Antibody-Catalyzed Asymmetric Intramolecular Michael Addition of Aldehydes and Ketones to Yield the Disfavored Cis-Product

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The world’s first commercially available catalytic antibody, 38C2 (Ab38C2), is perhaps the most promiscuous antibody catalyst generated to date. The antibody was found to efficiently catalyze aldol and retro-aldol reactions of a remarkably broad range of substrates with excellent enantioselectivity through an enamine class I aldolase mechanism. The antibody was raised against the β-diketone hapten 1, which served as a chemical trap to imprint a unique lysine residue with a pKa of 5.8 (Scheme 1). The X-ray structure of the antibody binding site suggests an interaction between a tyrosine residue and one of the carbonyl groups of the hapten (Scheme 1). Antibody 38C2 is also capable of catalyzing retro-Michael reactions of β-alkoxy ketones, a direct Michael addition of acetone to a maleimide derivative, 9a and mimics the classic organocatalytic Wieland–Miescher ketone synthesis.9

The recent report by List10 of chiral imidazolidinone-catalyzed intramolecular Michael reactions has prompted us to determine whether antibody 38C2 could catalyze the intramolecular Michael addition of aldehydes and ketones to enones. To our delight, incubation of formyl-enone I (R’ = H, R = H) or methyl ketone-enone (R’ = H, R = CH₃) with antibody 38C2 in phosphate-buffered saline (PBS), pH 7.4, indeed generated the expected Michael product II (Scheme 2).

The substrates for the antibody-catalyzed reactions were synthesized through a Wittig coupling in a biphasic solvent system using methylene chloride and 2 N sodium hydroxide as shown in Scheme 3. The Wittig salt III was dissolved in the aqueous phase and quickly formed the corresponding ylide. The keto-aldehyde (R = CH₃) or the dialdehyde (R = H) were added in methylene chloride. The reaction was mixed for several hours, and after workup the enone product was purified by standard column chromatography techniques. Racemic reference type II products were prepared by incubation of the enones with piperidine in DMF.

Several aldehyde-enones and methyl ketone-enones were suitable substrates for the antibody-binding site and afforded the intramolecular Michael products with excellent diastereoselectivity (Table 1). The cis/trans ratio and the enantioselectivity were determined by standard RP-HPLC and chiral-phase HPLC (AD-RH column), respectively.

Interestingly, the antibody reaction product was, in all examples, predominantly the thermodynamically unfavorable cis-diestereoisomer. In contrast, the asymmetric intramolecular Michael reaction catalyzed by imidazolidinones of formyl-enone 3 afforded almost exclusively the trans-diestereoisomer (S)-proline gave a 2:1 trans/cis ratio with only 15% ee of trans-3a.10 As presented in Table 1, antibody 38C2 also catalyzed the intramolecular Michael addition of methyl ketones 6–9 with very high enantio- and diastereoselectivity. Incubation of MacMillan imidazolidinone with ketone-enone 6 in THF for 3 days did not affford any Michael addition product.

The kcat and Km were determined from Lineweaver–Burk plots using Michaelis–Menten analysis (Table 2). Full graphical data are available in the Supporting Information. While the ketones reacted at a relatively moderate rate, aldehyde 3 yielded the intramolecular Michael product 3a with a kcat of 8.73 min⁻¹ (Table 2). To date, this is the highest measured rate for antibody catalysis of a C–C bond-forming reaction. The antibody-catalyzed reaction was up to 5 orders of magnitude faster than the background reaction in buffer alone. This relatively high ratio will be adequate for preparative-scale antibody reactions to generate enantiomerically pure products.12 In general, the aldehyde-enones were less stable (or more reactive) under these buffer conditions than the methyl ketone-enones, and their reactions had higher kcat values. Ketone-enone 6 exhibited the highest rate enhancement with a kcat/kmoot ratio of 350,000, very high cis/trans product selectivity (90/1), and an excellent enantiomeric excess value of 97%.

Scheme 4 illustrates our proposed mechanism for the antibody catalysis of the intramolecular Michael addition. The ε-amino group of the lysine residue (IV) presumably reacts with the formyl or the methyl ketone to form a nucleophilic enamine (V), that then reacts with the enone to generate the Michael addition product (VI). The imine in VI is hydrolyzed to release a carbonyl moiety (VII) and to regenerate the free ε-amino-lysine residue. The X-ray structure...
of antibody 33F12, a very similar antibody to 38C2, shows a tyrosine residue near the active lysine. In our proposed mechanism, the tyrosine hydroxyl residue acts as a Brønsted acid catalyst that activates the enone toward the Michael addition of the nucleophilic enamine. The antibody binding site tolerates a variety of ketone–aldehyde and aldehyde-enone substrates with different para substituents on the aromatic ring. However, the substrates with the fastest reaction rates among the aldehydes and the ketones were the nonsubstituted enones and aldehyde-enone substrates with different para substituents on the aromatic ring. This phenomenon is not observed in ketones 6a–9a.

Table 2. Kinetic Parameters for Intramolecular Michael Addition Catalyzed by Antibody 38C2

<table>
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<tr>
<th>Substrate</th>
<th>Product</th>
<th>cis/trans</th>
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<td>3a</td>
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</tr>
<tr>
<td>9</td>
<td>9a</td>
<td>90/1</td>
<td>94%</td>
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*Antibody reactions were performed in PBS (50 mM buffer, 100 mM sodium chloride), pH 7.4 with 5% acetonitrile at room temperature.

in our proposed mechanism, of antibody 33F12, a very similar antibody to 38C2, shows a tyrosine residue near the active lysine. In our proposed mechanism, the tyrosine hydroxyl residue acts as a Brønsted acid catalyst that activates the enone toward the Michael addition of the nucleophilic enamine. The antibody binding site tolerates a variety of ketone–aldehyde-enone substrates with different para substituents on the aromatic ring. However, the substrates with the fastest reaction rates among the aldehydes and the ketones were the nonsubstituted enones and aldehyde-enone substrates with different para substituents on the aromatic ring. This phenomenon is not observed in ketones 6a–9a.

Table 2. Kinetic Parameters for Intramolecular Michael Addition Catalyzed by Antibody 38C2

<table>
<thead>
<tr>
<th>Substrate</th>
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<th>$k_{cat}$ (min$^{-1}$)</th>
<th>$k_{cat}/K_m$</th>
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*Antibody reactions were performed in PBS (50 mM buffer, 100 mM sodium chloride), pH 7.4 with 5% acetonitrile at room temperature.

with high ee values and cis/trans ratios. To the best of our knowledge, no other Michael-addition methods offer such selectivity for the thermodynamically unfavored cis-diastereoisomers.

While organocatalytic Michael addition products are known, antibody 38C2 is the only catalyst known to act on both aldehyde and ketone substrates to produce enantiomeric pure cis-cyclopentane products. This study highlights the dynamic interplay between bioorganic chemistry and organocatalysis.

Acknowledgment. Financial support has been provided by Tel-Aviv University and the Israel Science Foundation.

Supporting Information Available: Full experimental details, characterization data of all new compounds and antibody assay conditions, full graphical data used to generate Table 2. This material is available free of charge via the Internet at http://pubs.acs.org.

References


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Supporting Information

Antibody-Catalyzed Asymmetric Intramolecular Michael Addition of Aldehydes and Ketones to Yield the disfavored Cis-Product

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Experimental

\textbf{General methods.} Thin layer chromatography (TLC): silica gel plates Merck 60 F\textsubscript{254}; compounds were visualized by irradiation with UV light and/or by treatment with a solution of 25 g phosphomolybdic acid, 10 g Ce(SO\textsubscript{4})\textsubscript{2}-H\textsubscript{2}O, 60 mL conc. H\textsubscript{2}SO\textsubscript{4} and 940 mL H\textsubscript{2}O followed by heating and/or by staining with a solution of 12 g 2,4-dinitrophenylhydrazine in 60 mL conc. H\textsubscript{2}SO\textsubscript{4}, 80 mL H\textsubscript{2}O and 200 mL 95% EtOH followed by heating. – Flash chromatography (FC): silica gel Merck 60 (particle size 0.040-0.063 mm), eluent given in parentheses. \textsuperscript{1}H NMR spectra were measured using Bruker Avance operated at 200 MHz or 400MHz as mentioned. \textsuperscript{13}C NMR spectra were measured using Bruker Avance operated at 50 MHz or 100MHz as mentioned. The chemical shifts are expressed in \(\delta\) relative to TMS (\(\delta = 0\) ppm) and the coupling constants \(J\) in Hz. The spectra were recorded in CDCl\textsubscript{3} as solvent at room temperature.
unless stated otherwise. All general reagents, including salts and solvents, were purchased from Aldrich (Milwaukee, MN). All reactions were carried out at room temperature unless stated otherwise.

**Abbreviations.** OsO₄ – Osmium tetraoxide, NMO – 4-Methylmorpholine N-Oxide, DCM- Dichloromethane, DMF- Dimethylformamide, EtOAc- Ethyl acetate, Hex- n-Hexanes, PBS- Phosphate buffer saline, THF – Tetrahydrofuran, NaOH – Sodium hydroxide.

8-(4-Methoxy-phenyl)-8-oxo-oct-6-enal (4)

(4’-Metoxyphenacyl)triphenylphosphonium bromide (500 mg, 1.02 mmol, 1.02 eq) was dissolved in a mixture of DCM (15 mL) and NaOH 2N (10 mL). The reaction was monitored by TLC (EtOAc:Hex 1:1) until complete disappearance of the phosphonium salt (0.5-3 hours). After completion, EtOAc was added and the solution was washed with brine. The organic phase was dried over sodium-sulphate, filtered and evaporated to yield the phosphonium ylide in an almost quantitative yield. The phosphonium ylide (410 mg, 1.00 mmol, 1 eq) was dissolved in DCM (3 mL) in a pressure tube, and Hexanedial (171 mg, 1.5 mmol, 1.5 eq) was added. The reaction was heated to 50°C and stirred for 3 hours. The reaction was monitored by TLC (EtOAc:Hex 1:2) for disappearance of the phosphonium salt. After completion, the solvent was evaporated and the crude product was purified by FC (EtOAc:Hex 1:2) to yield enone 4 in 53% yield (133 mg, 0.53 mmol). **¹H NMR** (200MHz,CDCl₃): δ = 9.77 (1H, t, J = 1.6 Hz), 7.94 (2H, d, J = 8.9 Hz), 6.86-6.99 (4H, m), 3.86 (3H, s), 2.48 (2H, dt, J = 7.0, 1.5 Hz), 2.33 (2H, q, J = 7.0 Hz), 1.52-1.78 (4H, m). **¹³C NMR**
(50MHz,CDCl₃): δ = 23.5, 29.6, 34.4, 45.6, 57.4, 115.7, 127.8, 132.6, 132.7, 149.7, 190.9, 204.1. CI-HRMS calcd for C₁₅H₁₈O₃ [MH⁺] m/z 247.1326, found 247.1333.

1-Phenyl-non-2-ene-1,8-dione (6)

Phenacyltriphenylphosphonium bromide (1175 mg, 2.55 mmol, 1.02 eq) was dissolved in a mixture of DCM (15 mL) and NaOH 2N (10 mL). The reaction was monitored by TLC (EtOAc:Hex 1:1) until complete disappearance of the phosphonium salt. After completion, EtOAc was added and the solution was washed with brine. The organic phase was dried over sodium-sulphate, filtered and evaporated to yield the phosphonium ylide in a quantitative yield. The phosphonium ylide (963 mg, 2.53 mmol, 1 eq) was dissolved in DCM (3 mL) in a pressure tube, and 6-Oxo-heptanal (1.5 eq) was added. The reaction was heated to 60°C, stirred for overnight and was monitored by TLC (EtOAc:Hex 1:2) for disappearance of the phosphonium salt. After completion, the solvent was evaporated and the crude product was purified by FC (EtOAc:Hex 1:2) to yield enone 6 in 85% yield (495 mg, 2.15 mmol). ¹H NMR (200MHz,CDCl₃): δ = 7.92 (2H, d, J = 7.9 Hz), 7.41-7.60 (3H, m), 7.04 (1H, dt, J = 15.4, 6.4 Hz), 6.88 (1H, d, J = 15.4 Hz), 2.47 (2H, t, J = 6.8 Hz), 2.33 (2H, q, J = 6.7 Hz), 2.04 (3H, s), 1.51-1.64 (4H, m). ¹³C NMR (50MHz,CDCl₃): δ = 23.2, 27.6, 29.9, 32.5, 43.3, 126.1, 128.4, 132.6, 137.8, 149.1, 190.8, 208.5. CI-HRMS calcd for C₁₅H₁₈O₂ [MH⁺] m/z 231.1377, found 231.1382.

8-Oxo-8-phenyl-oct-6-enal (3)

Aldehyde 3 was prepared in the same manner as 4, starting from phenacyltriphenylphosphonium bromide (1940 mg, 4.20 mmol, 1.01 eq), to yield 469 mg, 2.29 mmol (55%). Known compound (Registry 190522-49-7).
8-(4-Nitro-phenyl)-8-oxo-oct-6-enal (5)

Aldehyde 5 was prepared in the same manner as 4, starting from (4'-nitrophenacyl)triphenylphosphonium bromide (1520 mg, 3.00 mmol, 1.00 eq), to yield 510 mg, 1.95 mmol (65%). $^1$H NMR (400MHz,CDCl$_3$): $\delta$ = 9.79 (1H, t, $J$ = 1.4 Hz), 8.31 (2H, d, $J$ = 8.8 Hz), 8.05 (2H, d, $J$ = 8.8 Hz), 7.10 (1H, dd, $J$ = 15.4, 6.7 Hz), 6.87 (1H, d, $J$ = 15.4 Hz), 2.33-2.54 (4H, m), 1.48-1.66 (4H, m). $^{13}$C NMR (50MHz,CDCl$_3$): $\delta$ = 21.3, 27.4, 32.6, 43.5, 123.7, 125.7, 129.4, 142.6, 148.0, 150.7, 189.1, 201.9. CI-HRMS calcd for C$_{14}$H$_{15}$NO$_4$ [MH$^+$] m/z 262.1071, found 262.1079.

1-(4-Methoxy-phenyl)-non-2-ene-1,8-dione (7)

Ketone 7 was prepared in the same manner as 6, starting from (4'-methoxyphenacyl)triphenylphosphonium bromide (1823 mg, 3.71 mmol, 1.02 eq), to yield 549 mg, 2.11 mmol (58%). $^1$H NMR (200MHz,CDCl$_3$): $\delta$ = 7.95 (2H, d, $J$ = 8.9Hz), 6.85-7.06 (4H, m), 3.87 (3H, s), 2.45 (2H, t, $J$ = 6.8 Hz), 2.32 (2H, q, $J$ = 6.7 Hz), 2.14 (3H, s), 1.47-1.68 (4H, m). $^{13}$C NMR (100MHz,CDCl$_3$): $\delta$ = 25.2, 29.6, 31.8, 34.5, 57.4, 115.7, 127.7, 132.7, 150.0, 165.2, 190.9, 210.6. CI-HRMS calcd for C$_{16}$H$_{20}$O$_3$ [MH$^+$] m/z 261.1482, found 261.1492.

1-p-Tolyl-non-2-ene-1,8-dione (8)

Ketone 8 was prepared in the same manner as 6, starting from (4'-methylphenacyl)triphenylphosphonium bromide (600 mg, 1.26 mmol, 1.00 eq), to yield 200 mg, 0.82 mmol (65%). $^1$H NMR (200MHz,CDCl$_3$): $\delta$ = 7.82 (2H, d, $J$ = 8.2 Hz), 7.24 (2H, d, $J$ = 7.7 Hz), 7.01 (1H, dt, $J$ = 15.4, 6.4 Hz), 6.86 (1H, d, $J$ = 15.4 Hz), 2.44 (2H, t, $J$ = 6.9 Hz), 2.38 (3H, s), 2.30 (2H, q, $J$ = 6.6 Hz), 2.12 (3H, s), 1.49-1.62 (4H, m). $^{13}$C NMR (50MHz,CDCl$_3$): $\delta$ = 21.5, 23.2, 27.6, 29.8, 32.5, 43.3, 126.0,
Ketone 9 was prepared in the same manner as 6, starting from (4’-bromophenacyl)triphenylphosphonium bromide (600 mg, 1.11 mmol, 1.05 eq), to yield 227 mg, 0.73 mmol (69%). $^1$H NMR (400MHz,CDCl$_3$): $\delta$ = 7.79 (2H, d, $J = 8.6$ Hz), 7.60 (2H, d, $J = 8.6$ Hz), 7.05 (1H, dt, $J = 15.4$, 6.6 Hz), 6.83 (1H, dt, $J = 15.4$, 1.1 Hz), 2.46 (2H, t, $J = 6.8$ Hz), 2.33 (2H, q, $J = 6.6$Hz), 2.15 (3H, s), 1.48-1.70 (4H, m). $^{13}$C NMR (50MHz,CDCl$_3$): $\delta$ = 23.9, 29.5, 32.3, 35.1, 45.2, 115.6, 129.6, 131.9, 133.7, 138.5, 151.7, 191.8, 210.4. CI-HRMS calcd for C$_{15}$H$_{17}$O$_2$Br [MH+] $m/z$ 309.0482, found 309.0483.

cis-2-[2-(4-Methoxy-phenyl)-2-oxo-ethyl]-cyclopentanecarbaldehyde (4a)

Aldehyde 4 (165 mg, 0.67 mmol, 1 eq) was dissolved in DMF (1 mL), and piperidine (28 mg, 0.33 mmol, 0.5 eq) was added. The reaction was monitored by TLC (EtOAc:Hex 1:2). After completion (2 hours), DMF was removed under reduced pressure and the crude product was purified by FC (EtOAc:Hex 1:2) to yield the intramolecular Michael addition product 4a in 10% yield (16.5 mg, 0.067 mmol). $^1$H NMR (200MHz,CDCl$_3$): $\delta$ = 9.76 (1H, d, $J = 2.5$ Hz), 7.92 (2H, d, $J = 8.9$ Hz), 6.92 (2H, d, $J = 8.9$ Hz), 3.86 (3H, s), 3.17 (1H, dd, $J = 17.1$, 7.4 Hz), 3.02 (1H, dd, $J = 17.1$, 7.0 Hz), 3.00-3.09 (1H, m), 2.80-2.98 (1H, m), 1.42-1.99 (6H, m). $^{13}$C NMR (100MHz,CDCl$_3$): $\delta$ = 27.2, 33.5, 36.6, 41.1, 55.5, 57.4, 115.6, 131.8, 132.2, 199.7, 206.9. CI-HRMS calcd for C$_{15}$H$_{18}$O$_3$ [MH+] $m/z$ 247.1326, found 247.1334. The anti isomer was yielded as well in 85% yield (140 mg, 0.57 mmol). $^1$H NMR
(200MHz,CDCl3): δ = 9.66 (1H, d, J = 3.2 Hz), 7.92 (2H, d, J = 8.9 Hz), 6.92 (2H, d, J = 8.9 Hz), 3.86 (3H, s), 3.09 (1H, dd, J = 16.7, 7.1 Hz), 2.99 (1H, dd, J = 16.7, 6.7 Hz), 2.71 (1H, sext, J = 7.4 Hz), 2.42 (1H, ddd, J = 16.0, 8.0, 3.3 Hz), 1.25-2.11 (6H, m). $^{13}$C NMR (100MHz,CDCl3): δ = 28.7, 34.9, 38.8, 45.1, 57.4, 59.7, 115.7, 131.8, 132.3, 165.5, 199.6, 205.6. CI-HRMS calcd for C$_{15}$H$_{18}$O$_3$ [MH$^+$$]$ m/z 247.1326, found 247.1334.

cis-2-(2-Acetyl-cyclopentyl)-1-phenyl-ethanone (6a)

Ketone 6 (240 mg, 1.05 mmol, 1 eq) was dissolved in DMF (1mL) and piperidine (44 mg, 0.52 mmol, 0.5 eq) was added. The reaction was heated to 60°C, stirred for overnight and was monitored by TLC (EtOAc:Hex 1:2). After completion, DMF was removed under reduced pressure and the crude product was purified by FC (EtOAc:Hex 1:2) to yield the intramolecular Michael addition product 6a in 40% yield (98 mg, 0.42 mmol). $^1$H NMR (200MHz,CDCl3): δ = 7.94 (2H, d, J = 6.9 Hz), 7.42-7.56 (3H, m), 3.15-3.27 (1H, m), 3.06 (2H, m), 2.65-2.93 (1H, m), 2.15 (3H, s), 1.57-1.93 (6H, m). $^{13}$C NMR (50MHz,CDCl3): δ = 24.6, 28.8, 29.6, 32.5, 38.1, 43.9, 58.2, 128.1, 128.5, 133.0, 136.8, 199.6, 210.8. CI-HRMS calcd for C$_{15}$H$_{18}$O$_2$ [MH$^+$$]$ m/z 231.1377, found 231.1394. The anti isomer was yielded as well in 45% (115 mg, 0.48 mmol). $^1$H NMR (200MHz,CDCl3): δ = 7.97 (2H, d, J = 6.9 Hz), 7.43-7.57 (3H, m), 3.14 (1H, dd, J = 15.8, 6.0 Hz), 2.93 (1H, dd, J = 15.8, 7.3 Hz), 2.58-2.80 (2H, m), 2.20 (3H, s), 1.66-2.07 (6H, m). $^{13}$C NMR (50MHz,CDCl3): δ = 24.8, 25.3, 30.1, 34.1, 40.1, 41.4, 55.8, 129.8, 130.5, 135.0, 139.0, 201.9, 214.1. CI-HRMS calcd for C$_{15}$H$_{18}$O$_2$ [MH$^+$$]$ m/z 231.1377, found 231.1392.
**cis-2-(2-Oxo-2-phenyl-ethyl)-cyclopentanecarbaldehyde (3a)**

Aldehyde 3a was prepared in the same manner as 4a, starting from aldehyde 3 (145 mg, 0.67 mmol, 1 eq), to yield 24 mg, 0.11 mmol (9%) of 3a. 

$^1$H NMR (200MHz,CDCl$_3$): $\delta =$ 9.77 (1H, d, $J =$ 2.5 Hz), 7.93 (2H, d, $J =$ 6.8 Hz), 7.40-1.60 (3H, m), 3.01-3.30 (2H, m), 2.81 (1H, sext, $J =$ 7.1 Hz), 1.39-2.01 (6H, m). 

$^{13}$C NMR (50MHz,CDCl$_3$): $\delta =$ 25.4, 32.1, 38.2, 39.5, 53.4, 127.9, 128.5, 133.2, 136.7, 199.2, 204.9. CI-HRMS calcd for C$_{14}$H$_{16}$O$_2$ [MH$^+$] $m/z$ 217.1220, found 217.1222. The trans isomer was obtained as well in 17% (13 mg, 0.06 mmol) yield. 

$^1$H NMR (200MHz,CDCl$_3$): $\delta =$ 9.68 (1H, d, $J =$ 3.3 Hz), 7.94 (2H, d, $J =$ 6.9 Hz), 7.40-7.60 (3H, m), 3.10 (1H, dd, $J =$ 6.8, 4.0 Hz), 2.73 (1H, sext, $J =$ 7.6 Hz), 2.43 (1H, dd, $J =$ 8.0, 3.2 Hz), 1.97-2.09 (1H, m), 1.83-1.92 (2H, m), 1.65-1.79 (2H, m), 1.37-1.45 (2H, m). 

$^{13}$C NMR (100MHz,CDCl$_3$): $\delta =$ 26.7, 28.7, 34.9, 36.6, 45.5, 59.7, 190.0, 130.6, 135.1, 138.7, 201.0, 205.5. CI-HRMS calcd for C$_{14}$H$_{16}$O$_2$ [MH$^+$] $m/z$ 217.1220, found 217.1223.

**cis-2-[2-(4-nitro-phenyl)-2-oxo-ethyl]-cyclopentanecarbaldehyde (5a)**

Aldehyde 5a was prepared in the same manner as 4a, starting from aldehyde 5 (70 mg, 0.26 mmol, 1 eq), to yield 5 mg, 0.07 mmol (10%) of 5a. 

$^1$H NMR (200MHz,CDCl$_3$): $\delta =$ 9.75 (1H, d, $^3$J=$2.26Hz$), 8.27-8.33 (2H, m), 8.06-8.12 (2H, m), 3.28-3.40 (1H, dd, $J =$ 18.0, 7.5 Hz), 3.04-3.17 (2H, m), 2.72-2.88 (1H, m), 1.4-2.0 (6H, m). 

$^{13}$C NMR (50MHz,CDCl$_3$): $\delta =$ 23.5, 25.7, 32.0, 37.8, 40.2, 53.1, 123.8, 128.9, 141.3, 150.3, 197.8, 204.7. CI-HRMS calcd for C$_{14}$H$_{15}$ONO$_4$ [MH$^+$] $m/z$ 262.1071, found 262.1077. The trans isomer was obtained as well in 43% (30 mg, 0.29 mmol) yield. 

$^1$H NMR (200MHz,CDCl$_3$): $\delta =$ 8.29 (2H, d, $J =$ 7.0 Hz), 8.09 (2H, d, $J =$ 7.0 Hz), 3.13 (2H, t, $J =$ 6.6 Hz), 2.63-2.82 (1H, m), 2.36-2.50 (1H, m), 1.27-
2.12 (6H, m). $^{13}$C NMR (100MHz,CDCl$_3$): $\delta$ = 28.8, 31.6, 32.9, 34.9, 46.0, 59.6, 125.7, 125.8, 131.0, 143.0, 152.3, 199.5, 208.9. CI-HRMS calcd for C$_{14}$H$_{15}$ONO$_4$ [MH$^-$] m/z 262.1071, found 262.1078.

cis-2-(2-Acetyl-cyclopentyl)-1-(4-methoxy-phenyl)-ethanone (7a)

Ketone 7a was prepared in the same manner as 6a, starting from ketone 7 (265 mg, 1.02 mmol, 1 eq), to yield 46 mg, 0.17 mmol (17%) of 7a. $^1$H NMR (400MHz,CDCl$_3$): $\delta$ = 7.91 (2H,d, $J$ = 8.9 Hz), 6.91 (2H, d, $J$ = 8.9 Hz), 3.86 (3H, s), 3.22 (1H, d, $J$ = 7.4 Hz), 3.01 (1H, dd, $J$ = 17.2, 7.2 Hz), 2.93 (1H, dd, $J$ = 17.2, 7.0 Hz), 2.75-2.83 (1H, m), 2.13 (3H, s), 1.54-1.63 (6H, m). $^{13}$C NMR (50MHz,CDCl$_3$): $\delta$ = 23.3, 28.0, 31.3, 32.1, 38.4, 39.0, 53.9, 55.4, 113.6, 130.2, 130.3, 163.4, 198.5, 212.2. CI-HRMS calcd for C$_{16}$H$_{20}$O$_3$ [MH$^-$] m/z 261.1482, found 261.1495. The trans isomer was obtained as well in 22% (59 mg, 0.23 mmol) yield. $^1$H NMR (400MHz,CDCl$_3$): $\delta$ = 7.94 (2H, d, $J$ = 8.9 Hz), 6.93 (2H, d, $J$ = 8.9 Hz), 3.86 (3H, s), 3.06 (1H, dd, $J$ = 15.5, 6.1 Hz), 2.85 (1H, dd, $J$ = 15.5, 7.6 Hz), 2.66-2.75 (1H, m), 2.59-2.65 (1H, m), 2.19 (3H, s), 1.97-2.00 (2H, m), 1.67-1.71 (4H, m). $^{13}$C NMR (50MHz,CDCl$_3$): $\delta$ = 26.5, 30.8, 31.6, 34.5, 40.4, 45.6, 57.4, 60.3, 115.6, 131.9, 132.4, 165.4, 200.1, 212.9. CI-HRMS calcd for C$_{16}$H$_{20}$O$_3$ [MH$^-$] m/z 261.1482, found 261.1495.

cis-2-(2-Acetyl-cyclopentyl)-1-p-tolyl-ethanone (8a)

Ketone 8a was prepared in the same manner as 6a, starting from ketone 8 (167 mg, 0.68 mmol, 1.00eq), to yield 41 mg, 0.17 mmol (25%) of 8a. $^1$H NMR (400MHz,CDCl$_3$): $\delta$ = 7.82 (2H, d, $J$ = 8.2 Hz), 7.23 (2H, d, $J$ = 8.0), 3.22 (1H, q, $J$ = 7.4 Hz), 3.04 (1H, dd, $J$ = 17.4, 7.2 Hz), 2.95 (1H, dd, $J$ = 17.4, 7.0 Hz), 2.80 (1H,
sept, \( J = 7.1 \) Hz), 2.39 (3H, s), 2.13 (3H, s), 1.57-1.89 (6H, m). \(^{13}\)C NMR (100MHz,CDCl\(_3\)): \( \delta = 21.5, 23.3, 29.6, 31.2, 32.1, 38.2, 39.2, 53.8, 128.2, 129.1, 134.6, 143.7, 199.5, 212.1 \). CI-HRMS calcd for C\(_{16}H_{20}O_2\) [MH\(^+\)] \( m/z \) 245.1533, found 245.1550. The trans isomer was obtained as well in 26% (44 mg, 0.18 mmol). \(^1\)H NMR (400MHz,CDCl\(_3\)): \( \delta = 7.86 (2H, d, J = 6.6 \) Hz), 7.25 (2H, d, \( J = 6.6 \) Hz), 3.08 (1H, dd, \( J = 15.6, 6.0 \) Hz), 2.88 (1H, dd, \( J = 15.6, 7.3 \) Hz), 2.56-2.58 (2H, m), 2.40 (3H, s), 2.18 (3H, s), 1.90- 2.01 (2H, m), 1.64-1.72 (4H, m). \(^{13}\)C NMR (50MHz,CDCl\(_3\)): \( \delta = 21.5, 24.5, 28.8, 29.6, 32.5, 38.2, 43.8, 58.2, 128.2, 129.2, 134.3, 143.7, 199.2, 204.5 \). CI-HRMS calcd for C\(_{16}H_{20}O_2\) [MH\(^+\)] \( m/z \) 245.1533, found 245.1537.

**cis-2-(2-Acetyl-cyclopentyl)-1-(4-bromo-phenyl)-ethanone (9a)**

Ketone 9a was prepared in the same manner as 6a, starting from ketone 9 (320 mg, 1.03 mmol, 1 eq), to yield 126 mg, 0.41 mmol (39%) of 9a. \(^1\)H NMR (200MHz,CDCl\(_3\)): \( \delta = 7.79 (2H, d, J = 8.6 \) Hz), 7.58 (2H, d, \( J = 8.6 \) Hz), 3.11 (1H, dd, \( J = 17.4, 7.3 \) Hz), 2.92 (1H, dd, \( J = 17.4, 6.7 \) Hz), 2.70-2.85 (1H, m), 2.57-2.66 (1H, m), 2.13 (3H, s), 1.40-2.17 (6H, m). \(^{13}\)C NMR (100MHz,CDCl\(_3\)): \( \delta = 25.3, 30.2, 31.6, 34.1, 39.9, 41.4, 55.7, 129.6, 131.5, 133.8, 137.7, 200.9, 214.1 \). CI-HRMS calcd for C\(_{15}H_{17}O_2\)Br [MH\(^+\)] \( m/z \) 309.0482, found 309.0493.

The trans isomer was obtained as well in 56% (174 mg, 0.58 mmol) yield. \(^1\)H NMR (400MHz,CDCl\(_3\)): \( \delta = 7.82 (2H, d, J = 6.82 \) Hz), 7.60 (2H, d, \( J = 8.5 \) Hz), 3.07 (1H, dd, \( J = 15.8, 6.0 \) Hz), 2.85 (1H, dd, \( J = 15.8, 7.7 \) Hz), 2.69 (1H, sext, \( J = 7.9 \) Hz), 2.60 (1H, q, \( J = 7.9 \) Hz), 2.19 (3H, s), 1.97-2.03 (3H, m) 1.68-1.71 (3H, m). \(^{13}\)C NMR (100MHz,CDCl\(_3\)): \( \delta = 26.4, 30.8, 31.6, 34.4, 39.9, 45.9, 60.2, 130.1, 131.6, 133.8, 143.7, 199.5, 212.1 \).
137.4, 200.6, 212.7. CI-HRMS calcd for C\textsubscript{15}H\textsubscript{17}O\textsubscript{2}Br [MH+] m/z 309.0482, found 309.0483.

**Enantiomers separation**

All the *cis-* and *trans-* Michael addition products enantiomers, were separated by RP-HPLC (Hitachi LaChromeELITE equipped with an L-2000 series organizer box, L-2300 column-oven, L-2450 diode array detector, L-2200 autosampler and L-2130 pump) at various proportions of Acetonitrile : Water at 0.5 ml/min flow-rate.

**Table 1. Conditions for separation of intra molecular Michael products 3a-9a enantiomers.**

<table>
<thead>
<tr>
<th>Enone</th>
<th>Solvents ratio Acetonitrile : Water</th>
<th>Retention time <em>cis</em> enantiomers (min)</th>
<th>Retention time <em>trans</em> enantiomers (min)</th>
<th>[nm] λ Wavelength for monitoring enantiomeric separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>55% : 45%</td>
<td>16.57, 28.79</td>
<td>17.06, 17.66</td>
<td>244</td>
</tr>
<tr>
<td>4a</td>
<td>55% : 45%</td>
<td>20.76, 32.71</td>
<td>21.35, 24.75</td>
<td>274</td>
</tr>
<tr>
<td>5a</td>
<td>70% : 30%</td>
<td>15.14, 22.62</td>
<td>16.12, 17.14</td>
<td>266</td>
</tr>
<tr>
<td>6a</td>
<td>60% : 40%</td>
<td>9.36, 10.90</td>
<td>No separation. One pick 9.55</td>
<td>243</td>
</tr>
<tr>
<td>7a</td>
<td>55% : 45%</td>
<td>13.68, 15.56</td>
<td>13.06, 14.80</td>
<td>274</td>
</tr>
<tr>
<td>8a</td>
<td>55% : 45%</td>
<td>15.26, 16.41</td>
<td>15.62, 17.34</td>
<td>254</td>
</tr>
<tr>
<td>9a</td>
<td>60% : 40%</td>
<td>21.25, 24.15</td>
<td>19.66, 24.48</td>
<td>256</td>
</tr>
</tbody>
</table>

The enantiomeric excess of all *cis-* products of the reaction of the corresponding ketone or aldehyde with Ab38C2, was determined under the same conditions as mentioned in the appropriate entry in table 1. One example, the *cis-* product of ketone 7 with Ab38C2 (similar to 7a) was further purified under the same conditions, Acetonitrile 5µm (LiChroCART 250-4 Purospher® RP-18e column 250mm x 4mm, : Water 55% : 45%) used to separate the *cis-/trans-* stereoisomers. The enantiomeric excess of the purified *cis-* product was then determined.
Figure 1. Separation of racemic 7a enantiomers.

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Area</th>
<th>Area Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.687</td>
<td>21366960</td>
<td>49.8</td>
</tr>
<tr>
<td>15.540</td>
<td>21511123</td>
<td>50.2</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>42878083</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

In all other cis-enone products, a sample of the crude reaction solution was examined without further purification.

Figure 2. Separation of Ab38C2 reaction product 7a enantiomers.

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Area</th>
<th>Area Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.767</td>
<td>920136</td>
<td>2.3</td>
</tr>
<tr>
<td>15.687</td>
<td>38997299</td>
<td>97.7</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>39917435</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
Michaelis-Menten kinetic measurements

Lineweaver-Burk plots of Ab38C2-catalyzed intramolecular Michael addition of Aldehydes and Ketones

All reactions were carried out in phosphate buffered saline (PBS), pH 7.4 at 25°C. Reactions were typically carried out in concentrations ranging between 20-1250 μM. Antibody 38C2 was typically used in concentrations ranging between 0.05-1mg/ml. Antibody catalyzed reactions were monitored by RP-HPLC (Hitachi LaChromeELITE equipped with an L-2000 series organizer box, L-2300 column-oven, L-2450 diode array detector, L-2200 autosampler and L-2130 pump) using LiChroCART 250-4 Purospher® RP-18e column (250mm x 4mm, 5μm) at various proportions of Acetonitrile : Water (0.1% trifluoroacetic acid) at 1 ml/min flow-rate. Conditions for monitoring the reaction of formyl-enone and methylketone-enone substrates with Ab38C2 were: Acetonitrile : Water (55% : 45%), except for ketone 9: Acetonitrile : Water (60% : 40%).
Graph 1. Lineweaver-Burk plot of Ab38C2 catalysis for the reaction $3a \rightarrow 3a$. 

\[
y = 15.985x + 0.3433 \\
R^2 = 0.9931
\]

Graph 2. Lineweaver-Burk plot of Ab38C2 catalysis for the reaction $4a \rightarrow 4a$. 

\[
y = 216.82x + 0.9216 \\
R^2 = 0.9913
\]
.5 → 5a **Graph 3.** Lineweaver-Burk plot of Ab38C2 catalysis for the reaction

\[ y = 40.906x + 1.1276 \]

\[ R^2 = 0.9988 \]

\(1/\mu M\)

.6 → 6a **Graph 4.** Lineweaver-Burk plot of Ab38C2 catalysis for the reaction

\[ y = 392.72x + 1.1667 \]

\[ R^2 = 0.9917 \]

\(1/\mu M\)
Graph 5. Lineweaver-Burk plot of Ab38C2 catalysis for the reaction $7 \rightarrow 7a$

\[ y = 1292.3x + 1.8218 \]
\[ R^2 = 0.9963 \]

Graph 6. Lineweaver-Burk plot of Ab38C2 catalysis for the reaction $8 \rightarrow 8a$
Graph 7. Lineweaver-Burk plot of Ab38C2 catalysis for the reaction

\[ y = 323.44x + 7.2709 \]

\[ R^2 = 0.9838 \]

Table 2. \( K_{\text{uncat}} \) measurements for 3-9 in PBS:

<table>
<thead>
<tr>
<th>Reaction</th>
<th>( K_{\text{uncat}} ) [\text{min}^{-1}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ( \rightarrow ) 3a</td>
<td>( 3.5 \cdot 10^{-5} )</td>
</tr>
<tr>
<td>4 ( \rightarrow ) 4a</td>
<td>( 5.4 \cdot 10^{-5} )</td>
</tr>
<tr>
<td>5 ( \rightarrow ) 5a</td>
<td>( 5.7 \cdot 10^{-5} )</td>
</tr>
<tr>
<td>6 ( \rightarrow ) 6a</td>
<td>( 4.0 \cdot 10^{-7} )</td>
</tr>
<tr>
<td>7 ( \rightarrow ) 7a</td>
<td>( 3.0 \cdot 10^{-6} )</td>
</tr>
<tr>
<td>8 ( \rightarrow ) 8a</td>
<td>( 7.0 \cdot 10^{-7} )</td>
</tr>
<tr>
<td>9 ( \rightarrow ) 9a</td>
<td>( 1.0 \cdot 10^{-6} )</td>
</tr>
</tbody>
</table>

\( K_{\text{uncat}} \) measurements were made with the appropriate aldehyde or ketone at concentration in 500\( \mu \text{M} \) PBS solution at pH 7.4.