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PAPER

Accurate prediction of rate constants of Diels–Alder reactions and application to design of Diels–Alder ligation†

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Bioorthogonal reactions are useful tools to gain insights into the structure, dynamics, and function of biomolecules in the field of chemical biology. Recently, the Diels–Alder reaction has become a promising and attractive procedure for ligation in bioorthogonal chemistry because of its higher rate and selectivity in water. However, a drawback of the previous Diels–Alder ligation is that the widely used maleimide moiety as a typical Michael acceptor can readily undergo Michael addition with nucleophiles in living systems. Thus, it is important to develop a nucleophile-tolerant Diels–Alder system in order to extend the scope of the application of Diels–Alder ligation. To solve this problem, we found that the theoretical protocol M06-2X/6-31+G(d)//B3LYP/6-31G(d) can accurately predict the activation free energies of Diels–Alder reactions with a precision of 1.4 kcal mol^{-1} by benchmarking the calculations against the 72 available experimental data. Subsequently, the electronic effect and ring-strain effect on the Diels–Alder reaction were studied to guide the design of the new dienophiles. The criteria of the design is that the designed Diels–Alder reaction should have a lower barrier than the Michael addition, while at the same time it should show a similar (or even higher) reactivity as compared to the maleimide-involving Diels–Alder ligation. Among the designed dienophiles, three substituted cyclopropenes (*i.e.* 1,2-bis (trifluoromethyl)-, 1,2-bis(hydroxylmethyl)- and 1,2-bis(hydroxylmethyl)-3-carboxylcyclopropenes) meet our requirements. These substituted cyclopropene analogs could be synthesized and they are thermodynamically stable. As a result, we propose that 1,2-bis(trifluoromethyl)-, 1,2-bis (hydroxylmethyl)- and 1,2-bis(hydroxylmethyl)-3-carboxylcyclopropenes may be potential candidates for efficient and selective Diels–Alder ligation in living systems.

1. Introduction

Bioorthogonal reactions are organic transformations that can be carried out in living systems without disturbing the normal biological processes.[1–4] Such reactions should proceed with high efficiencies at low concentrations (10^{-9} to 10^{-3} mol L^{-1}) under mild conditions (*e.g.* in water, at neutral pH, and at room temperature). Besides this, these reactions should be inert to various biological electrophiles and nucleophiles in living systems. In the field of chemical biology and biotechnology, bioorthogonal reactions are useful tools to gain insights into the structure, dynamics, and function of biomolecules.[2,5–7] Up to now, several types of bioorthogonal reactions have been developed, such as the aldehyde/ketone-hydrazide/alkoxyamine condensation,[8–10] Staudinger reaction,[11–15] native chemical ligation,[16–18] and 1,3-dipolar cycloaddition[19–30] (click reactions). These reactions have been widely employed to study protein functions, ranging from visualization of protein expression and localization, measurement of protein activity, to identification of protein interaction sites in living cells.

Owing to the multifunctionality of biomolecules, the development of new bioorthogonal reaction is a continuing demand.[31–33] Because Diels–Alder reactions can take place in water with a fairly high rate and good selectivity,[34–39] they may present a useful type of bioorthogonal reactions (namely, Diels–Alder ligation).[31–33,40] In recent studies, the reactants of Diels–Alder ligation generally involve an electron-rich hexadiene (as a diene) and an electron-deficient maleimide (as a dienophile, Scheme 1). This ligation reaction has been found to be effective for the bioconjugation of peptides,[41] small molecules,[42,43] and oligonucleotide.[44,45] It has also been used for the bioconjugation of carbohydrate[46,47] to proteins. Unfortunately, a serious drawback of the previous Diels–Alder ligation is that the maleimide moiety as a typical Michael acceptor can readily undergo Michael addition with thiol groups or other nucleophiles in living systems. For example, the unprotected cysteine residues can react with maleimide rapidly, inhibiting the Diels–Alder cycloaddition

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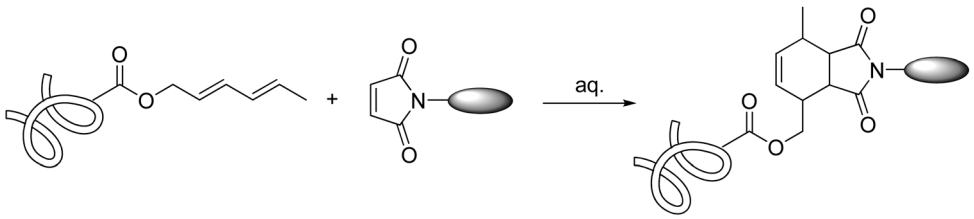
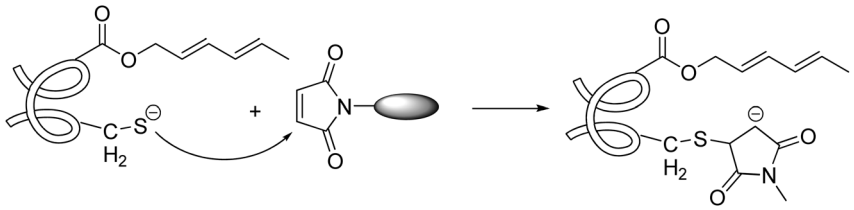
†Electronic supplementary information (ESI) available: Performance of different methods in calculating activation enthalpies, Cartesian coordinates of optimized structures and detailed thermodynamic data. See DOI: 10.1039/c2ob07079k



Scheme 1 Schematic representation of Diels–Alder ligation.



Scheme 2 Schematic representation of Michael addition with thiol groups.

(Scheme 2). Therefore, it is important to develop nucleophile-tolerant Diels–Alder ligation reactions.

In this context, we decided to carry out a systematic theoretical study on the design of new dienophiles in order to extend the scope of Diels–Alder ligation. To achieve this goal, we sought to use a theoretical method to guide the search. Thus we calibrated a theoretical method to accurately predict the barriers of Diels–Alder reactions by benchmarking the calculations against the available experimental data. With the aid of the calibrated theoretical method, we then studied the electronic effect and ring-strain effect on Diels–Alder reaction in order to guide the design of new dienophiles. Specifically, the designed Diels–Alder reaction must be as efficient as the maleimide-involved cycloaddition, while at the same time it should show a much lower reactivity towards Michael addition. Through our theoretical analysis, it is found that some substituted cyclopropenes may be potential candidates for the development of the next generation of Diels–Alder ligation.

2. Computational methods

Geometry optimization of all compounds in gas-phase without any constraint was conducted by the density functional theory method B3LYP with the basis set 6-31G(d).[48–57] Frequency calculation was conducted at the same level of theory as geometry optimization, to confirm the stationary points to be minima or saddle points. For saddle points, intrinsic reaction coordinate analysis (IRC)[58–60] was performed to verify that they connect the right reactants and products on the potential energy surface. Single-point energy calculation was performed by using M06-2X,[61,62] BMK,[63] MPWB1K,[63] M05-2X[62,64] method with the basis set 6-31+G(d) in the solvent. Single-point energy in the solvent corrected by the Gibbs free energy correction from frequency calculation was used to describe the reaction energetics throughout the study. The solvent effect was calculated with self-consistent reaction field (SCRF) method using SMD[65] model. All calculations were preformed with Gaussian 09 suite.[66] In the Gaussian 09 program, the reference state of the calculated free energies in gas phase is 1 atm. However, the calculated solvation free energies use a reference state of 1 mol L^{-1}. To convert the reference state, the equation[67–69] (G_{gas}(1 mol L^{-1}) = G_{gas} (1 atm) + RT ln(24.46)) is used. The systematic correction term is 1.90 kcal mol^{-1} at 298 K.

3. Results and discussion

3.1 Benchmarking the theoretical method

In 1997, Morokuma *et al.* studied the Diels–Alder reactions of acetylenic compounds by IMOMO(G2MS:MP2).[70] In 2003, Houk *et al.* calculated activation enthalpies of several Diels–Alder reactions with different methods (Table 1).[79] They found that CASPT2 and CBS-QB3 exhibited the best performance among various tested methods,[71] such as Hartree–Fock, MP2, CASSCF, CASPT2, density functional theory (B3LYP, BPW91, MPW1K, KMLYP, O3LYP) and CBS-QB3.[72–80] Their studies also show the possibility of accurately predicting the barriers of Diels–Alder reactions. However, CASPT2 and CBS-QB3 are expensive methods and not suitable for more complex molecular systems. In this study, we tried to find an alternative and cheaper theoretical method to evaluate the activation barriers of Diels–Alder reactions.

Initially, we collected the rate constants of 72 non-catalyzed Diels–Alder reactions, and then obtained their relevant activation barriers with the aid of Eyring–Polanyi equation (eqn (1)).

$$k = \frac{k_B T}{h} \exp\left(-\frac{\Delta G^{\neq}}{RT}\right) \quad (1)$$

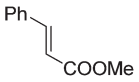
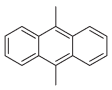
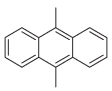
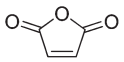
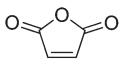
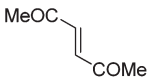
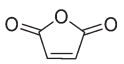
In the above equation, k is the rate constant, $\Delta G^{\neq}$ is the activation Gibbs free energy, k_B is Boltzmann's constant (*i.e.* 1.38 × 10^{-23} J K^{-1}), and h is Planck's constant (*i.e.* 6.6261 × 10^{-34} J s).

Next, eleven representative Diels–Alder reactions (Table 2, $\Delta G^{\neq}$ ranging from 13.0 to 33.0 kcal mol^{-1}) were selected to test the performance of different theoretical models (Table 2). Note that we used B3LYP/6-31G(d) to optimize geometries because

Table 1 The performance of different methods for calculating activation enthalpies of Diels–Alder reactions in ref. 79.

Methods	CASPT2	CBS-QB3	HF	MP2	CASSCF	B3LYP	BPW91	MPW1K	KMLYP	O3LYP
SD	1.6	1.6	5.4	2.3	9.7	1.9	2.6	2.1	2.0	2.6

Table 2 Theoretical activation free energies of Diels–Alder reactions by different methods

				$\Delta G^{\neq b}$ (cal.)					
Dienophile	Diene	k^a	$\Delta G^{\neq b}$ (exp.)	M06-2X[c]	B3LYP[c]	BMK[c]	MPWB1K[c]	M05-2X[c]	M06-2X[d]
		2.0×10^{-5}	33.2	32.2	52.3	43.6	40.6	31.9	33.7
		1.4×10^{-3}	29.1	28.5	41.3	36.8	34.5	26.8	29.1
		2.5×10^{-7}	26.5	25.7	33.5	32	29.9	24.3	27.7
		1.18×10^{-5}	23.8	23.3	30.7	27.8	25.7	22.1	24.8
		5.28×10^{-5}	23.3	22.0	29.8	27.2	25.9	20.3	23.9
		6.28×10^{-6}	24.1	25.2	34.5	30.7	28.2	23.9	26.8
		2.96×10^{-3}	20.5	20.0	30.0	25.8	23.2	18.1	21.2
		1.1×10^{-3}	16.7	15.7	24.0	20.2	17.8	12.9	17.3
		0.455	17.7	16.2	22.0	18.8	17.8	15.1	17.6
		4.8	16.2	14.7	23.5	18	17	12.7	16
		430	13.6	10.6	23.2	15.3	14.1	7.6	12
—	R^e		—	**0.9915**	0.9444	0.9911	0.9952	0.9893	0.9896
	RMSE[e]		—	**0.9**	3.8	2.7	2.1	1.4	1.0

[a] Data from ref. 86–91. Units: l mol^{-1} s^{-1}. [b] Units: kcal mol^{-1}. [c] Basis set: 6-31+G(d). [d] Basis set: 6-311++G(2d,2p). [e] Abbreviations: *R*, correlation coefficient; RMSE, root mean square error.

this method was recommended as a reliable DFT method for geometry optimization of Diels–Alder reaction by Houk *et al.*[79,81] Nonetheless, for single energy calculation, this B3LYP method was found to cause a large deviation of free energy barriers of the concerned Diels–Alder reactions. The root mean square error (RMSE) is 3.8 kcal mol^{-1}. We then examined four density functionals including M06-2X, BMK, MPWB1K, M05-2X which are well-documented for studying kinetics.[63,64,82–84] It was found that M06-2X gave the best result with an RMSE of 0.9 kcal mol^{-1}.[83,85] Furthermore, a larger basis set 6-311++g(2d, 2p) in combination with M06-2X was tested, whereas the RMSE increased to 1.0 kcal mol^{-1} (Table 2). It suggested that a more flexible basis set might not beneficial to the prediction of the barriers of Diels–Alder reactions.[77] Based on the above data, we concluded that M06-2X/6-31+G(d)//B3LYP/6-31G(d) method preformed the best for the eleven Diels–Alder reaction barriers with a precision of 0.9 kcal mol^{-1} (Fig. 1).

To further examine the reliability of M06-2X/6-31+G(d)// B3LYP/6-31G(d) method, we calculated the barriers of the other 62 experimentally measured Diels–Alder reactions. The results were listed in Table 3. We also examined the correlation between theoretical and experimental barriers. As shown in Scheme 1, the linear correlation coefficient is 0.9665 and the RMSE is 1.4 kcal mol^{-1} (Fig. 2). The results indicated that the current theoretical protocol could accurately predict the barriers of Diels–Alder reactions.

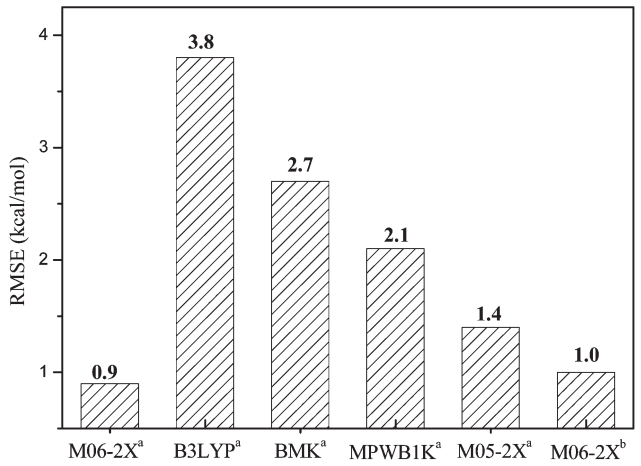


Fig. 1 The performance of different methods ([a]basis set: 6-31+G(d); [b]basis set: 6-311++G(2d,2p)).

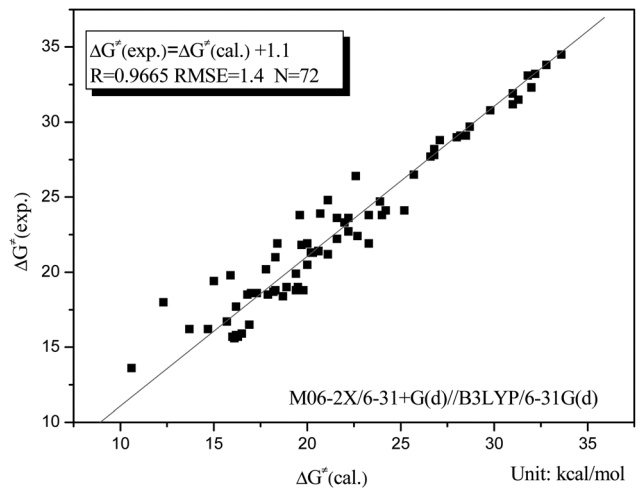


Fig. 2 Correlation between theoretical and experimental barriers of 72 Diels–Alder reactions.

3.2 New dienophile design

Maleimide-involving Diels–Alder reaction was successfully used for ligations of peptides and proteins as shown by Waldmann and co-workers in 2006[32,33] (Scheme 1). Waldmann's reaction proceeded smoothly at 25 °C in water with a conversion of 92% after 47 h.[32] Based on this information, we could estimate the barrier of Waldmann's Diels–Alder ligation *via* Eyring–Polanyi equation (eqn (1)). With the assumption that the half-life is 24 h and the initial concentration of the reactant (peptide) is 10 mM,[32] the barrier of Diels–Alder ligation is estimated to be +21.5 kcal mol^{-1}. Significantly, this value is almost identical to our computed value (+20.9 kcal mol^{-1}, shown in Table 4). It again demonstrates that the current theoretical protocol (*i.e.* M06-2X/6-31+G(d)//B3LYP/6-31G(d)) is a useful tool to predict Diels–Alder ligation system.

To develop new nucleophile-tolerant Diels–Alder ligations, the newly designed Diels–Alder reaction should have a lower barrier than the Michael addition, while at the same time it should show a similar (or better) Diels–Alder reactivity as

Table 3 Experimental and theoretical barriers of 72 Diels–Alder reactions

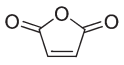
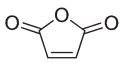
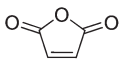
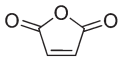
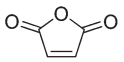
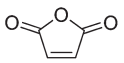
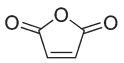
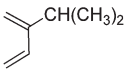
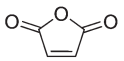
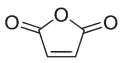
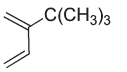
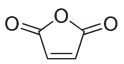
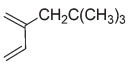
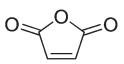
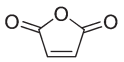
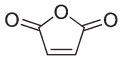
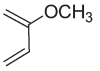
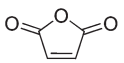
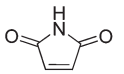
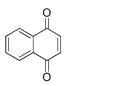
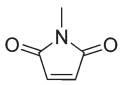
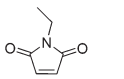
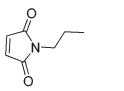
Entry	Dienophile	Diene	k^a	$\Delta G^{\neq b}$ (cal.)	$\Delta G^{\neq b}$ (exp.)
1			2.5×10^{-7}	25.7	26.5[c]
2			5.28×10^{-6}	23.9	24.7[c]
3			3.33×10^{-5}	22.2	23.6[c]
4			5.28×10^{-5}	22.0	23.3[c]
5			2.56×10^{-4}	22.7	22.4[c]
6			3.33×10^{-4}	21.6	22.2[c]
7			6.11×10^{-4}	19.7	21.8[c]
8			5.56×10^{-4}	20.0	21.9[c]
9			1.47×10^{-3}	20.2	21.3[c]
10			2.67×10^{-3}	18.3	21.0[c]
11			1.53×10^{-4}	22.2	22.7[c]
12			1.1×10^{-3}	15.7	16.7[d]
13			5.28×10^{-4}	23.3	21.9[c]
14			3.1×10^{-5}	19.6	23.8[e]
15			1.93×10^{-5}	24.2	24.1[e]
16			4.92	16.9	16.5[f]
17			14.2	16.5	15.9[f]
18			21.6	16.1	15.6[f]
19			16.8	16.2	15.8[f]
20			18.1	16.0	15.7[f]
21			20.3	16.3	15.7[f]

Table 3 *(Contd.)*

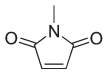
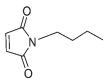
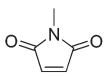
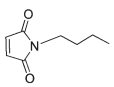
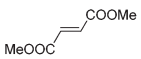
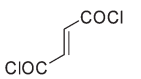
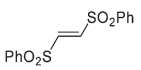
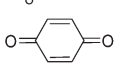
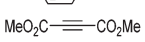
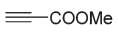
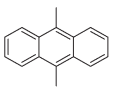
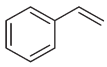
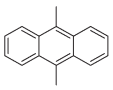
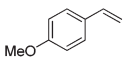
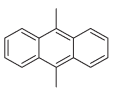
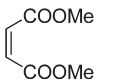
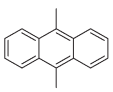
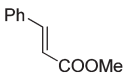
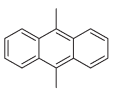
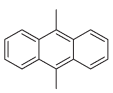
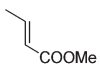
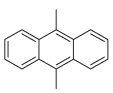
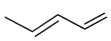
Entry	Dienophile	Diene	k^a	$\Delta G^{\neq b}$ (cal.)	$\Delta G^{\neq b}$ (exp.)
22			0.186	17.9	18.5[f]
23			0.149	17.3	18.6[f]
24			0.15	17.0	18.6[f]
25			0.161	16.8	18.5[f]
26			7.02×10^{-2}	19.5	19.0[f]
27			0.105	19.4	18.8[f]
28			9.46×10^{-2}	19.5	18.9[f]
29			0.11	19.8	18.8[f]
30			430	10.6	13.6[g]
31			4.8	14.7	16.2[g]
32			8.1×10^{-4}	20.3	21.3[g]
33			9.1×10^{-4}	21.1	21.2[g]
34			1.04×10^{-5}	24	23.8[g]
35			0.455	16.2	17.7[g]
36			7.4×10^{-4}	20.6	21.4[g]
37			4.958	13.7	16.2[g]
38			0.115	18.7	18.4[g]
39			7.05×10^{-2}	18.2	18.7[g]
40			8.97×10^{-3}	19.4	19.9[g]
41			3.13×10^{-4}	18.4	21.9[g]
42			5.56×10^{-2}	18.3	18.8[g]

Table 3 *(Contd.)*

Entry	Dienophile	Diene	k^a	$\Delta G^{\neq b}$ (cal.)	$\Delta G^{\neq b}$ (exp.)
43			3.95×10^{-2}	18.9	19.0[g]
44			1.18×10^{-5}	23.3	23.8[g]
45			6.28×10^{-6}	25.2	24.1[g]
46			2.96×10^{-3}	20.0	20.5[g]
47			1.41	21.6	23.6[h]
48			0.3	21.1	24.8[h]
49			4.35×10^{-2}	22.6	26.4[h]
50			8.4×10^{-3}	26.6	27.7[h]
51			7.38×10^{-3}	26.8	27.8[h]
52			4.2×10^{-3}	26.8	28.2[h]
53			2.15×10^{-3}	27.1	28.8[h]
54			1.60×10^{-3}	28.0	29.0[h]
55			1.52×10^{-3}	28.2	29.1[h]
56			1.45×10^{-3}	28.2	29.1[h]
57			1.40×10^{-3}	28.5	29.1[h]
58			7.18×10^{-4}	28.7	29.7[h]
59			2.05×10^{-4}	29.8	30.8[h]
60			1.48×10^{-4}	31.0	31.2[h]

Table 3 *(Contd.)*

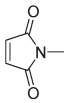
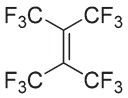
Entry	Dienophile	Diene	k^a	$\Delta G^{\neq b}$ (cal.)	$\Delta G^{\neq b}$ (exp.)
61			1.13×10^{-4}	31.3	31.5[h]
62			7×10^{-5}	31.0	31.9[h]
63			5×10^{-5}	32.0	32.3[h]
64			2.05×10^{-5}	31.8	33.1[h]
65			2.0×10^{-5}	32.2	33.2[h]
66			1.1×10^{-5}	32.8	33.8[h]
67			5.0×10^{-6}	33.6	34.5[h]
68			1×10^{-5}	20.7	23.9[i]
69			5.19×10^{-3}	17.8	20.2[i]
70			1.13×10^{-2}	15.9	19.8[i]
71			2.06×10^{-2}	15.0	19.4[i]
72			0.243	12.3	18.0[i]

[a] Units: 1 mol^{-1} s^{-1}. [b] Units: kcal mol^{-1}. [c] Conditions: 298 K in benzene.[86] [d] Conditions: 233 K in benzene.[86] [e] Conditions: 300 K in CD_3CN.[87] [f] Conditions: 298 K in water.[88,89] [g] Conditions: 293 K in dioxane.[90,91] [h] Conditions: 403 K in dioxane.[90,91] [i] Conditions: 293 K in dioxane.[90]

compared to the system involving maleimide (Scheme 3). To find possible candidates, we studied the electronic and ring-strain effect in Diels–Alder reactions. Thereafter, we were able to design new dienophiles by calculating the barriers in Diels–Alder and Michael reactions, respectively.

3.2.1 σ-Electron withdrawing substituents. In an early review, Sauer pointed out that electron rich dienes (such as 1,3-butadiene, cyclopentadiene or 9,10-dimethylanthracene) and electron-withdrawing substituents on the double bond of dienophiles accelerated the Diels–Alder reactions,[92] whereas electron-donating substituents decelerated the reaction rates. Our calculation results show the same trend (Table 3). For example, electron-withdrawing groups (cyano, or carbonyl groups) lead to lower barriers. However, it is important to note that such groups

Table 4 Calculated Diels–Alder barriers of σ-electron withdrawing group substituted alkenes

Entry	Diene	Dienophile	$\Delta G^{\neq a}$ (cal.)
3-1			20.9
3-2			29.8
3-3			29.0
3-4			24.5

[a] In water; units: kcal mol^{-1}.

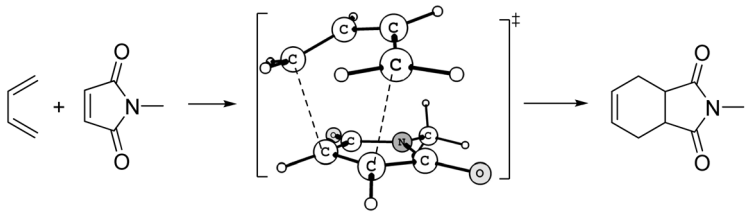
are all π-electron-withdrawing groups, which are typical Michael acceptors and can cause undesired Michael addition with thiol groups. Thus we considered σ-electron-withdrawing substituents (*e.g.* −F, $-CF_3$) in the following study (Table 4).

The barriers of Diels–Alder reactions between 1,3-butadiene and fluoro- and trifluoromethyl-substituted ethylenes were calculated and listed in Table 4. Unfortunately, all of them (*e.g.* entry 3-2, perfluoroethene, $\Delta G^{\neq}$ = +29.8 kcal mol^{-1}; entry 3-4, tetrafluoromethylethylene, +24.5 kcal mol^{-1}) have higher barriers than *N*-methylmaleimide (entry 3-1). Therefore, the promotion caused by σ-electron-withdrawing groups alone is not enough to meet our requirement for the energy barrier.

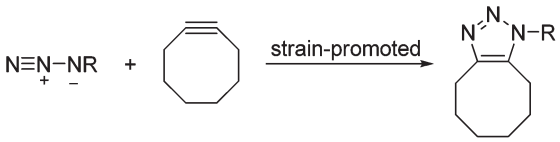
3.2.2 Ring-strain effect. In addition to the electronic factors, some structural factors, such as ring-strain effect can also affect the barriers.[74] For instance, Houk *et al.* used computational methods to study the 1,3-dipolar cycloaddition of phenyl azide with cyclic alkynes. They found that the energy barrier of the strained cyclooctyne with phenyl azide is much lower than the strain-free ones[74] (Scheme 4). Inspired by Houk's study, we examined the barriers of 1,3-butadiene and a series of cyclic olefins ranging from three to eight members (Table 5).

As shown in Table 4, cyclopropene (angle = 64.6°) is the most reactive dienophile with a Diels–Alder barrier of +21.5 kcal mol^{-1}. With the increase of the angle, the barriers of cyclobutene increases in the following sequence: cyclobutene (+30.8 kcal mol^{-1}) < cyclopentene (+34.2 kcal mol^{-1}) < cyclohexene (+39.0 kcal mol^{-1}). The Diels–Alder barrier for cyclohexene is the highest and then the barrier decreases for the larger homologs, *i.e.* cycloheptene (+37.9 kcal mol^{-1}) and cyclooctene (+34.8 kcal mol^{-1}). The strain relief during the cycloaddition process may be responsible for the barrier differences. According to the calculations, only the highly strained cyclopropenes meet our requirement for the energy barrier.

3.2.3 Substituted cyclopropenes. It should be noted that unsubstituted cyclopropene was reported to be thermodynamically unstable[93] and therefore, cannot be used for Diels–Alder ligation. Fortunately, some substituted cyclopropenes were reported to be stable compounds even in the presence of air and



Scheme 3 Diels–Alder reactions between 1,3-butadiene and *N*-methylmaleimide.



Scheme 4 Strain-promoted 1,3-dipolar cycloaddition.

Table 5 Calculated Diels–Alder barriers of cyclic alkenes

Entry	Dienophile	Angle (α)	$\Delta G^{\neq a}$ (cal.)
Cyc3		64.6	21.5
Cyc4		94.4	30.8
Cyc5		112.1	34.2
Cyc6		123.5	39.0
Cyc7		127.9	37.9
Cyc8		121.5	34.8

[a] 1,3-Butadiene as the diene; in water; unit: kcal mol^{-1}.

water.[94–96] To this end, we examined a series of substituted cyclopropenes.

As for substituted cyclopropenes, initial studies were preformed on the Diels–Alder reaction of 1,2-bimethylcyclopropene with 1,3-butadiene. However, the barrier is +26.8 kcal mol^{-1}, which is rather high for achieving the Diels–Alder reactions under mild conditions. Subsequently, a series of σ-electron-withdrawing group substituted 1,2-bimethylcyclopropenes were examined and the results are shown in Table 6. The barriers for the Diels–Alder reactions of 1,2-bis(fluoromethyl)-, 1,2-bis(trifluoromethyl)- and 1,2-bis(hydroxylmethyl)cyclopropene with 1,3-butadiene are +24.9, +16.6 and +22.3 kcal mol^{-1}, respectively. The low energy barriers suggest that Diels–Alder reactions using these dienophiles could be fairly fast at ambient temperature. In addition, we tested the substitution of 1,2-bis(fluoromethyl)cyclopropene at C3 position. The fluoro-group leads to a higher barrier of the Diels–Alder reaction possibly due

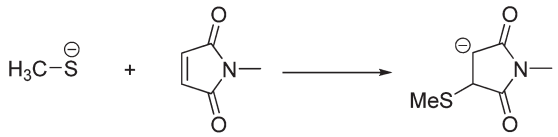
Table 6 Calculated barriers of Diels–Alder and thiol addition reactions of designed alkenes

			$\Delta G^{\neq}$ (cal.)[b] (kcal mol^{-1})	
Entry	Diene	Dienophile	Diels–Alder	Thiol addition
5-1			20.9	3.4
5-2			21.5	—
5-3			26.8	—
5-4			24.9	20.1
5-5			16.6 (−65.0)[a]	Not obtained computationally
5-6			22.3 (−57.1)[a]	27.1
5-7			29.5	—
5-8			24.2 (−49.1)[a]	26.2

[a] Gibbs free energy of the Diels–Alder reaction. [b] Either the activation free energy or the activation energy causes the same result. The detail about activation enthalpies was shown in ESI.†

to the steric hindrance on the *endo*-transition state. Fortunately, carboxyl substituted cyclopropene at C3-position offers a barrier of +24.2 kcal mol^{-1}. According to the calculations, the barrier of 1,2-bis(trifluoromethyl)cyclopropene with 1,3-butadiene (+16.6 kcal mol^{-1}) is much lower than that of *N*-methylmaleimide (+20.9 kcal mol^{-1}). The lower energy barrier suggests that when the initial concentration of the ligation partners is 10 mM, the half life would be only 22 seconds for the Diels–Alder ligation using 1,2-bis(trifluoromethyl)cyclopropene in water at 25 °C.

To gain effective and nucleophile-tolerant dienophiles, we should also take into account the barrier of thiol addition. In other words, the barriers of the new dienophiles involved thiol addition should be higher than those of Diels–Alder reactions. As shown in Scheme 5, methanethiol was used as model nucleophile. The thiol addition involving methanethiol with *N*-methylmaleimide has a considerably low barrier of +3.4 kcal mol^{-1}. It indicates that the reaction between π-electron-withdrawing group substituted olefins and thiol is very fast, interfering the Diels–Alder reaction between the olefins and dienes. On the other hand, according to our calculations, the thiol addition barriers of σ-electron-withdrawing-group-substituted cyclopropenes are higher (+27.1 and +26.2 kcal mol^{-1} for 1,2-bis(hydroxylmethyl)- and 1,2-bis(hydroxylmethyl)-3-carboxylcyclopropene, respectively, Fig. 3). As to 1,2-bis(trifluoromethyl) cyclopropene, we made great efforts to optimize the transition state of thiol addition, but failed. Furthermore, the Diels–Alder reactions of the newly designed substituted cyclopropenes were very exothermic (−65.0, −57.1 and −49.1 kcal mol^{-1} for 1,2-bis(hydroxylmethyl)-, 1,2-bis(trifluoromethyl)- and 1,2-bis(hydroxylmethyl)-3-carboxylcyclopropene, respectively). Therefore, the Diels–Alder reactions of these cyclopropenes were irreversible and their products were stable. Thus, our data suggest that the σ-electron-withdrawing-group-substituted cyclopropenes may be good candidates for Diels–Alder ligation without side reactions with nucleophiles.



Scheme 5 The computed model of thiol addition.

Based on the above calculations, we found that 1,2-bis(trifluoromethyl)-, 1,2-bis(hydroxylmethyl)- and 1,2-bis(hydroxylmethyl)-3-carboxylcyclopropenes might act as good dienophile candidates of new Diels–Alder ligation. However, one might wonder whether the designed compounds could be synthesized. In this context, we found that some analogs of these compounds had been produced, such as **A–F** (Scheme 6).

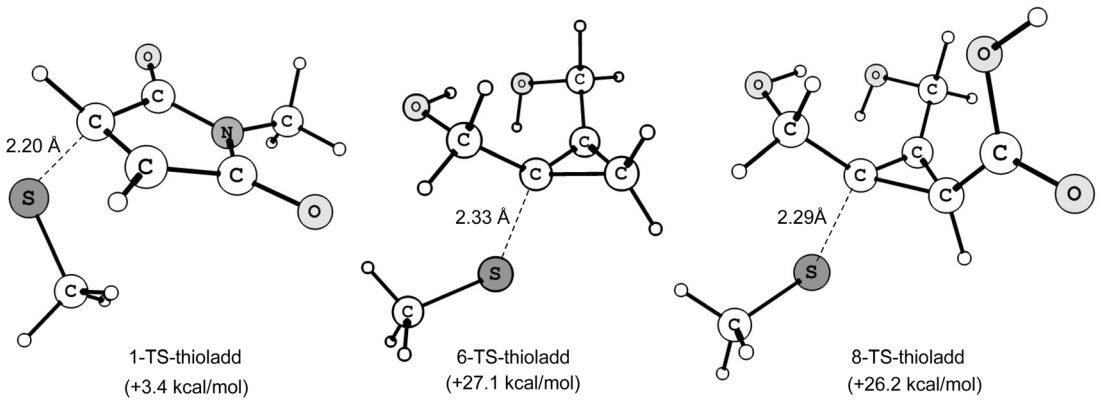
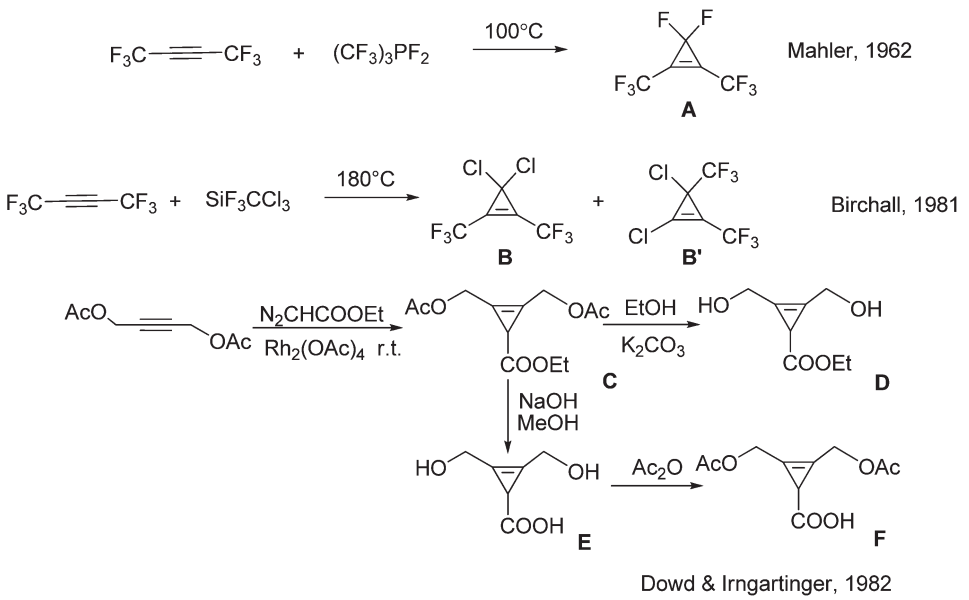


Fig. 3 Key transition states of thiol additions.



Scheme 6 Examples of substituted cyclopropenes.

In detail, Mahler reported that compound **A** was generated by heating hexafluoro-2-butyne and $(CF_3)PF_2$ at 100 °C in 1962;[94] Birchall reported that compound **B** could be synthesized from hexafluoro-2-butyne and SiF_3CCl_3 under harsh condition in 1981;[95] more recently, trifluoromethylation reactions catalyzed by transition metals (*e.g.* Pd, Cu) have also been intensively studied.[97,98] Using such reactions, 1,2-bis(trifluoromethyl)cyclopropene might be obtained. Dowd *et al.* reported that compound **C** could be obtained though a $Rh_2(OAc)_4$-catalyzed reaction of 1,4-diacetoxy-2-butyne and ethyl diazoacetate at room temperature.[96] Moreover, compound **C** was converted to compound **D** and **E** (1,2-bis(hydroxylmethyl)-3-carboxylcyclopropene) by treatments with different bases, respectively. Compound **F** was transformed from **E** by using Ac_2O.[96] The above mentioned compound **A–F** are structurally close to the substituted cyclopropenes we proposed, and therefore, it is reasonable to believe that 1,2-bis(trifluoromethyl)-, 1,2-bis(hydroxylmethyl)- and 1,2-bis (hydroxylmethyl)-3-carboxylcyclopropene could be synthesized *via* similar methods.

Besides the synthesis of substituted cyclopropenes, another question is whether the compounds are stable under the conditions adopted for the bioconjugation. It is reported that compound **C–F** could be possible to isolate from water wash and chromatography.[96] Based on these experimental conditions, we believed that these substituted cyclopropenes are stable at room temperature and tolerant to water and air. Moreover, 1-methylcyclopropene is known to be excellent for keeping food fresh. It is not only stable and non-toxic, but also able to be employed in the living system. According to the above information, we deemed that 1,2-bis(trifluoromethyl)-, 1,2-bis(hydroxylmethyl)- and 1,2-bis(hydroxylmethyl)-3-carboxylcyclopropenes may be potential candidates for selective Diels–Alder ligation used in living systems.

4. Conclusion

The Diels–Alder reaction is a promising and attractive procedure for ligation in bioorthogonal chemistry because of its higher rate and selectivity in water. However, the maleimide moiety widely used in Diels–Alder ligation easily undergoes Michael addition with thiol groups or other nucleophilic groups during the Diels–Alder cycloaddition. Thus, it is important to establish an effective and nucleophile-tolerant Diels–Alder system. To solve this problem, we found that the theoretical method M06-2X/6-31+G(d)//B3LYP/6-31G(d) can accurately predict the activation free energies of Diels–Alder reactions with a precision of 1.4 kcal mol^{-1}. Subsequently, the electronic effect and ring-strain effect on Diels–Alder reaction were studied to guide the design of the new dienophiles. Among the designed dienophiles, three substituted cyclopropenes (*i.e.* 1,2-bis(trifluoromethyl)-, 1,2-bis(hydroxylmethyl)- and 1,2-bis(hydroxylmethyl)-3-carboxyl-cyclopropenes) present suitable Diels–Alder barriers of +16.6, +22.3 and +24.2 kcal mol^{-1}, respectively. It suggested that the Diels–Alder reactions of the new dienophiles could proceed smoothly under mild conditions. More importantly, the barriers of thiol addition with thiols for 1,2-bis(hydroxylmethyl)- and 1,2-bis(hydroxylmethyl)-3-carboxylcyclopropene are +27.1 and +26.2 kcal mol^{-1}, respectively. For 1,2-bis(trifluoromethyl) cyclopropene, the attempts to optimize the transition state of thiol addition failed. These results indicated that the thiol additions to the newly designed dienophiles were difficult. Finally, according to the analysis of previous literature, the substituted cyclopropene analogs could be synthesized and thermodynamically stable. As a result, we propose that 1,2-bis (trifluoromethyl)-, 1,2-bis(hydroxylmethyl)- and 1,2-bis(hydroxylmethyl)-3-carboxylcyclopropenes may be potential candidates for efficient and selective Diels–Alder ligation in living systems.

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85 We also calculated the activation enthalpies using different methods. We found that M06-2X/6-31+G(d)//B3LYP/6-31G(d) method gives the best results for the six available experimental activation enthalpies for Diels–Alder reaction with a precision of 0.8 kcal mol^{-1}. More details see ESI.†.
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