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Design and Synthesis of a Transition State Analogue for the Diels–Alder Reaction

Paramjit S. Arora,[a] Que N. Van,[a] Michael Famulok,[b,*] A. J. Shaka[a] and James S. Nowick[a,*]

[a]*Department of Chemistry, University of California-Irvine, Irvine, CA 92697-2025, USA*
[b]*Institute of Biochemistry, Genzentrum of the Ludwig-Maximilian-University of Munich, Feodor-Lynen Strasse 25, D81375 Munich, Germany*

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Abstract—This paper describes the design and synthesis of a tricationic transition state analogue (TSA **1**) for the Diels–Alder reaction. TSA **1** contains a bicyclo[2.2.1]heptene ring system that mimics the boat conformation of the Diels–Alder transition state and is designed to bind tightly to antibodies, nucleic acids, and imprinted polymers by means of hydrogen bonds and salt-bridges. This paper also describes the syntheses of the Diels–Alder reaction substrates (diene **2** and dienophile **3**) and a sensitive HPLC assay to monitor the formation of Diels–Alder product **4**. In contrast to previously reported TSAs and dienophiles for the Diels–Alder reaction that are based upon maleimides, TSA **1** and dienophile **3** are based upon fumaramide. The fumaramide system should destabilize the initially formed boat conformer of Diels–Alder product **4** and stabilize a half-chair conformer. The conversion of the initially formed boat conformer to the half-chair conformer is designed to help prevent Diels–Alder product **4** from binding strongly to catalysts selected to strongly bind TSA **1**. This feature should minimize product inhibition, which can be a problem in the catalysis of the Diels–Alder reaction.

Introduction

The development of catalysts for carbon–carbon bond forming reactions has become an important goal in the past two decades. One approach to developing catalysts involves the use of transition state analogues (TSAs).[1] Transition state analogues are stable compounds that mimic the transition state of a reaction and can be used to elicit antibodies,[2] nucleic acids,[3] and imprinted polymers[4] that tightly bind the transition state for that reaction. By binding to the transition state, these macromolecules lower the energy of activation and accelerate the reaction.[5] TSAs have been employed to elicit antibodies that catalyze acyl transfer,[6] amide bond formation,[7] mechanistically disfavored epoxide opening,[8] *syn*-eliminations,[9] and various other reactions. Recently, TSAs have been used to generate nucleic acids that catalyze biphenyl isomerization[10] and porphyrin metalation.[11,12] Imprinted polymers that catalyze ester hydrolysis have also been prepared using this approach.[13] Carbon–carbon bond forming reactions have proven particularly attractive targets for supramolecular catalysis using TSAs,[2c] and catalytic antibodies for reactions such as the aldol condensation,[14] the Claisen rearrangment,[15] and the Diels–Alder reaction,[16–18] have been reported.

The Diels–Alder reaction is one of the most powerful carbon–carbon bond forming reactions, because it generates two new carbon–carbon bonds and four stereogenic centers simultaneously. For this reason, the Diels–Alder reaction has become a popular target for supramolecular catalysis. In 1989, Hilvert and co-workers reported a bicyclo[2.2.1]heptene TSA that elicits catalytic antibodies for the Diels–Alder reaction of a tetrachlorothiophene dioxide and *N*-ethylmaleimide.[16a] The next year, Schultz and co-workers reported a [2.2.2]bicyclooctene TSA that elicits catalytic antibodies for the reaction of an acyclic diene and an *N*-arylmaleimide.[16b] Several other systems have been reported subsequently.[16c–g]

Product inhibition can be a serious concern in the catalysis of the Diels–Alder reaction, because the cyclohexene product resembles the Diels–Alder transition state.

*Corresponding authors.

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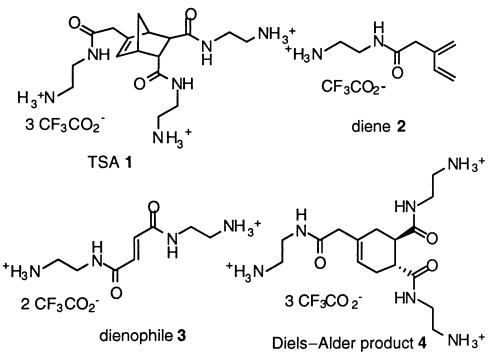
As the product forms, it can bind to the catalyst, thus preventing the substrates from binding and inhibiting catalysis. One of the challenges of the TSA approach is to generate catalysts that tightly bind to the transition state analogue and the transition state, but not to the product. The Hilvert system circumvents the product inhibition problem, because the initially formed Diels–Alder product loses sulfur dioxide to form a cyclohexadiene derivative. In the Schultz system, the antibody binds the product more tightly than the substrates.

This paper describes the design and synthesis of a tricationic transition state analogue (TSA **1**) for the Diels–Alder reaction that addresses the problem of product inhibition by means of conformational effects. It also describes the syntheses of the Diels–Alder reaction substrates (diene **2** and dienophile **3**) and a sensitive HPLC assay to monitor the formation of Diels–Alder product **4**.

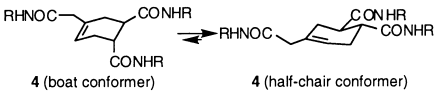


Results and Discussion

Design of the TSA

We designed TSA **1** to mimic the geometry of the Diels–Alder transition state and to non-covalently bind macromolecules. The transition state for the Diels–Alder reaction resembles a cyclohexene ring in a boat conformation.[19] The bicyclo[2.2.1]heptene ring of TSA **1** duplicates this conformation and mimics the transition state for the Diels–Alder reaction of diene **2** and dienophile **3**. The amide and ammonium groups of the tricationic TSA should form hydrogen bonds and salt-bridges with the polar and charged groups of antibodies, nucleic acids, and imprinted polymers.

To address the problem of product inhibition, we employed a fumaramide derivative (**3**) as the dienophile, instead of a maleimide derivative. Molecular modeling studies indicate that Diels–Alder adducts of maleimide favor a boat conformer that resembles the Diels–Alder transition state; a search of the Cambridge Structural Database corroborates the modeling studies.[20] For this reason, maleimide-based systems should be particularly prone to product inhibition. In contrast to the maleimide Diels–Alder adducts, the fumaramide Diels–Alder adduct **4** should relax from the initially formed boat conformer to the half-chair conformer (eq (1)).[16d] The product is not expected to severely inhibit catalysis, because catalysts selected against TSA **1** should bind tightly to the boat conformer, but not to the half-chair conformer.



(1)

Molecular mechanics calculations establish that TSA **1** closely resembles the transition state for the Diels–Alder reaction. The TSA and transition state were modeled using MacroModel V5.5 and the MM2* force field.[21] Houk and co-workers have developed empirical force-field parameters for the transition state of the Diels–Alder reaction, which are available within MacroModel.[22] Because parameters are only available for a limited set of substituents on the diene and dienophile, the transition state was modeled by the reaction of 2-methylbuta-1,3-diene with fumaraldehyde and TSA **1** was modeled as the corresponding bicyclo[2.2.1]heptene dialdehyde (Fig. 1). When the TSA and transition state are superimposed, the analogous carbon and oxygen atoms overlay with an RMS deviation of 0.41 Å. The TSA bears considerably less resemblance to the half-chair conformer of the product. When the TSA and the

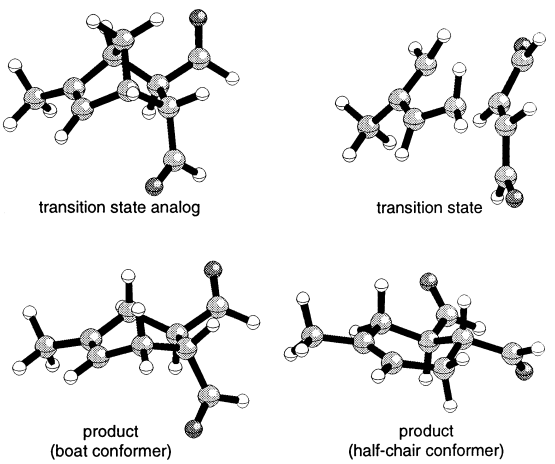


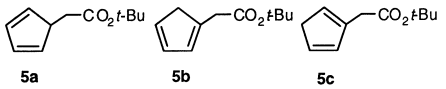
Figure 1. Molecular models of the transition state analogue, transition state, and product. The transition state was modeled by the reaction of 2-methylbuta-1,3-diene with fumaraldehyde, the transition state analogue was modeled as the corresponding bicyclo[2.2.1]heptene dialdehyde, and the product was modeled as the corresponding cyclohexene dialdehyde. Calculations were performed using MacroModel V5.5 and the MM2* force field.[21,22]

half-chair conformer are superimposed, the analogous carbon and oxygen atoms overlay with an RMS deviation of 1.02 Å.

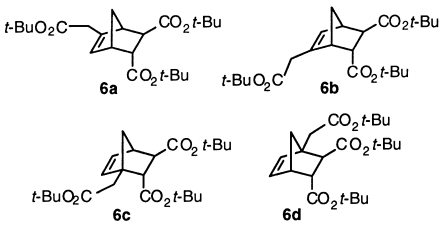
The half-chair conformer of the product is much more stable than the boat conformer. Modeling of the dialdehyde product indicates the boat conformer to be 6.9 kcal/mol higher in energy than the half-chair conformer (Fig. 1).[23] This energy difference should be sufficient to force the product to adopt the half-chair conformer, thus minimizing product inhibition.

Synthesis of TSA 1

Transition state analogue **1** was prepared as outlined in Scheme 1. Alkylation of sodium cyclopentadienide with *tert*-butyl bromoacetate affords cyclopentadiene **5**. Cyclopentadiene **5** comprises a mixture of three regioisomers (**5a–c**), which interconvert rapidly by a series of 1,5-sigmatropic shifts.[24]



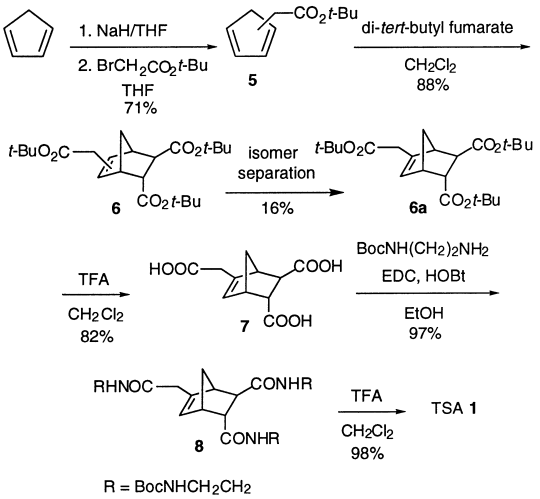
^{1}H NMR spectroscopy shows isomers **5b** and **5c** to be the major components of this mixture.[25] Diels–Alder reaction of **5** with di-*tert*-butyl fumarate[26] generates triester **6** as a mixture of four isomers (**6a–d**). The mixture



was separated by repeated column chromatography.[27] The separation proved difficult, because all of the diastereomers have similar R_f values. Nevertheless, isolation of the leading edge of the product band afforded diastereomer **6a** in 93% diastereomeric purity. Treatment of the of the diastereomeric triester **6a** with trifluoroacetic acid (TFA), to remove the *tert*-butyl ester groups, yielded triacid **7**. Triacid **7** was coupled with *N*-Boc-ethylenediamine[28] to produce carbamate **8**. Removal of the Boc protective groups with TFA provided TSA **1**.

Stereochemistry of triester 6a, triacid 7, and TSA 1

The stereochemistry of the Diels–Alder adduct **6a**, which was isolated by column chromatography, was determined by ^{1}H NMR spectroscopic studies of triacid



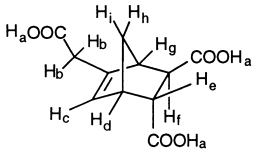
Scheme 1. Synthesis of transition state analogue **1**.

7. The triacid exhibits nine distinct resonances in the ^{1}H NMR spectrum in DMSO-d_6 (Table 1). Four of these resonances (1.40, 1.46, 5.75, and 12.24 ppm) are readily assigned to H_i, H_h, H_c, and H_a on the basis of their chemical shifts. The remaining five resonances are too similar in chemical shift (2.54–3.17 ppm) to be assigned unambiguously.

The assignment of these resonances is central to the determination of the stereochemistry of **7** and was performed using DPFGSE (double pulsed-field-gradient spin-echo) NOE[29] studies (Table 1). The DPFGSE NOE technique provides a substantial advantage over the conventional steady-state difference NOE method, because it is free of subtraction artifacts. The olefinic proton (H_c) gives an NOE to the resonance at 3.05 ppm, which was assigned to H_d. The resonance at 3.17 gives an NOE with H_d and was assigned as H_e. The resonance at 2.54 gives an NOE to H_e and was assigned as H_f. H_e exhibits an NOE to the resonance at 1.46 ppm (H_h), while H_f does not. The presence of an NOE between H_e and H_h establishes that H_e is *exo* and H_f is *endo*.

^{1}H NMR coupling constant studies further confirm this stereochemical assignment. H_e appears as a triplet with a coupling constant of 4.0 Hz, while H_f appears as a doublet with a coupling constant of 4.3 Hz. To interpret these values, a molecular model was generated for **7** using MacroModel and the MM2* force field,[21] and coupling constants were calculated according to the Karplus relationship within the program.[30] Using this model, coupling constants were determined as follows: $J_{de} = 5.2$ Hz, $J_{ef} = 5.7$ Hz, $J_{fg} = 0.6$ Hz). These values are reasonably consistent with those seen experimentally.

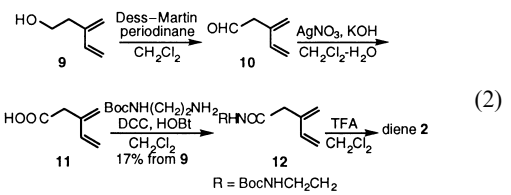
Table 1. [1]H NMR and NOE data for triacid **7**[a]



Assignment	Description	NOEs[b]
H_a	12.24 (br s, 3 H)	not measured
H_b	3.07 (s, 2 H)	not measured[c]
H_c	5.75 (s, 1 H)	H_b, H_d
H_d	3.05 (s, 1 H)	not measured[c]
H_e	3.17 (t, J = 4.0 Hz, 1 H)	H_d, H_f, H_h
H_f	2.54 (d, J = 4.3 Hz, 1 H)	H_b, H_e, H_g
H_g	2.94 (s, 1 H)	H_b, H_f, H_h, H_i
H_h	1.46 (d, J = 8.2 Hz, 1 H)	H_d, H_e, H_g, H_i
H_i	1.40 (d, J = 8.0 Hz, 1 H)	H_b, H_c, H_d, H_g, H_h

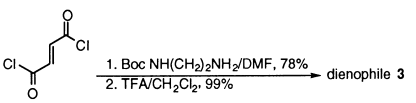
[a]^{1}H NMR data were recorded at 500 MHz in DMSO-d_6.
[b]NOEs were measured using the DPFGSE NOE technique.[29]
[c]Selective irradiation of this resonance was not possible.

Synthesis of diene 2, dienophile 3, and Diels–Alder product 4. Diene **2** was prepared from alcohol **9**[31] in four steps (eq (2)). The synthesis requires that alcohol **9** be oxidized to acid **11**, however, this transformation proved difficult. Attempts to oxidize **9** to **11** directly with Jones reagent yielded complex mixtures of products that could not be characterized. Oxidation of the alcohol first to aldehyde **10** and then to the acid proceeded in low yield. Among the reagents used to carry out this transformation, only the Dess–Martin reagent[32] provided aldehyde **10** with any success; other procedures (e.g. Swern oxidation[33] and TPAP oxidation[34]) resulted in migration of the diene into conjugation with the aldehyde group. Aldehyde **10** was further oxidized with silver oxide[35] to acid **11**. Acid **11** was coupled to *N*-Boc-ethylenediamine with DCC to obtain carbamate **12**; deprotection of carbamate **12** with TFA afforded diene **2**.



(2)

Dienophile **3** was prepared in two steps from fumaryl chloride (eq (3)). Reaction of fumaryl chloride with *N*-Boc-ethylenediamine in DMF provided carbamate **13**. The reactants were combined in a 1:2 ratio, and no additional base was used; when triethylamine or pyridine were added to the reaction mixture (to react with the HCl byproduct), black tar formed. Removal of the Boc group with TFA yielded dienophile **3**.



(3)

An authentic sample of Diels–Alder product **4** was required for the development of a kinetic assay for the Diels–Alder reaction between diene **2** and dienophile **3**. The Diels–Alder reaction of these compounds is slow, however, and it does not provide an efficient means of preparing **4** in sufficient quantity. To circumvent this slow reaction, we employed an alternative route. Thus, compound **4** was prepared by Diels–Alder reaction between alcohol **9** and di-*tert*-butyl fumarate, followed by oxidation of the alcohol group to the carboxylic acid, removal of the two *tert*-butyl groups with TFA, coupling of the three carboxylic acid groups with *N*-Boc-ethylenediamine, and removal of the three Boc protective groups with TFA.

Kinetic assay for the Diels–Alder reaction between diene 2 and dienophile 3

HPLC provides a convenient way to monitor the reaction between diene **2** and dienophile **3**. The primary amino groups of diene **2**, dienophile **3**, and Diels–Alder product **4** are readily converted to fluorescent isoindole groups by treatment of the Diels–Alder reaction mixture with *o*-phthaldialdehyde (OPT) and ethanethiol.[36,37]

The reaction was monitored by HPLC using both UV (229 nm) and fluorescence (excitation 243 nm, emission 460 nm) detection. Fluorescence detection provides greater sensitivity than UV and permits quantitation of **4** at micromolar concentrations. Benzylamine (100 μM) was used as an internal standard in these studies, and the concentration of **4** was determined by comparison of the areas of the peaks associated with the isoindole derivatives of these compounds. Studies in which known concentrations of **4** and benzylamine were derivatized, injected into the HPLC, and measured using fluorescence detection established that the peak area from **4** is 4.5 times that of benzylamine.[38] Kinetics studies indicate that the second-order rate constant for the reaction is $1.9 \pm 0.5 \times 10^{-2}\,M^{-1}\,h^{-1}$ at 298 K in aqueous buffer at pH 7.6.[39] The reaction can be run at millimolar concentrations and generates micromolar concentrations of product over the course of hours or days, without decomposition of the reactants.

Conclusion

Transition state analogue **1**, diene **2**, and dienophile **3** provide a set of tools for the development of macromolecular catalysts for the Diels–Alder reaction. These components add to the limited set of transition state analogues and reactants available for supramolecular catalysis of carbon–carbon bond formation. The cationic nature and conformational features of the present system should be particularly valuable in developing Diels–Alder catalysts with good binding properties, while minimizing problems associated with product inhibition. The ability of TSA **1** to elicit catalysts, as well as the cationic and conformational design elements of this system, remain to be evaluated experimentally.

Experimental

Materials and methods

Commercial grade reagents and solvents were used without further purification, except as indicated below. Dichloromethane was distilled from calcium hydride. Tetrahydrofuran was distilled from sodium and benzophenone under nitrogen. Sodium hydride (60% in mineral oil) was washed several times with hexanes and dried under a stream of nitrogen gas before use. 2-Methyl-2-propanol was distilled from sodium. All reactions were stirred magnetically in flame- or oven-dried glassware under a positive pressure of nitrogen gas.

Di-*tert*-butyl fumarate. A mixture of fumaryl chloride (1.03 g, 6.75 mmol), silver cyanide (1.31 mol, 32.7 mmol), 2-methyl-2-propanol (12.3 mL), and benzene (10 mL) was heated at reflux for 2 h.[40] The reaction mixture was allowed to cool to room temperature, filtered through Celite, and concentrated by rotary evaporation to afford a pale white solid. The solid was redissolved in 50 mL of CH_2Cl_2, and the solution was washed with 30 mL of saturated aqueous Na_2CO_3, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to obtain a 1.50 g (97%) of di-*tert* butyl fumarate as a white solid.[26]

Triester 6. A flame-dried, 250 mL, three-necked, round-bottomed flask, which was equipped with a nitrogen inlet adapter and a magnetic stirring bar, was charged with sodium hydride (5.99 g, 144 mmol) and THF (200 mL). The mixture was cooled with an ice bath, and cyclopentadiene (10 mL, 120 mmol) was added dropwise by a syringe over ca. 10 min. After the evolution of hydrogen gas ceased, 200 mL of THF was added. The reaction mixture was cooled to −78 °C with a dry ice-acetone bath, and *tert*-butyl bromoacetate (16.5 mL, 108 mmol) was added by syringe over 30 min. After 15 min, the dry ice–acetone bath was removed and the mixture was stirred for 30 min. The reaction mixture was quenched with 300 mL of water, and the aqueous phase was extracted with two 150 mL portions of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to afford a yellow oil. Column chromatography on silica gel (EtOAc:hexanes, 3:97) afforded 17.3 g (71%) of cyclopentadiene **5** as a colorless oil.

A solution of cyclopentadiene **5** (10.5 g, 56.9 mmol) and di-*tert*-butyl fumarate (10.0 g, 43.8 mmol) in CH_2Cl_2 (200 mL) was allowed to react under nitrogen for 20 h and then concentrated by rotary evaporation to afford a colorless oil. Column chromatography on silica gel (EtOAc:hexanes, 5:95) afforded 15.7 g (88%) of the triester **6** as a colorless oil. A single diastereomer (**6a**, 93% diastereomerically pure, 2.45 g) was isolated by repeated column chromatography on silica gel (EtOAc: hexanes, 5:95; $R_f = 0.15$). (The mixture of diastereomers and regioisomers elutes as a broad band, in which the first fractions are enriched in **6a**. These fractions were isolated, and later fractions containing **6a** were combined and subjected to further chromatography. ^1H NMR spectroscopy was used to analyze the isomeric purity of the fractions.): IR (neat) 1731 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.80 (s, 1H), 3.26 (t, $J = 4.2$ Hz, 1H), 3.12 (br s, 1H), 3.08 (s, 2H), 3.00 (s, 1H), 2.60 (dd, $J = 4.62$, 1.5 Hz, 1H), 1.61 (d, $J = 8.6$ Hz, 1H), 1.54 (dd, $J = 8.6$, 1.7 Hz, 1H), 1.47 (s, 9H), 1.46 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6, 172.2, 169.7, 144.4, 130.7, 80.5, 80.1, 79.5, 50.6, 50.3, 47.5, 47.1, 46.0, 36.8, 27.8; HRMS (FAB) *m/e* for $C_{23}H_{37}O_6$ $(M+H)^+$, calcd 409.2591, found 409.2592. Anal. calcd for $C_{23}H_{36}O_6$: C, 67.62; H, 8.88. Found: C, 67.69; H, 9.09.

Triacid 7. A solution of triester **6a** (1.60 g, 3.91 mmol) and trifluoroacetic acid (3.0 mL, 39 mmol) in CH_2Cl_2 (30 mL) was stirred for 20 h and then concentrated by rotary evaporation. The residual trifluoroacetic acid was removed by co-evaporation with three 10 mL portions of ether to afford 0.92 g of a pale-white solid. Column chromatography on silica gel (MeOH:CH_2Cl_2, 10:90), followed by recrystallization from EtOAc:CH_2Cl_2, afforded 0.70 g (82%) of triacid **7** as a white solid: IR (KBr) 3600–2300, 1704 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.24 (br s, 3H), 5.75 (s, 1H), 3.17 (t, J = 4.0 Hz, 1H), 3.07 (s, 2H), 3.05 (s, 1H), 2.95 (s, 1H), 2.54 (d, J = 4.3 Hz, 1H), 1.46 (d, J = 8.2 Hz, 1H), 1.40 (d, J = 8.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.5, 174.2, 171.9, 144.8, 130.4, 50.1, 49.2, 47.1, 46.4, 45.4, 35.1; HRMS (CI, NH_3) m/e for $C_{11}H_{16}NO_6$ $(M+NH_4)^+$, calcd 258.0977, found 258.0974. Anal. calcd for $C_{11}H_{12}O_6$: C, 55.00; H, 5.04. Found: C, 54.89; H, 5.15.

Carbamate 8. A solution of triacid **7** (0.100 g, 0.416 mmol), *N*-Boc-ethylenediamine[28] (0.333 g, 2.08 mmol), 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide hydrochloride (0.400 g, 2.08 mmol), and 1-hydroxybenzotriazole hydrate (0.056 g, 0.416 mmol) in ethanol (7 mL) was stirred for 8 h and then concentrated by rotary evaporation. The residue was purified by column chromatography on silica gel (MeOH:CH_2Cl_2, 5:95) to afford 0.270 g (97%) of carbamate **8** as a white solid: IR (KBr) 3700–3200, 3326, 1691, 1643 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 8.22 (br s, 1H), 8.06 (br s, 1H), 7.98 (br s, 1H), 6.66–6.60 (m, 3H), 5.87 (s, 1H), 3.36–3.06 (m, 16H), 2.87 (s, 1H), 2.60 (s, 1H), 1.60 (d, J = 8.0 Hz, 1H), 1.49–1.42 (m, 28H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.4, 174.4, 170.7, 156.4, 143.2, 133.8, 79.2, 79.1, 78.9, 54.2, 48.9, 48.4, 48.3, 48.0, 40.6, 40.2, 40.1, 40.0, 39.8, 38.4, 28.3; HRMS (FAB) m/e for $C_{32}H_{55}N_6O_9$ $(M+H)^+$, calcd 667.4030, found 667.4044. Anal. calcd for $C_{32}H_{54}N_6O_9$: C, 57.64; H, 8.16; N, 12.60. Found: C, 57.19; H, 7.93; N, 12.42.

TSA 1. A solution of carbamate **8** (1.56 g, 2.34 mmol) and trifluoroacetic acid (1.8 mL) in CH_2Cl_2 (7 mL) was stirred for 4 h and then concentrated by rotary evaporation. The residual trifluoroacetic acid was removed by co-evaporation with three 10 mL portions of ether to afford 1.63 g (98%) of TSA **1** as a light-yellow oil: IR (neat) 3700–2600, 3409, 3291, 3278, 2950, 1678 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 5.86 (s, 1H), 3.53–3.43 (m, 6H), 3.42–3.35 (m, 1H), 3.30 (t, J = 3.9 Hz, 1H), 3.22–3.04 (m, 8H), 2.90 (s, 1H), 2.58 (d, J = 4.7 Hz, 1H), 1.68 (d, J = 8.7 Hz, 1H), 1.55 (d, J = 8.8 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 177.3, 176.5, 174.5, 162.8 (q, J = 35 Hz), 144.9, 131.4, 116.4 (q, J = 292 Hz), 51.4, 50.2, 47.6, 47.5, 46.9, 39.2, 39.1, 37.1, 37.0, 36.8, 36.6, 36.5; HRMS (FAB) m/e for $C_{17}H_{31}N_6O_3$ $(M+H)^+$, calcd 367.2457, found 367.2449.

Carbamate 12. A solution of alcohol **9**[31] (1.30 g, 13.3 mmol) in CH_2Cl_2 (30 mL) was added to a solution of Dess–Martin reagent[32] (9.60 g, 22.6 mmol) in CH_2Cl_2 (50 mL) over 15 min. After 8 h, the reaction mixture was diluted with 50 mL of CH_2Cl_2 and poured into a mixture of 75 mL of 10% aqueous $Na_2S_2O_3$ and 75 mL of saturated aqueous $NaHCO_3$. The mixture was stirred to dissolve the solid, the layers were separated, and the aqueous phase was extracted with two 50 mL portions of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under a flow of nitrogen gas to a volume of 50 mL (aldehyde **10** in CH_2Cl_2). This solution of aldehyde **10** was added to a solution of silver nitrate[35] (5.21 g, 30.7 mmol) in H_2O (6 mL). The biphasic mixture was stirred for 5 min, and 0.1 M KOH (100 mL) was added in three portions over ca. 15 min. (CAUTION: the reaction is exothermic.) The thick suspension was stirred for 10 h and then filtered through Celite. The precipitate was washed with two 25 mL portions of CH_3OH, and the filterate was washed with CH_2Cl_2 (50 mL), acidified to pH 2 with concd HCl, and extracted with three 100 mL portions of diethyl ether. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to afford 0.92 g (62%, impure) of acid **11** as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 10.59 (br s,1H), 6.44 (dd, J = 17.6, 10.7 Hz,1H), 5.27–5.13 (m,4H), 3.29 (s,2H).

A solution of acid **11** (0.90 g, 8.04 mmol), *N*-Boc-ethylenediamine[28] (1.54 g, 9.65 mmol), dicyclohexyl carbodiimide (1.99 g, 9.65 mmol), and 1-hydroxybenzotriazole hydrate (0.22 g, 1.61 mmol) in THF (7 mL) was stirred for 8 h. The resulting suspension was filtered, and the filtrate was concentrated by rotary evaporation to afford 1.93 g of a white solid. Column chromatography on silica gel (MeOH:CH_2Cl_2, 3:97) yielded 0.59 g (17% from **9**) of carbamate **12** as a white solid: IR (KBr) 3350, 3302, 1689, 1659 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.43 (dd, J = 17.6, 10.8 Hz, 1H), 6.35 (br s, 1H), 5.28–5.15 (m, 4H), 4.99 (br s, 1H), 3.32 (app q, J = 5.6 Hz, 2H), 3.23 (app q, J = 5.5 Hz,2H), 3.19 (s,2H), 1.44 (s,9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 156.8, 140.5, 137.7, 120.8, 115.5, 79.5, 40.8, 40.6, 40.3, 28.5; HRMS (FAB) m/e for $C_{13}H_{23}N_2O_3$ $(M+H)^+$, calcd 255.1709, found 255.1713. Anal. calcd for $C_{13}H_{22}N_2O_3$: C, 61.39; H, 8.72; N, 11.01. Found: C, 60.92; H, 8.88; N, 10.76.

Diene 2

A solution of carbamate **12** (0.100 g, 0.393 mmol) and trifluoroacetic acid (1.00 mL) in CH_2Cl_2 (3 mL) was stirred for 30 min and then concentrated by rotary evaporation. The residual trifluoroacetic acid was removed by co-evaporation with three 10 mL portions of ether to afford 0.106 g (103%) of diene **2** as a yellow oil: IR

(neat) 3600–2600, 1674 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 6.48 (dd, J = 17.7, 10.8 Hz, 1H), 5.33–5.15 (m, 4H), 3.32 (t, J = 5.9 Hz,2H), 3.26 (s, 2H), 2.89 (t, J = 6.0 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 175.3, 163.0 (q, J = 35 Hz), 139.5, 137.8, 121.0, 116.3 (q, J = 289 Hz), 114.7, 39.0, 38.9, 36.9; HRMS (CI, isobutane) m/e for $C_8H_{15}N_2O$ $(M+H)^+$, calcd 155.1184, found 155.1190.

Carbamate 13. Fumaryl chloride (1.43 g, 9.35 mmol) was added to an ice-cooled solution of *N*-Boc-ethylenediamine[28] (3.11 g, 18.7 mmol) in DMF (25 mL). The ice bath was removed, and the reaction mixture was stirred at room temperature for 1 h. CH_2Cl_2 (50 mL) was added, and the resulting white precipitate was isolated by filtration and washed with CH_2Cl_2 to afford 2.91 g (77%) of carbamate **13** as a white solid: IR (KBr) 3351, 3313, 1691, 1632 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.43 (app t, J = 5.0 Hz, 2H), 6.83 (app t, J = 5.4 Hz, 2H), 6.77 (s, 2H), 3.18–3.11 (m, 4H), 3.01–2.95 (m, 4H), 1.38 (s, 18H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.8, 155.6, 132.6, 77.7, 39.9, 39.3, 28.2; HRMS (FAB) m/e for $C_{18}H_{33}N_4O_6$ $(M+H)^+$, calcd 401.2399, found 401.2392. Anal. calcd for $C_{18}H_{32}N_4O_6$: C, 53.99; H, 8.05; N, 13.99. Found: C, 54.06; H, 8.32; N, 13.84.

Dienophile 3. A solution of carbamate **13** (0.100 g, 0.250 mmol) and trifluoroacetic acid (0.5 mL) in CH_2Cl_2 (3 mL) was stirred for 1 h and then concentrated by rotary evaporation. The residual trifluoroacetic acid was removed by co-evaporation with three 10 mL portions ether, followed by trituration with hot EtOAc, to afford 0.106 g (99%) of dienophile **3** as a white solid: IR (neat) 3500–2600, 3288, 3143, 1674, 1657 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 6.90 (s, 2H), 3.60 (t, J = 5.9 Hz, 4H), 3.18 (t, J = 5.9 Hz, 4H); ^{13}C NMR (125 MHz, D_2O) δ 167.3, 162.9 (q, J = 35 Hz), 132.5, 116.9 (q, J = 291 Hz), 39.0, 37.1; HRMS (FAB) m/e for $C_8H_{17}N_4O_2$ $(M+H)^+$, calcd 201.1351, found 201.1350. Anal. calcd for $C_{12}H_{18}F_6N_4O_6$: C, 33.65; H, 4.24; N, 13.08. Found: C, 33.74; H, 4.42; N, 13.03.

Kinetic assay for the Diels–Alder reaction. *o*-Phthaldialdehyde (OPT) reagent was prepared as a solution composed of 500 mM OPT, 900 mM ethanethiol, and 1.5 M *N*,*N*-diisopropylethylamine in methanol.[36,37] This solution was freshly prepared each day, prior to use. In a typical experiment, 40 μL of a solution comprising 5.0 mM diene **2**, 5.0 mM dienophile **3**, and 0.10 mM benzylamine (internal standard) in aqueous buffer at pH 7.6[39] was incubated at 25 °C in an 0.5 mL Eppendorf tube. Aliquots (1 μL) were withdrawn periodically and mixed with acetonitrile (10 μL) and OPT reagent (1 μL). After 1 min, a 10 μL portion of the mixture was injected into an HPLC apparatus equipped with both UV and fluorescence detectors (C_{18} reverse-phase column, 77:23, CH_3CN:H_2O, 1.0 mL/min). Under these conditions, the following retention times were measured: 4.98 min (isoindole derivative of diene **2**), 6.57 min (isoindole derivative of dienophile **3**), 12.27 min (isoindole derivative of benzylamine), and 17.93 min (isoindole derivative of Diels–Alder product **4**).

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20. A search of the Cambridge Structural Database yielded 27 examples of Diels–Alder adducts of maleimide. Adducts containing [2.2.1]bicycloheptene and [2.2.2]bicyclooctene systems were excluded from the search. The cyclohexene rings of twenty-six of the adducts adopt a boat conformation (Cambridge Structural Database refcodes: BARDUT, BETDUZ, BIGSIT, EXMZPE, FEBDOF10, GEPHIS, GEPHOY, HEJDUV, JENSOK, JENTAX, JEXPIL, KERTEG, KOHRUU, KOHSAB, KOHSEF, MZPROD, PAFBIH, SIGLAV, TEDRAV, TULVAX, WEPHII, WEWCAC10, WISHEL, YUCWOI, YUVDEY, YUZVOE). In the remaining structure (refcode: CAFLIE), the cyclohexene ring adopts neither a boat nor a half-chair conformation, instead five of the cyclohexene carbons lie in one plane.

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37. The standard procedure for derivatization of amines with *o*-phthaldialdehyde (OPT) and a thiol in aqueous buffer (ref 35) was modified, because the derivatized diene **2**, dienophile **3**, and Diels–Alder product **4** are not soluble in aqueous solution. In the modified procedure, methanol and acetonitrile were used as solvents and *N*,*N*-diisopropylethylamine was used as a base.

38. The isoindole derivative of Diels–Alder product **4** exhibits a linear response in HPLC peak area between 1 and 50 μM; at higher concentrations a nonlinear response was observed.

39. A buffer at pH 7.6 consisting of 0.05 M Tris, 0.75 M NaCl, 0.010 $MgCl_2$, and 0.5 % Triton X-100 was used for these studies.

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