Peptidomimetic Catalysts as Chemical Probes of Weak Intermolecular Forces: An Insight into The N-H···Cl-C H-Bonding Interaction

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ABSTRACT

Peptide bond isosteres have been studied extensively in drug discovery. However, despite their widespread use, there is only a limited understanding of their functional mimicry of amides, in particular their hydrogen bonding capability that significantly affects peptide conformation and molecular recognition. With experimental and computational approaches, we have explored the hydrogen bonding acceptor potential of the chlorine substituent in chloroalkene dipeptide isosteres (CADIs) that have shown unique potential as backbone surrogates of cyclic peptides and β-turn peptides. The (*Z*)-chloroalkene and (*E*)-methylalkene analogues of a peptide catalyst, which engage in substrate-catalyst H-bonding for molecular recognition, were synthesized and employed as probe molecules to assess the H-bonding acceptor capabilities of CADIs. These peptidomimetic studies provide experimental evidence supporting the existence of an intermolecular H-bonding interaction between the chlorine substituent in CADIs and the amide proton of the carbamate substrate, an interaction that is capable of catalyzing asymmetric epoxidation reaction with moderate enantioselectivity. These findings show the capability of peptide catalysts to experimentally evaluate weak intermolecular forces. DFT calculations further elucidated that the chlorine substituent in CADIs prefers to form p-type hydrogen bonding interactions on the lateral portions of the chlorine atom, which is different from the carbonyl oxygen of amides which predominantly forms s-type H-bonds in peptide secondary structures.

INTRODUCTION

Replacement of a peptide bond with a peptide bond isostere is an established approach to increasing metabolic stability, potency and selectivity.[1] In particular, haloalkenes are regarded as robust surrogates for peptide bonds in drug discovery due to their high mimicry of the structural and electronic features of a peptide bond (Figure 1).[2] Due to the highly electronegative fluorine substituent as an equivalent to the carbonyl oxygen, fluoroalkene dipeptide isosteres (FADIs) have been thought to be one of the most ideal isosteres with which to mimic the shape and function of peptide bonds in their ground state.[3] These features have inspired much effort toward the development of the synthetic methods of FADIs[4] and the application of such isosteres to bioactive peptides[5] has been studied extensively.

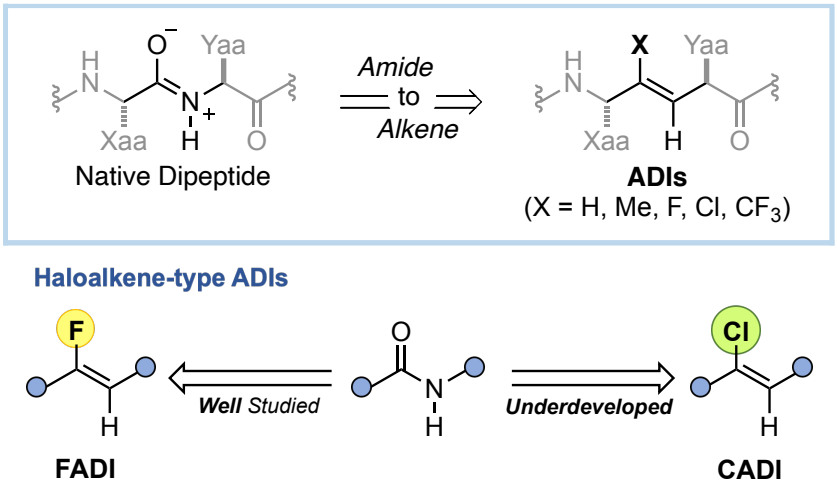


Figure 1. Native dipeptide and alkene dipeptide isosteres (ADIs).

Similarly, CADIs, which have a chlorine atom as the carbonyl oxygen equivalent can be a good mimic of dipeptides. Despite the advantages of the chlorine substituent as a bioisostere for many functional groups,[6] CADIs have received less attention than FADIs. The utility of CADIs was identified in 1996 as a highly potent analogue of hPTH (1-36).[7] After CADIs were overlooked for 20 years by ADI chemistry, Tamamura et al. reported in 2018 that CADIs can be a key structural component of cyclic pentapeptides such as an $\alpha V\beta 3$ integrin antagonist[8] and an amyloid-β aggregation inhibitor.[9] Our own efforts unveiled the utility of CADIs as a β-turn mimics superior to other alkene-type isosteres,[10,11] including methylalkene dipeptide isostere (MADI) that has been reported to show a high preference for a β-turn structure.[12] Incorporation of Gly-Gly-type CADI in a flexible 14-mer RGG peptide resulted in the preferred formation of a β-turn structure.[10] In addition, we revealed in 2022 that the interplay of the steric and stereoelectronic effects around the chloroalkene moiety contributes significantly to the stabilization of β-turn structures.[11]

While the potential of CADIs as a peptide bond isostere is being uncovered, there have been no reports about the functional mimicry of CADIs, including their behavior as H-bonding acceptors. Since the hydrogen bond is one of the most important non-covalent interactions affecting peptide conformation[13] and molecular recognition,[14,15] the elucidation of the capability of the chlorine substituent in CADIs as an H-bonding acceptor is a critical aspect of the use of CADIs in drug discovery and chemical biology.

Although there are several reports of theoretical calculations of the hydrogen-bonding interactions of neutral chlorines,[16] it is known that the strength of the interaction varies greatly depending on the calculation methods. Thus, it would be highly desirable to be able to evaluate the correct strength of the interactions experimentally. The relatively weaker strength of chlorine-mediated hydrogen-bonding interactions, however, has severely limited their experimental evaluation.

Herein, we report peptidomimetic studies toward this goal which involved comparison of CADI-types with other alkene-types, including FADI- and MADI-types. The key to this approach is the use of the enantioselectivity of the asymmetric peptidic catalysis as the parameter with which to evaluate the H-bonding acceptor ability of the chlorine substituent of CADIs (Figure 2). Our peptidomimetic studies revealed that the chlorine substituent of the (*Z*)-chloroalkene moiety can interact with H-bonding donor substrates *via* intermolecular H-bonding interaction in the transition state, while the H-bonding acceptor ability of the chlorine substituent is weaker than that of the fluorine substituent of FADI or the amide carbonyl oxygen. To the best of our knowledge, intermolecular recognition in the asymmetric reaction by N-H···Cl-C H-bonding interaction has not been reported. Although this approach requires the synthesis of the several peptidomimetics of the peptide catalyst, it will enable the experimental and quantitative evaluation of weak intermolecular forces, such as the H-bonding acceptor ability of chlorine atoms. Density functional theory (DFT) calculations have provided further insights, revealing that the chlorine substituent of CADI exhibits a preference for engaging in p-type hydrogen bonding interactions along the lateral regions of the chlorine atom. This behavior contrasts with that of the carbonyl oxygen in amides, which predominantly forms s-type hydrogen bonds in peptide secondary structures.

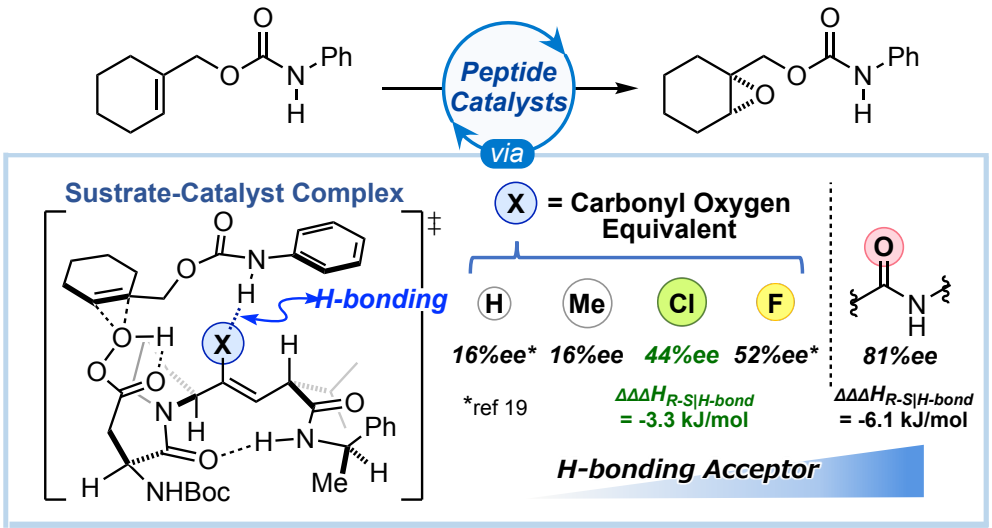


Figure 2. Asymmetric epoxidation reactions catalyzed by a Miller's peptide catalyst and its ADI analogues.

Design of a System to Evaluate the H-bonding Acceptor Ability of CADIs.

In our investigation of the H-bonding ability of the chlorine substituent in CADIs that is supposed to be a weak acceptor,[17] we considered the possibility of the peptide-catalyzed asymmetric reactions in which substrate-catalyst H-bonds are often important in molecular recognition. We hoped to compare and quantify the H-bonding acceptor ability of CADIs against that of FADIs and amides. These concerns guided us to a Miller's peptide catalyst (**3**)[18] as a probe compound to evaluate the H-bonding acceptor ability of CADIs. The peptide catalyst (**3**) is an aspartic acid-containing tetrapeptide forming a β-turn structure and highly enantioselectively catalyzing the asymmetric epoxidation. Functional analysis of the catalyst revealed that the H-bond between the carbonyl oxygen of the Pro-D-Val peptide bond and the carbamate proton of the substrate contributes significantly to the high enantioselectivity (81% ee) of the epoxidation. In contrast, substrates lacking H-bonding capability show low enantioselectivity (~10% ee). Importantly, the reactions with peptidomimetics, in which the Pro-D-Val peptide bond was replaced with either a (Z)-fluoroalkene or an (*E*)-alkene isostere, gave lower enantioselectivities (52% ee for a FADI analogue (**4**) and 16% ee for an EADI analogue (**5**), respectively).[19] This suggests a good correlation between the H-bonding acceptor ability and the enantioselectivity observed in the reaction (Figure 3). Based on this type of data, we sought to design a CADI analogue in which the Pro-D-Val peptide bond was replaced by (*Z*)-chloroalkene isostere so that the resulting enantioselectivity could be compared. In addition, the MADI analogue containing Pro-D-Val-type (*E*)-methylalkene isostere was synthesized as a control catalyst lacking H-bonding ability but with high structural mimicry.

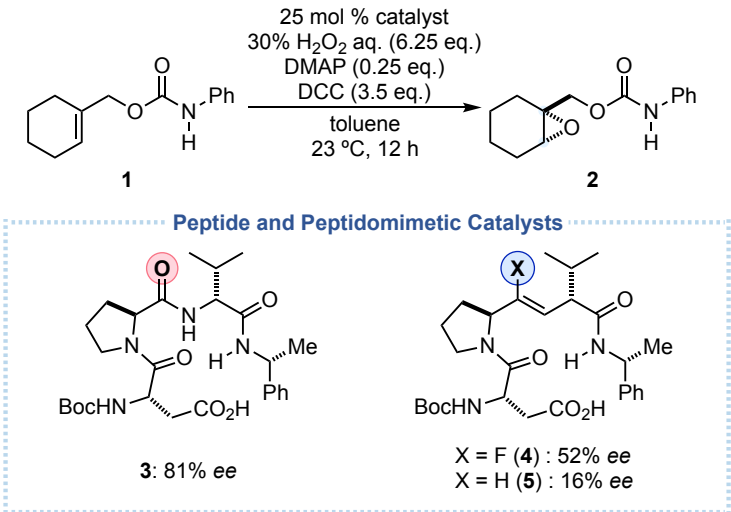
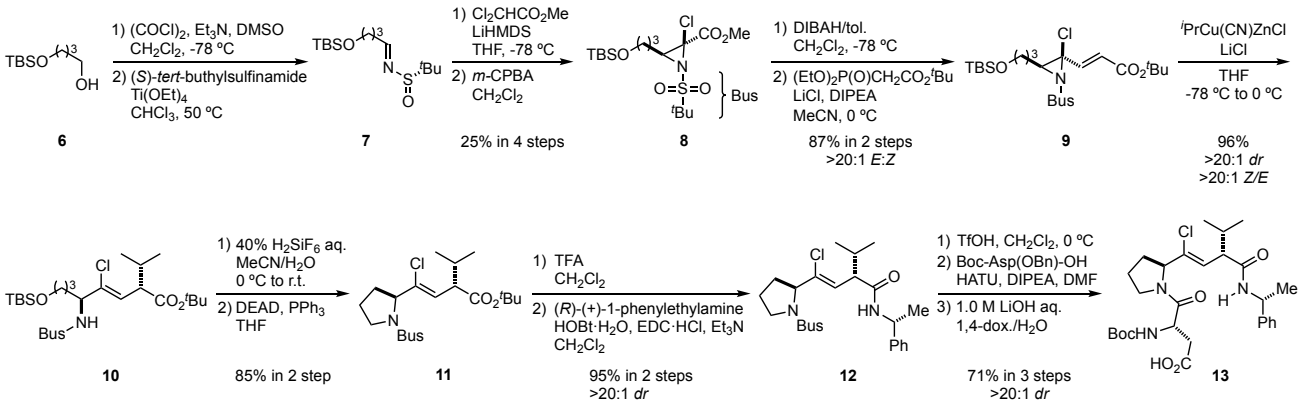


Figure 3. Asymmetric epoxidation reactions catalyzed by Miller's peptide and peptidomimetic catalysts.

Synthesis of a CADI analogue.

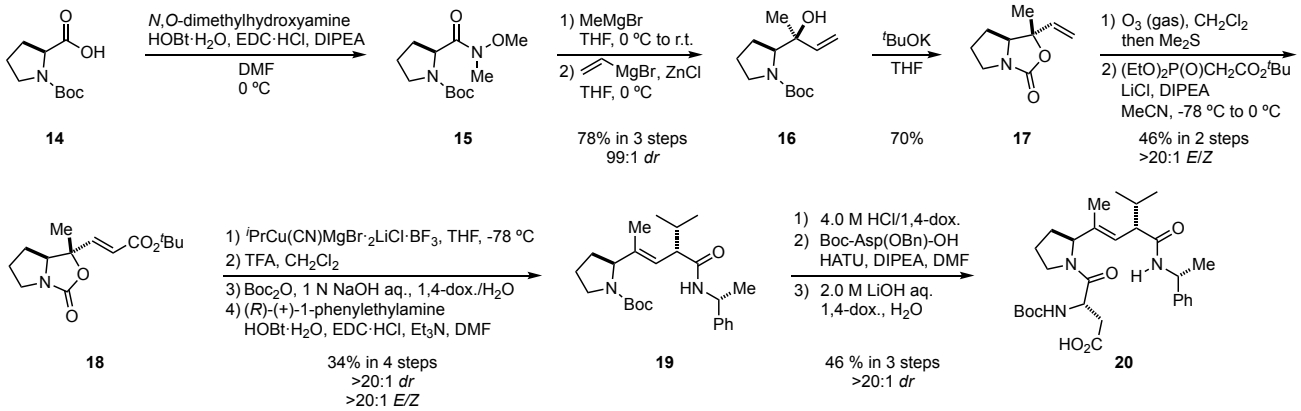
Starting from mono-TBS protected 1,4-butandiol (**6**), Swern oxidation and subsequent condensation with (*S*)-*tert*-butylsulfinamide gave the chiral N-sulfinyl aldimine (**7**), which was treated with the lithium enolate of methyl dichloroacetate followed by *m*-CPBA oxidation to afford the N-*tert*-butylsulfonyl (Bus)-protected aziridine (**8**) in 25% isolated yield over 4 steps (Scheme 1). Treatment of **8** with DIBALH and a subsequent Horner-Wadsworth-Emmons reaction produced the (E)-enoate (**9**). The α-stereogenic center was constructed by diastereoselective allylic alkylation through an anti-S_N2' mechanism with an isopropyl zinc-copper reagent, yielding the ester (**10**) in 96% yield with excellent *Z*-selectivity and diastereoselectivity. Cleavage of the TBS group by aqueous H_2SiF_6 provides the corresponding alcohol, which was subsequently subjected to the intramolecular cyclization to give the Pro-D-Val-type CADI (**11**) in 85% yield (2 steps). Deprotection of the *tert*-butyl ester with TFA and coupling with (*R*)-1-phenylethylamine gave the corresponding amide (**12**). Removal of the Bus group with TfOH and coupling with Boc-Asp(OBn)-OH followed by basic saponification of the benzyl ester in the Asp side chain gave the desired CADI analogue (**13**) of peptide catalyst (**3**).



Scheme 1. Synthesis of the CADI analogue of **3**.

Synthesis of the MADI analogue.

The synthesis of (*E*)-methylalkene-type analogue (Scheme 2) began with N-Boc-protected proline (**14**). Condensation of **14** with *N,O*-dimethylhydroxylamine gave the Weinreb amide (**15**). The *syn*-allyl alcohol (**16**) was formed stereoselectively by treatment of **15** with methylmagnesium bromide followed by chelation-controlled addition of vinylmagnesium bromide in the presence of $ZnCl_2$ (syn:anti = >20:1).[20] Intramolecular cyclization of **16** with *tert*-BuOK provided the oxazolidinone (**17**) in 70% yield. Harries ozonolysis of **17**, followed by Horner-Wadsworth-Emmons reaction produced the (*E*)-enoate (**18**). Successive treatment of **18** with the organocyanocuprate-BF_3 complex, *i*-PrCu(CN)MgCl•BF_3, TFA for deprotection of *tert*-butyl ester, and Boc_2O for N-protection, followed by coupling with (*R*)-1-phenylethylamine gave the corresponding amide (**19**). The expected MADI analogue (**20**) was obtained by sequential manipulation similar to that performed with the chloroalkene analogue (**13**).



Scheme 2. Synthesis of the MADI analogue of **3**.

Structural analysis of ADI analogues.

Since the β-turn conformation of the peptide catalyst is important for the enantioselectivity, conformational analysis by ^{1}H-NMR of the CADI and MADI analogues was performed. Figure 4A shows the ^{1}H-NMR spectra of **3**, **13**, and **20** in $CDCl_3$ from δ 2.4-7.0 ppm.[19] For the chloroalkene-type analogue (**13**), only a single conformation was observed in this condition, which is consistent with the spectra of peptide catalyst **3**. While the methylalkene-type isostere is known as a β-turn promotor,[12] the MADI analogue (**20**) is an approximately 5:1 conformational mixture that could be the trans- and cis-isomer of the prolyl amide. This is probably because the n)(π Pauli repulsion[21] works against the trans-amide formation which is suitable for the β-turn structure. Efforts to elucidate the effects of CADI and MADI on the conformational preference of the prolyl amide are in progress, inspired by Raines's work with FADIs.[21] In addition, spectra of **13** and **20** derived from nuclear overhauser effect spectroscopy (NOESY) were collected. As is shown in Figure 4B, the observed NOEs for **13** and the major conformer of **20** are similar to that of peptide catalyst **1**, with coincidence between: (1) the C_α-H of Asp and the C_δ-H of Pro, (2) the C_α-H of Pro and the amide proton of Val or the vinyl proton of alkene moieties, and (3) the C_α-H of Val and C-terminal amide proton. All of these are characteristic NOEs found in β-turn peptides.[19,22] These data suggest that both the CADI analogue (**13**) and the major conformer in the MADI analogue (**20**) adopt the β-turn structures similar to that of **3**. It is significant that combining our results with those of Miller results in the (*E*)-alkene- and fluoroalkene-type analogues appear as a 3.1 and 9:1 mixtures in solution[19] and suggests that the sequence of β-turn mimicry of ADIs can be defined as CADI > FADI > MADI > EADI with respect to the ratio of the conformers.

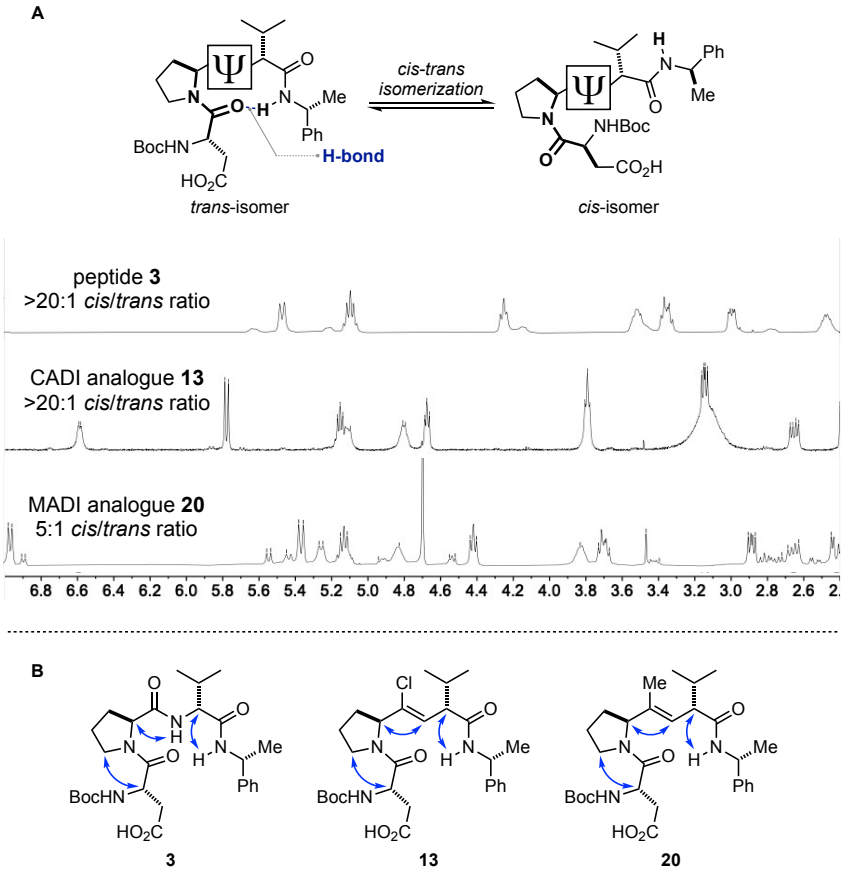


Figure 4. (A) Partial ^{1}H NMR spectra of catalysts **3**, **13**, and **20**. (B) Representative NOE contacts from the major conformational isomers of catalysts **3**, **13**, and **20**.

Enantioselectivity of asymmetric epoxidation reactions

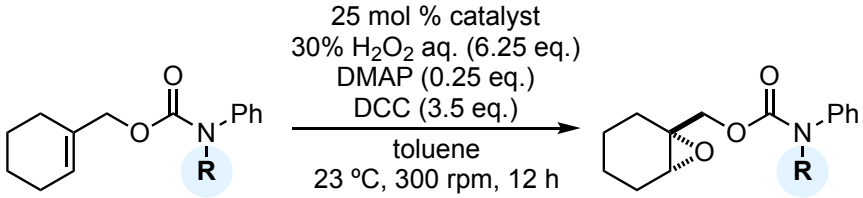
Encouraged by the conformational analogy of **13** and **20** with the original catalyst (**3**), we explored the asymmetric epoxidation promoted by ADI analogues in an effort to evaluate the H-bonding acceptor ability of the chloroalkene-type isostere (Table 1). Since the peptide-catalyzed asymmetric epoxidations proceed in heterogeneous conditions that could affect the enantioselectivity, we checked the reproducibility of our conditions which included 0.25 equiv. catalyst, 6.25 equiv. 30% H_2O_2, 0.25 equiv. DMAP, 3.5 equiv. DCC, 0.4 M toluene at 23 °C for 12 h stirred at 300 rpm. Confirmation of the reproducibility of our conditions was obtained by the reaction with peptide **3** that provided almost identical results in terms of the conversion (62%) and enantioselectivity (80 % ee) with Miller's data (63% conv., 81% ee).[19] Furthermore, full conversion (>95% conv.) was obtained by an extended reaction time (38 h) without any decrease in the enantioselectivity. These results allowed direct comparison of the enantioselectivity observed in our conditions with the published results.

The asymmetric epoxidation catalyzed by the CADI analogue (**13**) provided an exciting result, in which the product was delivered with 44% ee, a value higher than that of the MADI analogue (**20**) without a H-bonding acceptor (16% ee). Furthermore, the reaction by **13** with *N*-methyl carbamate substrate without a H-bonding donor provided much less enantioselectivity (3% ee). This intriguing result shows

that the chlorine substituent in **13** is involved in the transition state as an H-bonding acceptor which interacts with H-bonding donor substrates.

The moderate enantioselectivity observed in **13** provides valuable insight into the H-bonding ability of ADIs. The observed enantioselectivity in **13** is lower than that of the peptide catalyst (**3**) (80% ee) or the FADI analogue (52% ee),[19] but is higher than that of the control catalysts (**13**) and (**20**) which lack an H-bonding acceptor. These results indicate that the sequence of H-bonding acceptor ability in this reaction is peptide > FADI > CADI with respect to the enantioselectivity. It is also important that the low enantioselectivity was observed in the reaction catalyzed by **20**, and the enantioselectivity was the same as EADI analogue **5**.[19] Considering that the major components of the two analogues form similar β-turn structures, this suggests that non-covalent interactions other than the H-bond, for example π-π interactions, may play a role in organizing the substrate-catalyst complex.

Table 1. Catalytic performance of **3**, **13**, and **20**.

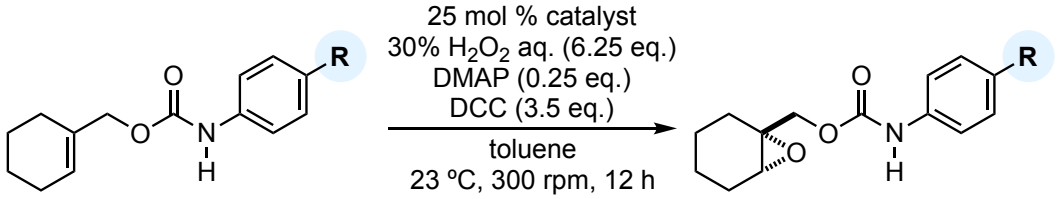


catalytic compounds	Substrate	enatiomeric excess	ref.
amide-type **3**	R = H, **1**	80% *ee*	This work
FADI analogue **4**	R = H, **1**	52% *ee*	19
CADI analogue **13**	R = H, **1**	44% *ee*	Thiswork
MADI analogue **20**	R = H, **1**	16% *ee*	This work
EADI analogue **5**	R = H, **1**	16% *ee*	19
amide-type **3**	R = Me, **21**	23% *ee*	This work
CADI analogue **13**	R = Me, **21**	3% *ee*	This work

To gain insight into the H-bonding acceptor ability of **13**, the sensitivity of H-bonding acceptor was examined by its reactions with carbamates bearing electron-donating and electron-withdrawing substituents on the *para*-position of the aromatic ring (Table 2). In contrast to the results with peptide catalysts showing that electronic substitution did not significantly affect the enantioselectivity, the CADI analogue was highly sensitive to this substitution. While electron-donating groups had small effect on the enantioselectivities (40% ee for MeO (**23**) and 42% ee for Me (**24**), respectively), introduction of electron-withdrawing groups at this *para*-position resulted in higher enantioselectivities (51% ee for Br (**25**) and 75% ee for CF3 (**26**)) than the *N*-Ph substrate (**1**). There is a good correlation between the enantiomeric

excess and the Hammet substituent constant (σ_p), which represents the acidity of carbamate hydrogen atom. These results support the N-H···Cl-C H-bonding interaction being responsible for the observed enantioselectivity.

Table 2. *para*-Substituent effects on the catalytic performance of **3** and **13**.



(0.15 mmol)

catalytic compounds	R (σ_p)	enatiomeric excess	catalytic compounds	R (σ_p)	enatiomeric excess
13	OMe (-0.27), **23**	40% *ee*	**3**	OMe (-0.27), **23**	78% *ee*
13	Me (-0.17), **24**	42% *ee*	**3**	Me (-0.17), **24**	75% *ee*
13	H (0.00), **1**	44% *ee*	**3**	H (0.00), **1**	80% *ee*
13	Br (0.23), **25**	51% *ee*	**3**	Br (0.23), **25**	82% *ee*
13	CF_3 (0.54), **26**	75% *ee*	**3**	CF_3 (0.54), **26**	83% *ee*

Energetics of H-bonding interactions in catalysis

Given the catalytic ability of the CADI analogue (**13**), the energy of the H-bonding interaction of **13** was determined thermodynamically, applying the Eyring plot (equation 1).[23,24] The term of the natural logarithm of relative rate constants is converted to a term using enantiomeric excess, as shown in equation 2.[24,25]

$$R\ln(k_R/k_S) = \Delta\Delta S^{\ddagger}_{R\text{-}S} - \Delta\Delta H^{\ddagger}_{R\text{-}S}/T \qquad (1)$$

$$R\ln(k_R/k_S) = R[\ln(100+\%ee)/(100-\%ee)] \qquad (2)$$

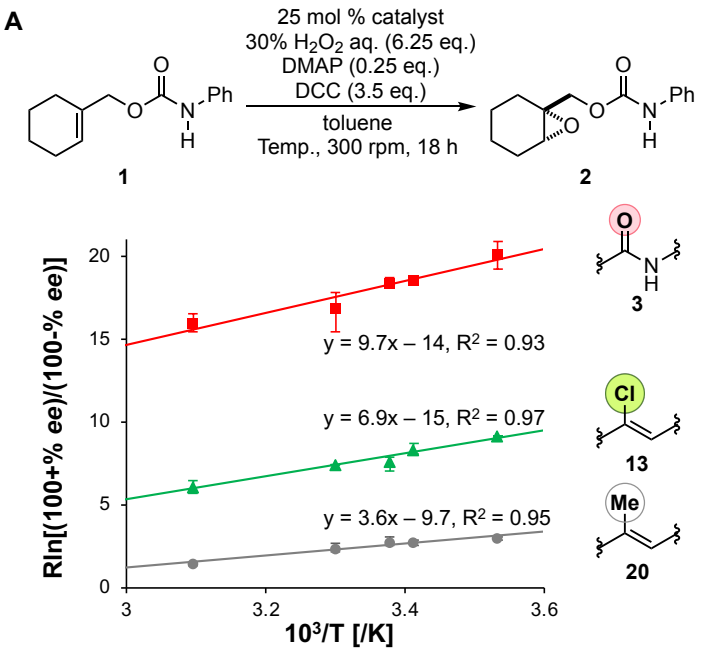
The temperature dependence of the enantioselectivity was determined by chiral HPLC analysis and plotted as a straight-line using equation 1 for each reaction with **3**, **13**, and **20** (Figure 5). With these plots, the activation enthalpy ($\Delta\Delta H^{\ddagger}_{R\text{-}S} = \Delta H^{\ddagger}_{R} - \Delta H^{\ddagger}_{S}$) contributing to non-covalent interactions was calculated. For **3**: $\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{amide}}$ = -9.7 kJ/mol, for **13**: $\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{CADI}}$ = -6.9 kJ/mol, and for **20**: $\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{MADI}}$ = -3.6 kJ/mol. Since the methylalkene moiety in **20** has no H-bonding possibility, $\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{MADI}}$ is perceived as other factors common to **3** and **13** with the exception of the H-bonding interactions. When $\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{MADI}}$ is subtracted from $\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{CADI}}$ and $\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{amide}}$ (equations 3 and 4) this gives the difference of energies of the H-bonding interactions between the transition states leading to the formation of the major (*R,R*)- and minor (*S,S*)-products as $\Delta\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{H-bond|amide}}$ = -6.1 kJ/mol for **3** and $\Delta\Delta\Delta H^{\ddagger}_{R\text{-}S\ \text{H-bond|CADI}}$= -3.3 kJ/mol for **13**, respectively. Consequently, the chlorine substituent in the CADI analogue can participate in H-bonding with a minimum energy of 3.3 kJ/mol.

$$\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{amide}} - \Delta\Delta H^{\ddagger}_{R\text{-}S|\text{MADI}}$$
$$= (\Delta H^{\ddagger}_{R|\text{amide}} - \Delta H^{\ddagger}_{S|\text{amide}}) - (\Delta H^{\ddagger}_{R|\text{MADI}} - \Delta H^{\ddagger}_{S|\text{MADI}})$$
$$= (\Delta H^{\ddagger}_{R|\text{amide}} - \Delta H^{\ddagger}_{R|\text{MADI}}) - (\Delta H^{\ddagger}_{S|\text{amide}} - \Delta H^{\ddagger}_{S|\text{MADI}})$$
$$= \Delta\Delta H^{\ddagger}_{R|\text{H-bond|amide}} - \Delta\Delta H^{\ddagger}_{S|\text{H-bond|amide}}$$
$$= \Delta\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{H-bond|amide}} \quad (3)$$

$$\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{CADI}} - \Delta\Delta H^{\ddagger}_{R\text{-}S|\text{MADI}}$$
$$= (\Delta H^{\ddagger}_{R|\text{CADI}} - \Delta H^{\ddagger}_{S|\text{CADI}}) - (\Delta H^{\ddagger}_{R|\text{MADI}} - \Delta H^{\ddagger}_{S|\text{MADI}})$$
$$= (\Delta H^{\ddagger}_{R|\text{CADI}} - \Delta H^{\ddagger}_{R|\text{MADI}}) - (\Delta H^{\ddagger}_{S|\text{CADI}} - \Delta H^{\ddagger}_{S|\text{MADI}})$$
$$= \Delta\Delta H^{\ddagger}_{R|\text{H-bond|CADI}} - \Delta\Delta H^{\ddagger}_{S|\text{H-bond|CADI}}$$
$$= \Delta\Delta\Delta H^{\ddagger}_{R\text{-}S|\text{H-bond|CADI}} \quad (4)$$

While the absolute value of the entropic term of the catalysis for **13** is comparable to that for **3**, the absolute value of the entropic term of the catalysis with **20** is significantly lower than those of **3** and **13**. This shows that the transition states providing (*R*,*R*)- and (*S*,*S*)-products have almost the same degrees of freedom, probably due to the absence of the catalyst-substrate H-bonding interaction.

A



B

catalytic compounds	$\Delta\Delta H^{\ddagger}_{R\text{-}S}$ (kJ/mol)[a,b]	$T\Delta\Delta S^{\ddagger}_{R\text{-}S}$ (kJ/mol)[a,b]	$\Delta\Delta\Delta H^{\ddagger}_{R\text{-}S\|H\text{-bond}}$ (kJ/mol)[b]
amide-type **3**	-9.7	-4.3	-6.1[c]
CADI analogue **13**	-6.9	-4.6	-3.3[d]
MADI analogue **20**	-3.6	-2.9	—

[a] From equation 1 and 2. [b] The values listed in the Table are those at 298 K.
[c] From equation 3. [d] From equation 4.

Figure 5. (**A**) Temperature dependence of the enantioselectivity and (**B**) Activation parameters for the asymmetric epoxidation reactions catalyzed by peptide **3** and ADI analogues **13** and **20**.

DFT calculation of transition states of the CADI- and amide-type catalysts.

To investigate the nature of the H-bonding acceptor ability of CADI, the transition states of the asymmetric epoxidation reactions with CADI and amide catalysts were investigated computationally. Using models in which the N-Boc group was changed to the *N*-acetyl group (chloroalkene-type complex: **27**, amide-type complex: **28**) (Figure 6), the transition states were calculated by density functional theory (DFT) methods using several parameters, including dispersion-corrected functionals (ωB97XD, and M06-2X). The functional screening revealed that the calculated results obtained at the ωB97D/6-311G(d,p) level of theory are reasonable for this system, and single-point energies were calculated at the B97D/6-311++G(d,p) level using the polarizable continuum model (toluene) based on the structures optimized by the ωB97XD method.

In the chloroalkene-type complex (**27**), the distance between the carbamate hydrogen atom of substrate and the chlorine atom of the CADI catalyst in **27** is 2.70 Å, which is less than the sum of the van der Waals radii for hydrogen and chlorine (3.0 Å).[26] This supports the theory that the chlorine substituent of CADI can serve as a H-bonding accepter in the transition state. The length of N-H···Cl-C H-bonding interaction in **27** is longer than that of the corresponding H-bond (1.99 Å) in **28**, indicating that the chlorine substituent of CADI catalyst can interact with H-bond donors at a long-range due mainly to the large van der Waals radius of chlorine. It should be noted that there is a characteristic difference in the bond angles of H-bonding interactions. The bond angle of the Cl-mediated H-bonding interaction in **27** is 103.0°, which is smaller than that of the corresponding H-bond (136.1 °). This geometrical feature around the chlorine substituent in **27** is probably due to the negative electrostatic potential developed on the lateral portions of the chlorine atom. This observed geometry of the Cl-mediated H-bonding interaction has been observed in crystallographically[27] and by calculations[16c], suggesting that it is one of the favorable geometries of the chlorine-mediated molecular interactions.

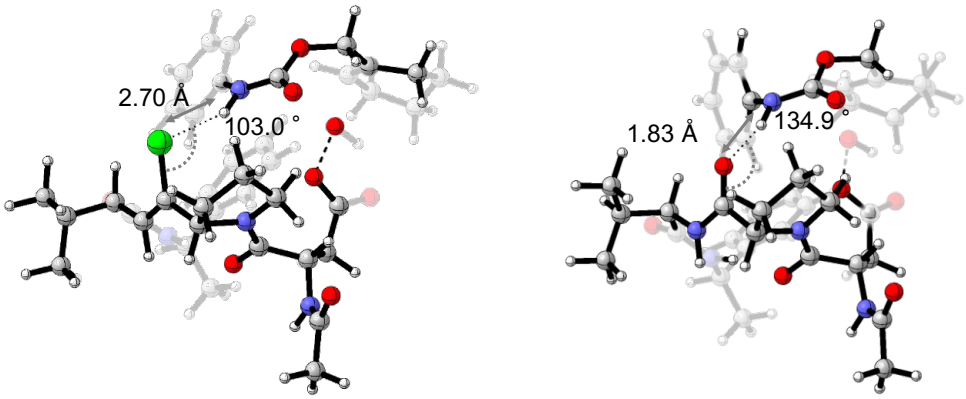


Figure 6. Comparison of the transition states for the asymmetric epoxidation reactions with CADI and

amide catalysts.

To investigate whether the geometries of **27** and **28** enable H-bonding, Natural Bonding Orbital (NBO) analyses[28] were carried out at the ωB97XD/6-311G** level. This NBO analysis revealed that the chlorine substituent in **27** has two types of lone pairs. One is characterized by an *s*-type orbital ($n_{s\,(Cl)}$) along the C-Cl bond and the second by *p*-type orbitals ($n_{p\,(Cl)}$) and oriented orthogonally with respect to one another (Figures 7A-7C). The calculated interaction energy of *p*-type lone pairs is 4.72 kcal/mol, which is 6.3 times higher than that of *s*-type lone pair ($n_{s\,(Cl)} \rightarrow \sigma^*_{(N\text{-}H)}$, 0.75 kcal/mol). Consequently, in the chloroalkene-type complex, *p*-type orbitals are more likely to participate in the N-H···Cl-C H-bonding interaction than the *s*-type orbital. For the amide-type complex, an *s*-type lone pair is more likely to participate in the H-bond than the p-type carbonyl lone pair (Figures 8A and 8B). The interaction energy of the *s*-type lone pair is 8.43 kcal/mol, which is 5 times higher than that of the *p*-type lone pair ($n_{p\,(O)} \rightarrow \sigma^*_{(N\text{-}H)}$, 1.67 kcal/mol), and this is consistent with the observation that in the secondary structures such as the α-helix or the β-sheet, amide hydrogens often approach carbonyl oxygens along the axis of the carbonyl bond to form H-bonds.[13c] These calculations suggest that the orientation of the Cl-mediated H-bonding interactions is possibly different from that of the H-bonds involving the carbonyl oxygen of amides. Although the chlorine substituent of CADIs is a weaker H-bond acceptor than the carbonyl oxygen of amides, the substitution effects on the orientation of H-bonds may play important roles in determining the conformations and molecular recognitions of peptidomimetics containing CADIs.

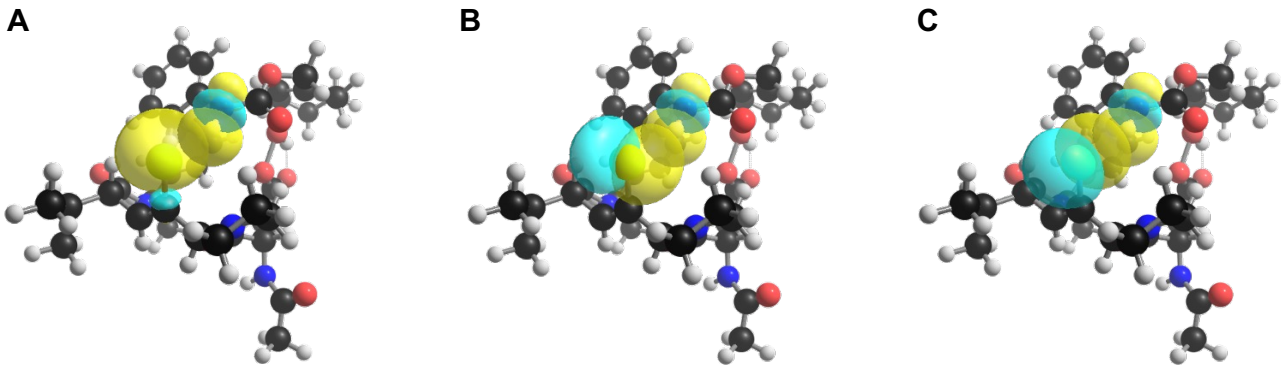


Figure 7. Selected overlap interactions of **27** between $n_{s(Cl)}$ and $\sigma^*_{(N\text{-}H)}$ (**A**), between $n_{p1(Cl)}$ and $\sigma^*_{(N\text{-}H)}$ (**B**), and between $n_{p2(Cl)}$ and $\sigma^*_{(N\text{-}H)}$ (**C**).

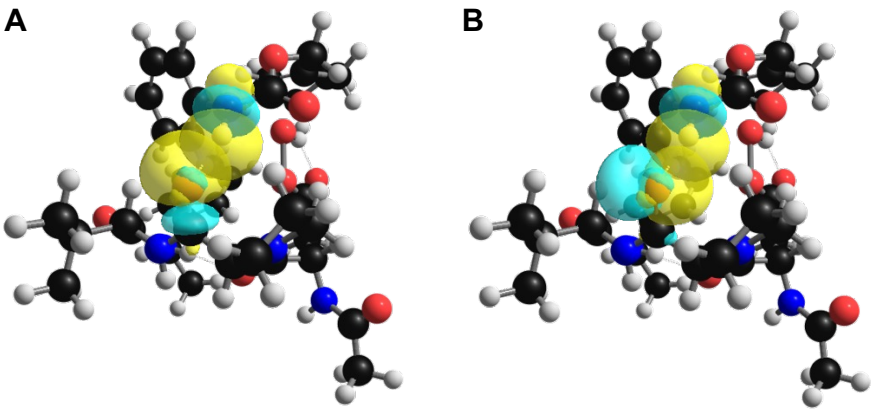


Figure 8. Overlap interactions of **28** between $n_{s(O)}$ and (**A**) $\sigma^*_{(N\text{-}H)}$, and (**B**) between $n_{p(O)}$ and $\sigma^*_{(N\text{-}H)}$.

CONCLUSION

We have investigated experimentally and computationally, the ability of the chlorine substituent in CADIs to perform as an H-bonding acceptor. The (*Z*)-chloroalkene and (*E*)-methylalkene analogues of the peptide catalyst for the asymmetric epoxidation reaction were synthesized and used as probe molecules to evaluate the H-bonding acceptor ability of CADIs. Peptidomimetic studies indicated that the chlorine substituent of CADIs can form intermolecular H-bonding interactions and is more sensitive to the acidity of donor hydrogen atoms than that of the carbonyl oxygen of amides. Our study also showed that the H-bonding acceptor ability follows the order: amide > FADI > CADI. Computational studies also revealed the unique ability of the chlorine substituent of CADIs to form H-bonding interactions preferentially on the lateral portions of the chlorine atom. Since H-bonds play a crucial role in determining molecular structures and recognitions, these finding provide valuable insights into the rational design of peptidomimetics containing CADIs.

Acknowledgements

This research was supported by a Grant-in-Aid for Scientific Research (B) (20H03363) from The Ministry of Education, Culture, Sports, Science and Technology, Japan and in part by the Takeda science foundation. We are grateful to Dr. Yusuke Sasano (Tohoku University) for assistance in DFT calculation.

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