bar of ≤1.6 kcal/mol (note: chemical accuracy = 1 kcal/mol). Therefore, the ONIOM(G3B3:B3LYP) method constitutes a reliable as well as feasible method for further theoretical study on the Crich ligation.

SCHEME 2. Possible Substitutions That Can Affect the Efficiency of the Crich Ligation



3. Effect of Substitution on the Efficiency of Crich Ligation

The Crich ligation principally attaches an allyl group to a free thiol in a biomacromolecule. The thiol groups tested in Crich's study included the cysteine residues and the thiolated carbohydrates.[10] Meanwhile, the allyl groups tested by Crich et al. mostly carried one or two substituents at the R_1 position (Scheme 2). A thorough understanding of the effect of substitution at the R_S and R_1 positions on the efficiency of the ligation is needed at the present time. It also is relevant and interesting to study how the substitution at the R_2 and R_3 positions will affect the same ligation.[31]

Here we systematically explore, for the first time, the effects of every possible substitution on the efficiency of the ligation by using the established ONIOM(G3B3:B3LYP) method. The mechanistic origins of the substituent effects are also discussed in depth. Note that in the following study we only consider the *trans*-olefins for both the starting material and the product of the rearrangement.

3.1. Effect of R_S Substitution. The thiol groups tested in Crich's study included the cysteine residues and the thiolated carbohydrates.[10] It was found that thiolated carbohydrates (secondary thiols) underwent ligation at 80 °C but the ligation of cysteine residues (primary thiols) only required room temperature. Here we have calculated the free energy barriers for the [2,3]-sigmatropic rearrangement of S_1-substituted disulfides carrying Me, Et, *i*-Pr, *t*-Bu, Bn, Ph, and β-D-glucopyranosyl groups (Table 3).

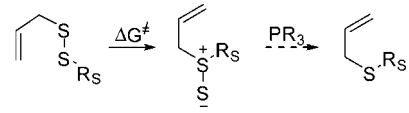
The results indicate that the free energy barrier of the ethyl-substituted disulfide **4** (21.2 kcal/mol) is slightly lower than that of the methyl-substituted compound **1** (22.2 kcal/mol) by 1.0 kcal/mol (note that the temperature in Table 3 is 25 °C and different from that in Table 2). The benzyl-substituted disulfide **7** exhibits an even lower barrier of +19.6 kcal/mol. These observations may be attributed to the fact that ethyl and benzyl are better electron donors than the methyl group. On the other hand, the isopropyl and *tert*-butyl-substituted disulfides **5** and **6** show barriers of 22.0 and 22.8 kcal/mol. These values are higher than that for **4**, indicating that a disulfide carrying more bulky substituents at R_S disfavors the rearrangement. Furthermore, the barriers of the phenyl-substituted disulfide **8** (22.7 kcal/mol) and the thiolated carbohydrate **9** (23.2 kcal/mol) are also higher than that of **1**.

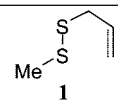
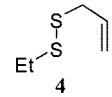
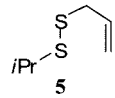
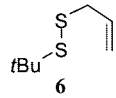
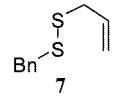
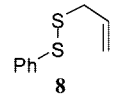
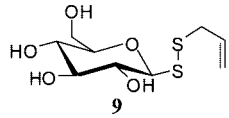
(29) Problems of using MP2 to study pericyclic reactions were previously discussed. See: Ess, D. H.; Houk, K. N. *J. Phys. Chem. A* **2005**, *109*, 9542.

(30) Review: Dapprich, S.; Komiromi, I.; Byun, K. S.; Morokuma, K.; Frisch, M. J. *THEOCHEM* **1999**, *461*−*462*, 1.

(31) Note: earlier studies by Hoefle and Baldwin on the equilibrium between alkyl allyl disulfides and thiosulfoxides indicated that increased bulk at R_3 reduced the concentrations of the thiosulfoxides. See ref 24.

TABLE 3. Free Energy Barriers ($\Delta G^{\neq}$), Intrinsic Barriers ($\Delta G_0^{\neq}$), Thermodynamic Barriers ($\Delta G_{thermo}^{\neq}$), and Reaction Free Energies (ΔG_R) for the [2,3]-Sigmatropic Rearrangement of R_S-Substituted Disulfides in Methanol (in kcal/mol, 25 °C)



Entry	Disulfide	$\Delta G^{\neq}$	$\Delta G_0^{\neq}$	$\Delta G_{thermo}^{\neq}$	ΔG_R
1	 **1**	22.2	16.8	5.4	9.9
2	 **4**	21.2	16.5	4.7	9.1
3	 **5**	22.0	15.7	6.3	11.6
4	 **6**	22.8	16.7	6.1	11.2
5	 **7**	19.6	16.1	3.5	8.4
6	 **8**	22.7	15.8	6.9	12.5
7	 **9**	23.2	16.4	6.8	12.5

The above results show that substituents produce effects on the efficiency of Crich ligation. To further understand the origin of these effects in terms of steric hindrance or thermodynamic factors, we decided to utilize the Marcus theory[32] to separate the intrinsic and thermodynamic contributions to the calculated free energy barriers. Briefly, the Marcus theory can be described by the following equation

$$\Delta G^{\neq} = \Delta G_0^{\neq} + \frac{1}{2}\Delta G_R + \frac{(\Delta G_R)^2}{16\Delta G_0^{\neq}} = \Delta G_0^{\neq} + \Delta G_{thermo}^{\neq} \quad (1)$$

where the free energy barrier ($\Delta G^{\neq}$) of a nondegenerate reaction is the sum of the intrinsic barrier ($\Delta G_0^{\neq}$) and the thermodynamic contribution ($\Delta G_{thermo}^{\neq}$). The intrinsic barrier corresponds to a hypothetical thermoneutral process (i.e., a degenerate transformation). The thermodynamic contribution is an estimate of the change in the activation energy due to the variation of reaction thermodynamics, which is based on an assumption that the hypersurface of potential energy behaves like two overlapping parabolas representing reactant and product energies. Originally the Marcus theory was developed for the electron-transfer reactions.[32] More recently the Marcus theory has also been successfully applied to several organic reactions including the [1,5]-hydrogen shifts[33] and radical cyclizations.[34]

Using the Marcus theory it is straightforward to calculate the intrinsic barrier ($\Delta G_0^{\neq}$):

$$\Delta G_0^{\neq} = \frac{1}{2}\left[\Delta G^{\neq} - \frac{1}{2}\Delta G_R + \sqrt{(\Delta G^{\neq})^2 - \Delta G^{\neq}\cdot\Delta G_R}\right] \quad (2)$$

With the intrinsic barrier in hand, we are then able to calculate the thermodynamic contribution to the overall free energy barrier using the following equation.

$$\Delta G_{thermo}^{\neq} = \Delta G^{\neq} - \Delta G_0^{\neq} \quad (3)$$

An important advantage of using Marcus theory is that we can now quantitatively analyze the mechanism of substituent effects. The Marcus theory allows us to separate the intrinsic contributions under a thermoneutral condition (for example, steric hindrance in the transition state) from the thermodynamic reasons (i.e., reactivity change because the reaction is more exothermic or endothermic).

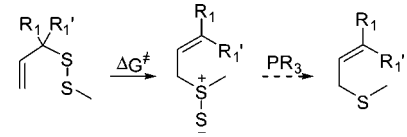
The results in Table 3 show that from entry 1 to 7 the intrinsic barriers (i.e., $\Delta G_0^{\neq}$) range from 15.7 to 16.8 kcal/mol, whereas the thermodynamic barriers range more significantly from 3.5 to 6.9 kcal/mol. The higher reactivity of the ethyl- and benzyl-substituted disulfides stems mainly from the thermodynamic factors because their thermodynamic barriers are 0.7 and 1.9 kcal/mol lower than that of **1**. On the other hand, the lower reactivity of disulfide **6**, **8**, and **9** is consistent with the

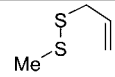
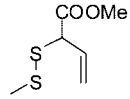
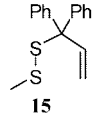
(32) (a) Marcus, R. A. *J. Chem. Phys.* **1956**, *24*, 966. (b) Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 891.

(33) Alabugin, I. V.; Manoharan, M.; Breiner, B.; Lewis, F. L. *J. Am. Chem. Soc.* **2003**, *125*, 9329.

(34) (a) Alabugin, I. V.; Manoharan, M. *J. Am. Chem. Soc.* **2005**, *127*, 12583. (b) Alabugin, I. V.; Manoharan, M. *J. Am. Chem. Soc.* **2005**, *127*, 12583. (c) Wu, C. W.; Ho, J. J. *J. Org. Chem.* **2006**, *71*, 9595. (d) Yu, Y. Y.; Fu, Y.; Xie, M.; Liu, L.; Guo, Q. X. *J. Org. Chem.* **2007**, *72*, 8025. (e) Shi, J.; Chong, S.-S.; Fu, Y.; Guo, Q.-X.; Liu, L. *J. Org. Chem.* **2008**, *73*, 974.

TABLE 4. Free Energy Barriers ($\Delta G^{\neq}$), Intrinsic Barriers ($\Delta G_0^{\neq}$), Thermodynamic Barriers ($\Delta G_{thermo}^{\neq}$), and Reaction Free Energies (ΔG_R) for the [2,3]-Sigmatropic Rearrangement of R_1-Substituted Disulfides in Methanol (in kcal/mol, 25 °C)



Entry	Disulfide	$\Delta G^{\neq}$	$\Delta G_0^{\neq}$	$\Delta G_{thermo}^{\neq}$	ΔG_R
1	 **1**	22.2	16.8	5.4	9.9
2	 **2**	19.2	16.1	3.1	5.9
3	 **10**	18.4	15.9	2.5	4.8
4	 **11**	14.8	13.9	0.9	1.7
5	 **12**	14.6	14.0	0.6	1.3
6	 **13**	16.4	13.9	2.5	4.9
7	 **14**	14.9	15.1	-0.2	-0.3
8	 **15**	10.3	13.9	-3.6	-7.7

observation that their thermodynamic barriers are all above +6 kcal/mol. As a result, the effect of R_S-substiution stems mainly from the thermodynamic factors. To support this argument, the reaction free energies for compounds **1**, **4**, and **7** are +8−9 kcal/mol whereas the reaction free energies for compounds **6**, **8**, and **9** are +11−12 kcal/mol.

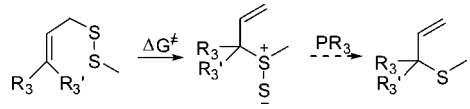
3.2. Effect of R_1 substitution. Crich et al. have examined several allyl disulfides carrying one or two alkyl substituents at the R_1 position.[10] These substrates are potentially useful for the functionalization of proteinogenic thiols with biological groups such as the farnesyl chains. Here we examine the effect of more types of R_1 groups on the efficiency of the ligation where R_1 does not have to equal an alkyl group (Table 4).

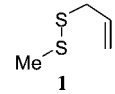
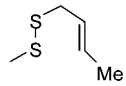
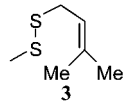
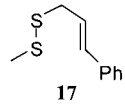
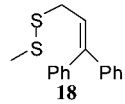
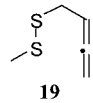
The results show that the introduction of a single methyl or ethyl group at the R_1 position will reduce the free energy barrier by 3−4 kcal/mol for compounds **2** and **10**. This dramatic free energy reduction is consistent with Crich's observation that secondary and tertiary, but not primary, allyl akyl disulfides were able to undergo the disulfurizative rearrangement at room temperature.[10] The introduction of a single ester or phenyl group at the R_1 position will further decrease the free energy barrier to 14.8 and 14.6 kcal/mol for compounds **11** and **12**. These substituents are conjugated with the double bond in the products but not in the reactants. Their remarkably low barriers are comparable to the free energy barrier (i.e., 14.9 kcal/mol) of the diethyl compound (i.e., **14**). The diphenyl compound (i.e., **15**) shows an even lower free energy barrier of 10.3 kcal/mol.

Through the Marcus theory analysis it can be readily recognized that the major driving force for the acceleration effect of the R_1 substitution is the thermodynamic factor. The intrinsic barrier ($\Delta G_0^{\ddagger}$) varies in the range from 13.9 to 16.8 kcal/mol. By comparison, the thermodynamic barrier ($\Delta G_{thermo}^{\ddagger}$) changes more dramatically from +5.4 kcal/mol for compound **1** to −3.6 kcal/mol for compound **15**.

3.3. Effect of R_3 Substitution. In earlier studies by Hoefle and Baldwin on the equilibrium between alkyl allyl disulfides and thiosulfoxides it was proposed that increased bulk at R_3 reduced the concentrations of the thiosulfoxides in the equilib-

TABLE 5. Free Energy Barriers ($\Delta G^{\neq}$), Intrinsic Barriers ($\Delta G_0^{\neq}$), Thermodynamic Barriers ($\Delta G_{thermo}^{\neq}$), and Reaction Free Energies (ΔG_R) for the [2,3]-Sigmatropic Rearrangement of R_3-Substituted Disulfides in Methanol (in kcal/mol, 25 °C)[a]



Entry	Disulfide	$\Delta G^{\neq}$	$\Delta G_0^{\neq}$	$\Delta G_{thermo}^{\neq}$	ΔG_R
1	 **1**	22.2	16.8	5.4	9.9
2	 **16**	23.0	15.2	7.8	14.0
3	 **3**	24.0	14.0	9.9	17.2
4	 **17**	29.0	17.7	11.3	19.8
5[a]	 **18**	-	-	-	-
6	 **19**	18.1	18.1	0.0	0.0

[a] Note: The transition state for the rearrangement of compound **18** cannot be stably located after many failed attempts.

rium.[24] Here we examine the [2,3]-sigmatropic rearrangement of alkyl allyl disulfides carrying one or two methyl and phenyl groups (Table 5).

The results show that the placement of one methyl group at R_3 will increase the free energy barrier by 0.8 kcal/mol, whereas two methyl groups at R_3 will increase the free energy barrier by 1.8 kcal/mol. These magnitudes of barrier increase will slow down the rearrangement by ca. 10-fold. By comparison, the placement of one phenyl group at R_3 will increase the free energy barrier by 6.2 kcal/mol. It is expected that these barriers are too high for the ligation to take place. Through the Marcus theory analysis it is evident that the high barriers for compounds **3**, **16**, and **17** can be attributed to the unfavorable thermodynamic barriers.

As a special R_3-substituted disulfide, compound **19** is interesting from the synthetic point of view. This substrate has an allene group that is compatible with most of the functional groups present in the biomacromolecules. Its [2,3]-sigmatropic rearrangement produces a diene moiety that may be further utilized for Diels−Alder ligation of proteins.[35] Significantly it is found that **19** has a free energy barrier of 18.1 kcal/mol, which is slightly lower than that of **2** (19.2 kcal/mol) and **10** (18.4 kcal/mol). Compounds with a substitution pattern very similar to that of **2** and **10** were known to undergo the Crich ligation at room temperature.[8] As a result, **19** should also be able to undergo the rearrangement readily at room temperature and, therefore, be useful in realistic applications. The low free energy barrier of **19** can be attributed to the favorable thermodynamics of the transformation ($\Delta G_{thermo}^{\neq}$ is 0.0 kcal/mol) from a cumulene into a conjugated 1,3-diene.

3.4. Effect of R_2 Substitution. The effect of R_2 substitution has not been investigated in depth previously. This type of substitution may affect the rearrangement through the electronic effect. Because it has been demonstrated that the S−S bond in the transition state is significantly polarized (Section 2.1), we are very curious about the possible effect of R_2 substitution on the efficiency of the ligation.

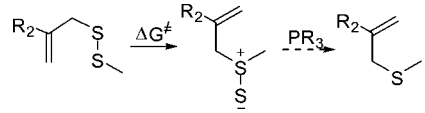
Unfortunately, the calculation results (Table 6) show that the R_2 effect is not as significant as expected. Most of the R_2 substituents (i.e., CF_3, F, Ph) change the free energy barrier by less than 1.5 kcal/mol. Electron-withdrawing substituents (such CN and CF_3) at R_2 appear to have an acceleration effect on the rearrangement. However, such substituents lead to electron-deficient olefins that may react with thiols through 1,4-addition complicating the ligation. By contrast, the OMe substitution at R_2 increases the free energy barrier by 3.2 kcal/mol.

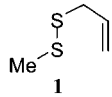
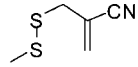
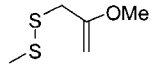
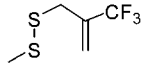
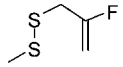
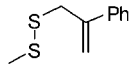
3.5. Intrinsic vs Thermodynamic Barriers. In the above sections it has been found that the efficiency of the rearrangement reaction is determined mainly by the thermodynamic factor. Here we further confirm this argument by plotting the free energy barriers against the intrinsic and thermodynamic barriers for all the compounds listed in Tables 3–6 (Figure 6).

The results evidently show that the free energy barriers do not have a strong dependence on the intrinsic barriers (Figure 6a). The acceleration or retardation of the reaction by the substitution cannot be interpreted as the result of either a lower or higher steric hindrance introduced by the substituents. On the other hand, we observe a strong linear dependence of the

(35) Dantas de Araujo, A.; Palomo, J. M.; Cramer, J.; Seitz, O.; Alexandrov, K.; Waldmann, H. *Chem. Eur. J.* **2006**, *12*, 6095.

TABLE 6. Free Energy Barriers ($\Delta G^{\neq}$), Intrinsic Barriers ($\Delta G_0^{\neq}$), Thermodynamic Barriers ($\Delta G_{\text{thermo}}^{\neq}$), and Reaction Free Energies (ΔG_R) for the [2,3]-Sigmatropic Rearrangement of C_2-Substituted Disulfides in Methanol (in kcal/mol, 25 °C)



Entry	Disulfide	$\Delta G^{\neq}$	$\Delta G_0^{\neq}$	$\Delta G_{\text{thermo}}^{\neq}$	ΔG_R
1	 **1**	22.2	16.8	5.4	9.9
2	 **20**	20.5	13.6	6.9	12.3
3	 **21**	25.4	20.0	5.4	10.2
4	 **22**	21.6	15.4	6.2	11.4
5	 **23**	23.4	17.0	6.4	11.7
6	 **24**	23.4	17.4	6.0	11.2

reaction free energy barriers on the thermodynamic barriers (Figure 6b, correlation coefficient = 0.9597). Due to this reason, the efficiency of the Crich ligation should be interpreted mainly in terms of reaction thermodynamics, namely, the equilibrium from the disulfide to the thiosulfoxide.

Note that the above conclusion cannot be readily drawn from any early or late transition state theory, because the rearrangement transition state (see Figure 1) is geometrically as well as chemically very different from both the starting material (disulfide) and the product (thiosulfoxide). Also remarkable is the slope of the correlation (1.13), which is close to unity and deviates from the earlier empirical relationships going back to Dimroth and Bronsted where $\Delta\Delta E^{\neq} = {}^1/_2\Delta\Delta E_{\text{rxn}}$.[36] The physical origin of this intriguing exothermodynamic relationship and its generalization to other types of [2,3]-sigmatropic rearrangement reactions are the subject of ongoing studies.

4. Conclusion

The Crich ligation represents a new interesting chemoselective method for the functionalization of peptides and proteins under biocompatible conditions. To more fully understand the detailed mechanism of the ligation and to explore the effect of substitutions on its efficiency, we carry out theoretical calculations to study this new ligation method. Through the study we can now make the following conclusions.

1. The MP2 method wrongly predicts the substituent effect on the ligation efficiency. The ONIOM(CCSD(T):B3LYP) method correctly predicts the substituent effects but overestimates the free energy barriers by ca. 4 kcal/mol. The ONIOM(G3B3:B3LYP) method is found to be reliable as well as feasible for modeling the Crich ligation reactions.

2. The rate-determining step of the Crich ligation is the [2,3]-sigmatropic rearrangement of the alkyl allyl disulfide, which is followed by a phosphine-mediated desulfurization step proceeding through a typical uncomplicated S_N2 pathway. The S−S bond is significantly polarized during the sigmatropic rearrangement and, therefore, the reaction proceeds more rapidly in polar solvents.

3. Theoretical calculations show that R_S and R_3 substitutions elevate the free energy barrier of the Crich ligation, whereas the R_2 substitution does not exert a useful effect. Only the substitution at R_1 can effectively reduce the free energy barrier of the reaction to less than 20 kcal/mol (a value required to allow the reaction to complete in minutes at 25 °C). Therefore, secondary and tertiary allyl alkyl disulfides can undergo the disulfurizative rearrangement at room temperature.

4. Through the Marcus theory analysis, it is concluded that the major factor for the retardation of the reaction by substituents at R_S and R_3 and for the acceleration of the reaction by substituents at R_1 is the thermodynamic equilibrium between the disulfide and thiosulfoxide instead of the steric effect introduced by the substituents. To shift the equilibrium to instigate the ligation under mild conditions, the placement of substituents at R_1 is obligatory for the allyl case.

5. It is proposed that alkyl buta-2,3-dienyl disulfides (i.e., allene-containing disulfides) may also undergo the Crich ligation at room temperature without the help of the R_1 substituent.

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5. Theoretical Methods

All the calculations were performed with the Gaussian 03 software.[37] Geometry optimization and frequency analysis were performed by using the B3LYP method with the 6-31G* basis set. All the reactants, intermediates, and products were optimized without any constraint and were confirmed to be local minima without any imaginary frequency. On the other hand, all the transition states had one and only one imaginary frequency corresponding to the reaction coordinate as confirmed by the IRC analysis.

The single-point energies were calculated by using a two-layered ONIOM method. The high layer was handled at the G3B3 level whereas the low layer was calculated at the B3LYP/6-311++G** or B3LYP/6-31G* level. Gibbs free energy corrections at 25 or 60 °C were obtained by using an ideal gas model under the rigid rotor/harmonic oscillator approximation. The polarized continuum solvation model (PCM) was used to calculate the free energies in solution at the geometries optimized in the gas phase with UA0 radii.[25] The Wiberg bond index analysis was calculated by using the NBO 3.1 program[38] implemented in Gaussian 03.

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Supporting Information Available: Detailed optimized geometries and free energies. This material is available free of charge via the Internet at http://pubs.acs.org.

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