Synthesis and inhibitory activities at mGluRs of 3-alkylated and N-alkylated cyclopentyl-glutamate analogues

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Abstract
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Keywords
inhibitory, 3, alkylated, n, cyclopentyl, glutamate, analogues, activities, synthesis, mglurs, CMMB

Disciplines
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Synthesis and Inhibitory Activities at mGluRs of 3-Alkylated and N-Alkylated Cyclopentyl-Glutamate Analogues

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Keywords: Cyclopentyl-glutamate analogues; mGluR2; agonists; antagonists; crystal structures.

ABSTRACT

The conformationally restricted glutamate analogues, 3-alkyl-1-amino-2-cyclopentene-1,3-dicarboxylates and N-alkylated analogues have been prepared in a regioselective and diastereoselective manner. From the biological studies of the 3-alkylated analogues, compound 13b was found to be the most potent antagonist (IC₅₀ 7.7 μM) at mGluR2. Amongst the N-alkylated analogues, compound 20 was found to be a partial agonist (EC₅₀ 9.5 μM) and as well as an antagonist (IC₅₀ 47 μM) at mGluR2.
1. Introduction

L-Glutamate (Glu) is the principal excitatory amino acid (EAA) neurotransmitter in the mammalian central nervous system (CNS) and operates through two main types of glutamate receptors.\(^1\) Those which form ligand-gated cation channels (ionotropic glutamate receptors, iGluRs) and those which are coupled \textit{via} G-protein to intercellular enzyme systems which influence the production of second messengers (metabotropic glutamate receptors, mGluRs).\(^2\) Both iGluRs and mGluRs play crucial roles in the healthy, as well as the diseased CNS and these receptors are therefore potential targets for therapeutic intervention.\(^3\) Considerable research efforts have been focused upon the development of selective agonists and antagonists for iGluRs and mGluRs.\(^4\)

A variety of cyclic, conformationally restricted glutamate analogues have been prepared in laboratories around the world but only a few are highly potent and sub-type selective. One such compound is the conformationally restricted glutamate analogue, \((1S,3R)-1\text{-aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD] 1},\) which selectively activates metabotropic glutamate receptors (mGluRs) over the ionotropic type. \((1S,3R)\)-ACPD 1 however is not selective for the individual eight mGluR subtypes.\(^5\) While its diastereomer \((1S,3S)\)-ACPD 2 is more potent and selective at mGluR2 (EC\(_{50}\) 1.2 \(\mu\text{M}\)) than at other subtypes.\(^6\)

\begin{center}
\begin{tikzpicture}

\node [draw, rounded corners] at (0,0) {\includegraphics[width=0.2\textwidth]{figure1a.png}};
\node [draw, rounded corners] at (0.5,0) {\includegraphics[width=0.2\textwidth]{figure1b.png}};
\node [draw, rounded corners] at (0,0.5) {\includegraphics[width=0.2\textwidth]{figure1c.png}};
\node [draw, rounded corners] at (0.5,0.5) {\includegraphics[width=0.2\textwidth]{figure1d.png}};

\node at (4,0) {\text{Figure 1. Conformationally restricted glutamate analogues.}};
\end{tikzpicture}
\end{center}
We previously reported the synthesis and biological activities of the conformational restricted cyclopentenyl-glutamate compound (S)-3, the dehydro-analogue of APCD. It was found to be an agonist at mGluR5 (EC\textsubscript{50} 18 \mu M) and mGluR2 (EC\textsubscript{50} 45 \mu M) receptors.\textsuperscript{7} We also reported the synthesis of the 4-phenyl substituted derivative, \textit{rac}-4, and its aryl substituted derivatives. Compound \textit{rac}-4 is a selective antagonist at mGluR2 with modest activity with an IC\textsubscript{50} value of 32 \mu M.\textsuperscript{8}

In order to further understand the SAR of the derivatives of \textit{rac}-3, we aimed to synthesise its 3-alkylated and \textit{N}-alkylated derivatives and investigate their biological activities.

The synthesis of the 3-alkylated compounds can be achieved by regioselective deprotonation at the\gamma-position of the\alpha,\beta–unsaturated ester moiety of protected derivatives of \textit{rac}-3 by a strong base to generate the corresponding extended enolate which undergoes regioselective alkylation at the\alpha-position to give 3-alkylated products 5 (Scheme 1).\textsuperscript{9}

![Scheme 1. Alkylation of\alpha,\beta–unsaturated ester.](image)

We report here the regioselective and diastereoselective synthesis of 3-alkylated and \textit{N}-alkylated derivatives of (S)-3. The biological activities of these compounds as agonists and antagonists at mGluR2 will also be discussed.

2. Results and discussion
2.1 Synthesis of compounds
2.1.1 Cyclopentenyl derivatives

Racemates 9a-d and their diastereoisomers 10a-d were obtained, according to Scheme 2. Treating the known racemate 6\textsuperscript{7} with two equivalent of KN(TMS)\textsubscript{2} at -78 °C,
followed by the addition of alkylating agents gave exclusively the $\alpha$–alkylated products 7 and 8 as the major and minor products, respectively. These outcomes concurred with the works reported by Katzenellenbogen et al., on the $\alpha$–alkylation of lithium dienolates derived from carbonyl esters. The ratio of the isolated diastereoisomers 7 and 8 was determined by HPLC analysis, to be within the range of 8:2.

Scheme 2. Synthesis of racemic 9 and 10. Reagents and conditions (a) KN(TMS)$_2$, THF, -78 °C; RI or RBr; (b) 10% aq. HCl, reflux.

Table 1 Isolated yields (%) of compounds 7 and 8.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>7 (%)</th>
<th>8 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (R = Me)</td>
<td>48</td>
<td>6</td>
</tr>
<tr>
<td>b (R = CH$_2$CHCH$_2$)</td>
<td>45</td>
<td>6</td>
</tr>
<tr>
<td>c (R = Bn)</td>
<td>77</td>
<td>11</td>
</tr>
<tr>
<td>d [R = (CH$_2$)$_3$Ph]</td>
<td>36</td>
<td>1.6</td>
</tr>
</tbody>
</table>
$^1$H NMR spectroscopic analysis of the two diastereomers, such as 7a and 8a indicated that the major diastereomer 7a exhibited a H-4 resonance ($\delta$ 5.89, d, $J = 4.2$ Hz) and H-5 resonance ($\delta$ 5.71, d, $J = 4.2$ Hz). The CH$_3$-3 resonance at $\delta$ 1.49 of the major isomer 7a appeared more downfield than the corresponding resonance ($\delta$ 1.32) of the minor isomer 8a. The relative stereochemistry of 7a was determined from NOESY experiments that showed significant cross-peaks between the signal for H-(Ar) and H-2$\alpha$, H-2$\alpha$ and H-(CH$_3$-3) and that for H-(Ar) and H-(CH$_3$-3). These cross-peaks are consistent with the cis orientation of the two carboxyl groups (Fig. 2). The relative stereochemistries of the compounds, 8a, 8b and 7c were further unequivocally determined by X-ray structure analysis (Figs. 3-5). Suitable crystals for X-ray analysis of compounds 8a, 8b and 7d were grown from a mixture of ethyl acetate and hexane.

Figure 2. NOESY correlations for compound 7a.
**Figure 3.** ORTEP diagram of the structure of compound 8a. Ellipsoids have been drawn at the 20% probability level.

**Figure 4.** ORTEP diagram of the structure of compound 8b. Ellipsoids have been drawn at the 30% probability level. Minor components of disordered atoms were omitted for clarity.
The stereoselectivity of compound 7 over 8 can be explained, *via* the complexation of the carboxyl groups to the potassium ion. As shown in intermediate A, addition of the alkyl group from the upper face to intermediate A, would then give compound 7 as the major product as shown in Figure 6.

Acid hydrolysis of 7a-d and 8a-d under similar conditions gave the hydrochloride salts of 9a-d and 10a-d, respectively in acceptable yields (Table 2).
Table 2 Isolated yields (%) of compounds 9 and 10.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (R = Me)</td>
<td>86</td>
<td>80</td>
</tr>
<tr>
<td>b (R = CH₂CHCH₂)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>c (R = Bn)</td>
<td>94</td>
<td>98</td>
</tr>
<tr>
<td>d [R = (CH₂)₃Ph]</td>
<td>63</td>
<td>76</td>
</tr>
</tbody>
</table>

2.1.2 Cyclopentyl derivatives

The racemic cyclopentyl derivatives, 13 and 14, were synthesised, according to Scheme 3. Removal of diphenyl methylene group from 7 and 8 by a mild acid hydrolysis followed by hydrogenation proceeded smoothly to give 11 and 12, respectively, in good yields.

Acid hydrolysis of 11a-c and 12c gave the hydrochloride salts of 13a-c and 14c, respectively (Table 3).

Scheme 3. Synthesis of racemic 13 and 14. Reagents and conditions (a) (i) 1 M HCl, ether, RT, 16 h, (ii) Pd/C, H₂, MeOH, RT, (b) 10% aq. HCl, reflux.
Table 3 Isolated yields (%) of compounds 11, 12, 13 and 14.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (R = Me)</td>
<td>83</td>
<td>na$^a$</td>
<td>85</td>
<td>na$^a$</td>
</tr>
<tr>
<td>b (R = Pr)</td>
<td>94</td>
<td>93</td>
<td>99</td>
<td>na$^a$</td>
</tr>
<tr>
<td>c (R = Bn)</td>
<td>66</td>
<td>70</td>
<td>86</td>
<td>85</td>
</tr>
</tbody>
</table>

$^a$na : not attempted

2.1.2 N, N-Dimethylated analogues

The synthesis of N-alkylated compound 16 is shown in Scheme 4. Treatment of racemic 15 with formaldehyde, NaBH$_3$CN, acetic acid in dry methanol afforded 16 in 70% yield which was hydrolysed in aqueous 10% HCl to give the hydrochloride salt of 17 in 98% yield.

With compound 16 in hand, it was further reduced using Mg turnings in dry methanol to give a mixture of diastereomers, cis 18 and the trans 19, as the major and minor diastereoisomers respectively, in the ratio of 4:1, respectively. The major diastereoisomer 18 was hydrolysed to afford 20 in a quantitative yield.

Scheme 4. Synthesis of racemic 17 and 20. Reagents and conditions (a) HCHO, NaBH$_3$CN, acetic acid, CH$_3$CN (b) 10% aq. HCl, reflux, (c) Mg turnings, dry CH$_3$OH, RT.
$^1$H NMR spectroscopic analysis of the two diastereomers 18 and 19 indicated that the major diastereomer 18 exhibited a H-3 resonance at $\delta$ 2.92 (dq) more downfield than the corresponding resonance ($\delta$ 2.84, dq) of the minor isomer 19.

The relative stereochemistry of compound 18 was determined by NOESY experiments that showed significant cross peaks between the signal for H-3$\alpha$ and H-2$\alpha$ and that for H-2$\alpha$ and H-(CH$_3$). These confirmed the cis orientation of the two carboxyl groups (Fig. 7).

![NOESY cross-peaks [Spartan Pro. generated structure (AM1)]](image)

**Figure 7.** NOESY correlations for compound 18.

Reduction of $\alpha,\beta$–unsaturated esters and other electron deficient alkenes using magnesium metal in dry methanol has been described by us and others to be a selective and efficient method to provide the desired cis 18 as the major product. The cis orientation of the two carboxylic groups in 18 is considered to be significant structural features for activities. The selectivity observed in the magnesium-methanol reduction of compound 16 might be due to the formation of the Mg(II) complex B, as shown in Figure 8, which upon stereoselective protonation would give compound 18 as the major product.
Figure 8. Suggested mechanism leading to the stereochemistry of compound 18.

2.1.3 N-alkylated analogues

The racemic N-alkylated compounds 22a-e were obtained according to Scheme 5, the imine intermediates 21a-e were obtained by treating the known 15 with various aldehydes in dry THF in the presence of powdered molecular sieves, then followed by the reduction in the presence of NaBH₃CN and acetic acid, in methanol to afford 21a-e in acceptable yields. Acid hydrolysis of 21a-e provided the hydrochloride salts of compounds 22a-e in high yields (Table 4).

Scheme 5. Synthesis of racemic 22. Reagents and conditions (a) (i) RCHO, THF, powdered molecular sieves (5 Å), RT, (ii) NaBH₃CN, acetic acid, MeOH; (b) 10% aq. HCl, reflux.

Table 4 Isolated yields (%) of compounds 21 and 22.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>21</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (R = Ph)</td>
<td>80</td>
<td>98</td>
</tr>
<tr>
<td>b (R = 2-pyridinyl)</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>c (R = 3-pyridinyl)</td>
<td>58</td>
<td>98</td>
</tr>
<tr>
<td>d [R = 3-(OH)C₆H₄]</td>
<td>81</td>
<td>92</td>
</tr>
<tr>
<td>e [R = 4-(OH)C₆H₄]</td>
<td>69</td>
<td>95</td>
</tr>
</tbody>
</table>
2.2 Biological studies: Signal transduction at cloned metabotropic glutamate receptors

Signal transduction experiments were performed with CHO cells heterologously expressing human mGlu2 receptors. Signalling at the mGlu2 receptor was measured by mean of a $[^{35}\text{S}]\text{GTP} \gamma \text{S}$ binding assay on membranes from these cells. The results for the agonist and antagonist activities are summarised in Table 5.

Table 5 Agonist and antagonist activities of synthesised compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>mGlu2-ago-AC, EC$<em>{50}$ ± SD in μM (E$</em>{\text{max}}$%)</th>
<th>mGlu2-ago-GTP, EC$<em>{50}$ ± SD in μM (E$</em>{\text{max}}$%)</th>
<th>mGlu2-antag-GTP, IC$<em>{50}$ ± SD in μM (E$</em>{\text{max}}$%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rac-3</td>
<td>73 ± 5 (73%)</td>
<td>55 ± 14 (71%)</td>
<td>&gt;300</td>
</tr>
<tr>
<td>(S)-3</td>
<td>nd$^b$</td>
<td>45 ± 10 (38%)</td>
<td>60 (39%)</td>
</tr>
<tr>
<td>rac-9a</td>
<td>387 (53%)</td>
<td>partial ago$^c$</td>
<td>partial anta$^c$</td>
</tr>
<tr>
<td>rac-13b</td>
<td>nd$^b$</td>
<td>&gt;1000</td>
<td>7.7 ± 2.1 (71%)</td>
</tr>
<tr>
<td>rac-14c</td>
<td>nd$^b$</td>
<td>nd$^b$</td>
<td>200 (67%)</td>
</tr>
<tr>
<td>rac-17</td>
<td>3 (51%)</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>rac-20</td>
<td>nd$^b$</td>
<td>9.7 ± 0.5 (34%)</td>
<td>47 (66%)</td>
</tr>
<tr>
<td>rac-22b</td>
<td>nd$^b$</td>
<td>&gt;100</td>
<td>75 ± 11 (100%)</td>
</tr>
<tr>
<td>rac-22c</td>
<td>nd$^b$</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

$^a$Values from reference 7. $^b$nd: not determined. $^c$No full dose response.

Three compounds, namely 9a, 17 and 20 were found to be mGlu2 agonists. Compound 17 was shown to exhibit mGluR2 agonism in the adenyl cyclase assay, with the potency of 3 μM with an E$_{\text{max}}$ of 51% of the maximal glutamate response. However, the agonist activity of 17 was not confirmed in the $[^{35}\text{S}]\text{GTP} \gamma \text{S}$ binding experiment, where this compound was expected to activate radioligand binding. Compound 17 was also shown to have inhibitory activity at the AMPA receptor in a glycine binding assay with an IC$_{50}$ value of 6.8 μM (data is not shown).
The most active agonist in this series was 20 with a potency of EC\textsubscript{50} 9.5 µM and an E\textsubscript{max} of 34% of the maximal glutamate response. This compound also had inherent partial antagonist activity with an IC\textsubscript{50} of 47 µM. The concentration-response curves illustrated that compound 20 is a partial agonist and as well as antagonist at mGluR2 receptors (Fig. 9).

**Figure 9.** Effect of 20 on human mGluR2: partial agonist and antagonist activity in $[^{35}\text{S}]$GTP\textsubscript{γ}S binding.

Full antagonistic activities were found amongst the saturated (13b and 20), as well as unsaturated (22b) ring structures. The most potent antagonist was 13b with an IC\textsubscript{50} of 7.7 µM. The concentration-response curve revealed that 13b was an antagonist with full inhibition of glutamate signalling in the $[^{35}\text{S}]$GTP\textsubscript{γ}S binding assay, obtained from two independent experiments (Fig. 10).
Figure 10. Antagonist activity of 13b and antagonist activity of 22b at mGluR2.

Only one compound in this series, namely 22c, was found to be an inhibitor at the glycine site of the NMDA receptor with an IC\(_{50}\) of 1.9 \(\mu\)M (data is not shown), as measured in rat cortical membranes by radio-ligand binding.

The other synthesised compounds in this series were found to be inactive on the tested receptors.

By comparing the activities of the saturated and unsaturated compound pairs, such as 9b and 13b, it would appear that reduction of the double bond results in an improvement in the antagonist potency. Compound 9b is not active in tested mGluR receptors, while 13b is a selective antagonist at mGluR2. Within the structures of the saturated active analogues, they were found to have the carboxylic groups in the \textit{cis} configuration. This was observed in the compound pair 17 and 20. The unsaturated analogue 17 showed no activity in the mGluR2 [\(^{35}\text{S}\)]GTP\(_\gamma\)S binding assay. Compound 20 was the most active agonist as well as a partial antagonist. This structural feature was also found important in \textit{rac}-4 and its active analogues and the well-known \((\text{\textit{I}S,\textit{3}S})\)-ACPA\(^6\). In summary, our results reveal that the ring flexibility and the \textit{cis} orientation of the two carboxylic groups appear to be important structural features for activities at the mGluR2 receptor.
3. Conclusions

In conclusion, through this undertaking we have successfully prepared 3-alkylated and N-alkylated derivatives of rac-3 in a regioselective and diastereoselective manner. In this series of compounds, we have discovered a number of novel mGluR2 agonists and antagonists with potency in the low μM range. Although, the conclusive structural activity relationship remains complex, we have however gained a clearer understanding of some of the key structure features required for a selective antagonist at mGluR2.

4. Experimental

4.1 Chemistry
Solvents and reagents were purchased from commercial sources and used without further purification unless otherwise stated. Unless specified, all NMR spectra were recorded at 300 MHz (1H NMR) or 75 MHz (13C NMR) in CDCl3 solution. 13C NMR assignments were based on DEPT experiments. Preparative HPLC was performed using a Waters Delta prep 4000 HPLC, on a normal phase Pre Nova-Pak® HR Silica 6 μm 60 (25 × 10 mm) column. All separations were carried out by isocratic elution, using eluents A and B (A, in petroleum spirit; B, ethyl acetate) in the ratio 98:2. The flow rate was at 20 mL/min. Compounds were detected using a Waters 486 tunable UV absorbance detector.

4.2 Biological Testing

[^35S]GTPγS (specific activity 37 MBq/ml) was obtained from Amersham (Little Chalfont, UK). Dulbecco’s modified Eagle medium (DMEM) and dialyzed fetal calf serum were from Life technologies (Gaithersburg, MD). Scintillation fluid Ultimaflo AF as well as the Unifilter-96 GF/B plates were from Packard (Meriden, CT). Guanosine-5’-diphosphate dilithium salt (GDP) was from Boehringer Manheim (Basel, Switzerland), Fluo 3-AM was from Molecular Probes (Leiden, The Netherlands). Probenecid was from Sigma (St. Louis, MO). Black 96-well plates were from Costar (Merck, Overijse Belgium).

4.3.1 (1R*,3R*)-3-Ethyl 1-methyl 1-(diphenylmethyleneamino)-3-methylcyclopent-4-ene-1,3-dicarboxylate (7a)
To a solution of compound 6 (0.552 g, 1.46 mmol) in dry THF (12 mL) at -78 °C was added a solution of KN(TMS)$_2$ (2.5 equiv., 7.3 mL, 3.66 mmol, 0.5 M in THF). After stirring for 1 h, a solution of methyl iodide (10 equiv., 0.9 mL, 14.6 mmol) in dry THF (2 mL) was added drop wise to the mixture and the stirring continued for another 1 h. The reaction mixture was allowed to warm to RT over 1 h then was diluted with a saturated aqueous solution of NaCl, and then extracted with ether. The combined organic extracts were dried over MgSO$_4$ and the solvent was removed to give a dark yellow residue which was purified by column chromatography (ethyl acetate/petroleum spirit, 1:9) to give a mixture of diastereomers (54%) in the ratio of 8:1 as determined by $^1$H NMR spectroscopic analysis. The two diastereomers were separated by HPLC (ethyl acetate/petroleum spirit, 5:95).

Major isomer 7a, a yellow solid (0.274 g, 48%). R$_f$ (5% ethyl acetate/ petroleum spirit) 0.28. $^1$H NMR $\delta$ 7.60-7.12 (m, 10H), 5.89 (d, $J = 4.2$ Hz, 1H), 5.71 (d, $J = 4.2$ Hz, 1H), 4.13-4.07 (m, 2H), 3.38 (s, 3H), 3.03 (d, $J = 9.9$ Hz, 1H), 2.31 (d, $J = 9.9$ Hz, 1H), 1.49 (s, 3H), 1.22 (t, $J = 7.2$ Hz, 3H). $^{13}$C NMR $\delta$ 175.8 (C), 173.2 (C), 168.3 (C), 140.3 (C), 137.7 (C), 133.5 (CH), 130.2 (CH), 128.7 (2CH), 128.6 (2CH), 128.4 (4CH), 127.9 (2CH), 78.3 (C), 60.7 (CH$_3$), 55.2 (C), 51.7 (CH$_2$), 49.3 (CH$_2$), 26.1 (CH$_3$), 14.1 (CH$_3$). MS (ES+) m/z 392 ([MH$^+$], 80%). HRESIMS m/z 392.1817 (MH$^+$), calcd for C$_{24}$H$_{26}$NO$_4$ (MH$^+$) 392.1817.

$(IR^*,3S^*)$-3-Ethyl 1-methyl 1-(diphenylmethyleneamino)-3-methylcyclopent-4-ene-1,3-dicarboxylate (8a)

Minor isomer 8a, a yellow solid (34 mg, 6%). R$_f$ (5% ethyl acetate/ petroleum spirit) 0.52. $^1$H NMR $\delta$ 7.60-7.12 (m, 10H), 5.90 (d, $J = 5.4$ Hz, 1H), 5.71 (d, $J = 5.4$ Hz, 1H), 4.12-4.06 (m, 2H), 3.42 (s, 3H), 2.82 (d, $J = 13.8$ Hz, 1H), 2.50 (d, $J = 13.8$ Hz, 1H), 1.32 (s, 3H), 1.26 (t, $J = 6.9$ Hz, 3H). $^{13}$C NMR $\delta$ 176.1 (C), 173.6 (C), 168.2 (C), 140.3 (C), 137.6 (C), 133.9 (CH), 130.2 (CH), 128.8 (2CH), 128.7 (2CH), 128.4 (4CH), 127.9 (2CH), 78.5 (C), 60.8 (CH$_3$), 55.3 (C), 51.9 (CH$_2$), 47.9 (CH$_2$), 25.4 (CH$_3$), 14.2 (CH$_3$). MS (ES+) m/z 392 ([M+H], 85%). HRESIMS m/z 392.1815 (MH$^+$), calcd for C$_{24}$H$_{26}$NO$_4$ (MH$^+$) 392.1817.
(1R*,3R*)-1-Ethyl 3-methyl 1-allyl-3-(diphenylmethyleneamino)cyclopent-4-ene-1,3-dicarboxylate (7b)

Major isomer 7b (0.274 g, 45%) yellow solid. Rf (5% ethyl acetate/ petroleum spirit) 0.36. 1H NMR δ 7.57-7.12 (m, 10H), 5.94 (d, J = 5.4 Hz, 1H), 5.76 (d, J = 5.4 Hz, 1H), 5.82-5.68 (m, 1H), 5.76 (d, J = 5.4 Hz, 1H), 5.14-5.10 (m, 1H), 5.09-5.07 (m, 1H) 4.15-4.08 (m, 2H), 3.39 (s, 3H), 2.96 (d, J = 13.7 Hz, 1H), 2.67-2.52 (m, 2H), 2.40 (d, J = 13.7 Hz, 1H), 1.22 (t, J = 6.9 Hz, 3H). 13C NMR δ 174.4 (C), 173.1 (C), 168.2 (C), 140.2 (C), 137.4 (C), 136.0 (CH), 134.2 (CH), 133.7 (CH), 132.3 (C), 130.2 (CH), 129.9 (CH), 128.7 (CH), 128.5 (CH), 128.3 (2CH), 128.2 (CH), 127.9 (2CH), 118.1 (CH2), 78.1 (C), 60.6 (CH2), 59.3 (C), 51.7 (CH3), 46.8 (CH2), 43.9 (CH2), 14.1 (CH3). MS (ES+) m/z 418.19 ([MH]+), 100.0%. HRESIMS m/z 418.2014 [MH]+, calcd for C26H28NO4 (MH+) 418.2012.

(1R*,3R*)-1-Ethyl 3-methyl 1-allyl-3-(diphenylmethyleneamino)cyclopent-4-ene-1,3-dicarboxylate (8b)

Minor isomer 8b, a yellow solid (36.6 mg, 6%). Rf (5% ethyl acetate/ petroleum spirit) 0.54. 1H NMR δ 7.57-7.12 (m, 10H), 5.93 (d, J = 5.6 Hz, 1H), 5.77 (d, J = 5.6 Hz, 1H), 5.73-5.62 (m, 1H), 5.06-5.04 (br d, J = 5.6 Hz, 1H), 5.02 (br s, 1H), 4.21-4.09 (m, 2H), 3.43 (s, 3H), 2.81 (d, J = 14.4 Hz, 1H), 2.58 (d, J = 14.4 Hz, 1H) 2.42 (d, J = 5.6 Hz, 2H), 1.25 (t, J = 6.8 Hz, 3H). 13C NMR δ 174.7 (C), 173.5 (C), 168.1 (C), 140.2 (C), 137.5 (C), 136.1 (CH), 134.5 (CH), 133.6 (CH), 130.2 (CH), 128.8 (CH), 128.6 (CH), 128.3 (2CH), 127.9 (3CH), 118.1 (CH2), 78.1 (C), 60.8 (CH2), 59.4 (C), 51.9 (CH3), 45.8 (CH2), 42.2 (CH2), 14.3 (CH3). MS (ES+) m/z 418.19 ([MH]+), 100.0%. HRESIMS m/z 418.2013 (MH+), calcd for C26H28NO4 (MH+) 418.2012.

4.3.2 General procedure for alkylation of 6

To a solution of compound 6 (0.552 g, 1.46 mmol) in dry THF (12 mL) at -78 °C was added a solution of KN(TMS)2 (2.5 equiv., 7.3 mL, 3.66 mmol, 0.5 M in THF). After
stirring for 1 h, a solution of alkyl bromide (2.5 equiv., 3.7 mmol) in dry THF (2 mL) was added drop wise to the mixture and the stirring continued for another 1 h. The reaction mixture was allowed to warm to RT over 1 h then was diluted with a saturated aqueous solution of NaCl, and then extracted with ether. The combined organic extracts were dried over MgSO₄ and the solvent was removed to give a dark yellow residue which was purified by column chromatography (ethyl acetate/petroleum spirit, 1:9) to give a mixture of diastereomers. The two diastereomers were separated by HPLC (ethyl acetate/petroleum spirit, 5:95).

(1R*,3R*)-1-Ethyl 3-methyl 1-benzyl-3-(diphenylmethyleneamino)cyclopent-4-ene-1,3-dicarboxylate (7c)
Major isomer 7c, a yellow solid (0.525 g, 77%). Rf (5% ethyl acetate/ petroleum spirit) 0.35. ¹H NMR δ 7.62-7.59 (m, 2H), 7.43-7.12 (m, 13H), 5.93 (d, J = 5.7 Hz, 1H), 5.75 (d, J = 5.7 Hz, 1H), 4.02 (q, J = 7.2 Hz, 2H), 3.38 (s, 3H), 3.28 (d, J = 13.2 Hz, 1H), 3.13 (d, J = 13.2 Hz, 1H), 2.91 (d, J = 13.5 Hz, 1H), 2.56 (d, J = 13.5 Hz, 1H), 1.14 (t, J = 7.2 Hz, 3H). ¹³C NMR δ 174.2 (C), 173.8 (C), 168.2 (C), 144.1 (C), 140.0 (C), 137.4 (C), 136.2 (CH), 133.8 (CH), 130.2 (2CH), 129.7 (2CH), 128.7 (4CH), 128.5 (2CH), 128.2 (2CH), 128.0 (CH), 127.9 (CH), 126.5 (CH), 77.9 (C), 60.7 (CH₂), 60.5 (C), 51.7 (CH₃), 47.4 (CH₂), 45.6 (CH₂), 13.9 (CH₃). MS (ES+) m/z 468.0 ([M+H], 100.0%), Anal. calcd for C₃₀H₂₉NO₄: C, 77.06; H, 6.25; N, 3.00. Found: C, 77.31; H, 6.38; N, 2.75.

(1R*,3S*)-1-Ethyl 3-methyl 1-benzyl-3-(diphenylmethyleneamino)cyclopent-4-ene-1,3-dicarboxylate (8c)
Minor isomer 8c, a yellow solid (75.0 mg, 11%). Rf (5% ethyl acetate/ petroleum spirit) 0.65. ¹H NMR δ 7.82-7.80 (m, 2H), 7.60-7.08 (m, 13H), 5.92 (d, J = 5.4 Hz, 1H), 5.80 (d, J = 5.4 Hz, 1H), 4.10-4.02 (m, 2H), 3.45 (s, 3H), 3.06 (d, J = 13.2 Hz, 1H), 2.96 (d, J = 13.2 Hz, 1H), 2.74 (s, 2H), 1.14 (t, J = 7.2 Hz, 3H). ¹³C NMR δ 174.1 (C), 173.6 (C), 168.3 (C), 144.3 (C), 139.8 (C), 137.3 (C), 136.2 (CH), 133.6 (CH), 130.1 (2CH), 129.4 (2CH), 129.0 (4CH), 128.8 (2CH), 128.3 (2CH), 128.1 (CH), 127.5 (CH), 126.1 (CH), 77.6 (C), 61.0 (C), 60.3 (CH₂), 52.0 (CH₃), 48.3 (CH₂), 45.8 (CH₂), 14.0 (CH₃). MS
(ES+) m/z 468.0 ([MH⁺], 100.0%). Anal. calcd for C_{30}H_{29}NO_{4}: C, 77.06; H, 6.25; N, 3.00. Found: C, 76.97; H, 6.47; N, 2.81.

(1R*,3R*)-3-Ethyl 1-methyl 1-(diphenylmethyleneamino)-3-(3-phenylpropyl)cyclopent-4-ene-1,3-dicarboxylate (7d)
Major isomer 7d, a yellow oil (0.260 g, 36%). R_f (5% ethyl acetate/ petroleum spirit) 0.36. \textsuperscript{1}H NMR δ 7.58-7.09 (m, 15H), 5.93 (d, J = 5.4 Hz, 1H), 5.71 (d, J = 5.4 Hz, 1H), 4.11-4.05 (m, 2H), 3.39 (s, 3H), 3.01 (d, J = 13.6 Hz, 1H), 2.68-2.56 (m, 2H), 2.33 (d, J = 13.6 Hz, 1H), 1.94-1.79 (m, 2H), 1.68-1.60 (m, 2H), 1.20 (t, J = 6.8 Hz, 3H). \textsuperscript{13}C NMR δ 174.8 (C), 173.0 (C), 168.1 (C), 141.7 (C), 140.0 (C), 137.2 (C), 136.1 (CH), 133.7 (CH), 130.1 (CH), 128.6 (CH), 128.4 (2CH), 128.2 (2CH), 128.1 (4CH), 127.8 (2CH), 127.7 (2CH), 125.6 (CH), 77.8 (C), 60.5 (CH₂), 59.2 (C), 51.6 (CH₃), 47.4 (CH₂), 39.1 (CH₂), 35.9 (CH₂), 27.0 (CH₂), 13.9 (CH₃). MS (ES+) m/z 496.0 ([MH⁺], 100.0%). HRESIMS m/z 496.2488 (MH⁺), calcd for C_{32}H_{34}NO₄ (MH⁺) 496.2493.

(1R*,3S*)-3-Ethyl 1-methyl 1-(diphenylmethyleneamino)-3-(3-phenylpropyl)cyclopent-4-ene-1,3-dicarboxylate (8d)
Minor isomer 8d, a yellow oil (11.6 mg, 1.6%). R_f (5% ethyl acetate/ petroleum spirit) 0.54. \textsuperscript{1}H NMR δ 7.56-7.11 (m, 15H), 5.93 (d, J = 5.4 Hz, 1H), 5.73 (d, J = 5.4 Hz, 1H), 4.18-4.07 (m, 2H), 3.39 (s, 3H), 2.83 (d, J = 13.5 Hz, 1H), 2.57-2.54 (m, 2H), 2.52 (d, J = 13.5 Hz, 1H), 1.76-1.67 (m, 2H), 1.61-1.51 (m, 2H), 1.22 (t, J = 6.9 Hz, 3H). \textsuperscript{13}C NMR δ 175.2 (C), 173.5 (C), 168.0 (C), 142.0 (C), 140.2 (C), 137.5 (C), 136.4 (CH), 134.0 (CH), 130.2 (CH), 128.7 (CH), 128.6 (2CH), 128.3 (4CH), 128.2 (2CH), 127.8 (4CH), 125.7 (CH), 78.1 (C), 60.6 (CH₂), 59.4 (C), 51.8 (CH₃), 46.6 (CH₂), 38.7 (CH₂), 35.9 (CH₂), 27.2 (CH₂), 14.2 (CH₃). MS (ES+) m/z 496.0 ([MH⁺], 100.0%). HRESIMS m/z 496.2488 (MH⁺), calcd for C_{32}H_{34}NO₄ (MH⁺) 496.2493.

4.3.3 General hydrolysis procedure for 7 and 8
Compound 7a (0.270 g, 0.70 mmol) in 10% aq. HCl (3 mL) was heated at 80 °C for 16 h.
After cooling, the reaction mixture was diluted with water (2 mL) and washed with ether (5 mL). The water was removed under reduced pressure to give the HCl salt of 9a as a white solid (0.133 g, 86%).

**(IR*,3R*)-1-Amino-3-methylcyclopent-4-ene-1,3-dicarboxylic acid (9a)**

A white solid (0.133 g, 86%). $^1$H NMR (D$_2$O) $\delta$ 6.14 (d, $J = 5.4$ Hz, 1H), 5.65 (d, $J = 5.4$ Hz, 1H), 3.01 (d, $J = 14.7$ Hz, 1H), 1.86 (d, $J = 14.7$ Hz, 1H), 1.30 (s, 3H). $^{13}$C NMR (D$_2$O) $\delta$ 176.2 (C), 169.9 (C), 141.1 (CH), 124.0 (CH), 66.3 (C), 53.8 (C), 40.9 (CH$_2$), 22.0 (CH$_3$). MS (ES+) $m/z$ 186.0 ([MH$^+$], 60%). HRESIMS $m/z$ 186.0766 (MH$^+$) calcd for C$_8$H$_{12}$NO$_4$ (MH$^+$) 186.0761.

**(IR*,3S*)-1-Amino-3-methylcyclopent-4-ene-1,3-dicarboxylic acid (10a)**

A white solid (0.124 g, 80%). $^1$H NMR (D$_2$O) $\delta$ 6.18 (d, $J = 5.4$ Hz, 1H), 5.72 (d, $J = 5.4$ Hz, 1H), 2.59 (d, $J = 15.3$ Hz, 1H), 2.35 (d, $J = 15.3$ Hz, 1H), 1.32 (s, 3H). $^{13}$C NMR (D$_2$O) $\delta$ 176.2 (C), 169.9 (C), 141.1 (CH), 124.0 (CH), 66.5 (C), 53.8 (C), 40.9 (CH$_2$), 22.0 (CH$_3$). MS (ES+) $m/z$ 186.0 ([MH$^+$], 60%). HRESIMS $m/z$ 186.0763 (MH$^+$), calcd for C$_8$H$_{12}$NO$_4$ (MH$^+$) 186.0761.

**(IR*,3R*)-1-Allyl-3-aminocyclopent-4-ene-1,3-dicarboxylic acid (9b)**

A pale yellow solid (0.170 g, 100%). $^1$H NMR (D$_2$O) $\delta$ 6.31 (d, $J = 5.7$ Hz, 1H), 5.80 (d, $J = 5.7$ Hz, 1H), 5.78-5.64 (m, 1H), 5.12 (br d, $J = 4.8$ Hz, 1H), 5.08 (br s, 1H), 3.03 (d, $J = 15.0$ Hz, 1H), 2.49 (br d, $J = 4.8$ Hz, 2H), 2.04 (d, $J = 15.0$ Hz, 1H). $^{13}$C NMR (D$_2$O) $\delta$ 174.7 (C), 169.3 (C), 139.1 (CH), 129.4 (CH), 124.5 (CH), 116.4 (CH$_2$), 66.0 (C), 57.5 (C), 39.6 (CH$_2$), 38.4 (CH$_2$). MS (ES+) $m/z$ 212 (M+H). HRESIMS $m/z$ 212.0919 (MH$^+$), calcd for C$_{10}$H$_{14}$NO$_4$ (MH$^+$) 212.0917.

**(IR*,3S*)-1-Allyl-3-aminocyclopent-4-ene-1,3-dicarboxylic acid (10b)**

A pale yellow solid (22.1 mg, 100%). $^1$H NMR (D$_2$O) $\delta$ 6.29 (d, $J = 4.3$ Hz, 1H), 5.81 (d, $J = 4.3$ Hz, 1H), 5.74-5.64 (m, 1H), 5.11-5.04 (m, 2H), 2.65-2.58 (m, 1H), 2.55 (dd, $J =$
15.0 Hz, 1H), 2.52 (d, J = 15.0 Hz, 2H), 2.47-2.41 (m, 1H). $^{13}$C NMR (D$_2$O) δ 177.6 (C), 172.9 (C), 143.5 (CH), 132.8 (CH), 127.9 (CH), 119.0 (CH$_2$), 69.5 (C), 60.4 (C), 41.5 (CH$_2$), 40.4 (CH$_2$). MS (ES+) m/z 212 ([MH$^+$], 100%). HRESIMS m/z 212.0932 (MH$^+$), calcd for C$_{10}$H$_{14}$NO$_4$ (MH$^+$) 212.0917.

(1R*,3R*)-1-Amino-3-benzylcyclopent-4-ene-1,3-dicarboxylic acid (9c)
A pale yellow solid (43.4 mg, 94%). $^1$H NMR (D$_2$O) δ 7.27-7.13 (m, 5H), 6.29 (d, J = 5.7 Hz, 1H), 5.74 (d, J = 5.7 Hz, 1H), 3.09 (d, J = 13.5 Hz, 1H), 3.01 (d, J = 14.7 Hz, 1H), 2.98 (d, J = 13.5 Hz, 1H). $^{13}$C NMR (D$_2$O) δ 174.5 (C), 169.1 (C), 138.9 (CH), 132.9 (C), 126.7 (2CH), 125.4 (2CH), 124.4 (CH), 124.2 (CH), 66.1 (C), 58.7 (C), 41.2 (CH$_2$), 38.6 (CH$_2$). MS (ES+) m/z 262 (M+H, 30%). HRESIMS m/z 262.1077 (MH$^+$), calcd for C$_{14}$H$_{16}$NO$_4$ (MH$^+$) 262.1074.

(1R*,3S*)-1-Amino-3-benzylcyclopent-4-ene-1,3-dicarboxylic acid (10c)
A white solid (29.0 mg, 98%). $^1$H NMR (D$_2$O) δ 7.25-7.15 (m, 5H), 6.25 (d, J = 5.7 Hz, 1H), 5.79 (d, J = 5.4 Hz, 1H), 3.18 (d, J = 13.5 Hz, 1H), 3.03 (d, J = 13.5 Hz, 1H), 2.64 (d, J = 15.0 Hz, 1H), 2.49 (d, J = 15.0 Hz, 1H). $^{13}$C NMR (D$_2$O) δ 174.5 (C), 169.1 (C), 138.9 (CH), 132.2 (C), 126.6 (2CH), 125.2 (2CH), 124.5 (CH), 124.3 (CH), 66.1 (C), 58.6 (C), 40.9 (CH$_2$), 38.8 (CH$_2$). HRESIMS m/z 262.1075 (MH$^+$), calcd for C$_{14}$H$_{16}$NO$_4$ (MH$^+$) 262.1074.

(1R*,3R*)-1-Amino-3-(3-phenylpropyl)cyclopent-4-ene-1,3-dicarboxylic acid (9d)
A white solid (37.3 mg, 63%). $^1$H NMR (D$_2$O) δ 7.25-7.15 (m, 5H), 6.25 (d, J = 5.7 Hz, 1H), 5.72 (d, J = 5.7 Hz, 1H), 3.02 (d, J = 14.4 Hz, 1H), 2.57-2.52 (m, 2H), 1.92 (d, J = 14.4 Hz, 1H), 1.78-1.20 (m, 4H). $^{13}$C NMR (D$_2$O) δ 175.1 (C), 169.5 (C), 139.4 (C), 139.1 (CH), 125.4 (4CH), 124.2 (CH), 122.9 (CH), 66.0 (C), 57.5 (C), 39.1 (CH$_2$), 35.1 (CH$_2$), 31.7 (CH$_2$), 23.2 (CH$_2$). MS (ES+) m/z 290 ([MH$^+$], 100%). HRESIMS m/z 290.1389 (MH$^+$), calcd for C$_{16}$H$_{20}$NO$_4$ (MH$^+$) 290.1387.
(IR*,3S*)-1-Amino-3-(3-phenylpropyl)cyclopent-4-ene-1,3-dicarboxylic acid (10d)
A white solid (22.7 mg, 76%). $^1$H NMR (D$_2$O) $\delta$ 6.95-6.80 (m, 5H), 5.94 (d, $J = 5.4$ Hz, 1H), 5.45 (d, $J = 5.4$ Hz, 1H), 2.30 (d, $J = 15.3$ Hz, 1H), 2.21 (t, $J = 7.5$ Hz, 2H), 2.12 (d, $J = 15.3$ Hz, 1H), 1.58-1.48 (m, 1H), 1.35-1.28 (m, 1H), 1.25-1.14 (m, 2H). $^{13}$C NMR (D$_2$O) $\delta$ 174.2 (C), 168.7 (C), 140.7 (CH), 138.5 (C), 124.9 (4CH), 123.9 (CH), 122.4 (CH), 66.0 (C), 57.0 (C), 57.4 (CH$_2$), 33.1 (CH$_2$), 31.4 (CH$_2$), 22.7 (CH$_2$). MS (ES$^+$) $m/z$ 290 (M+H, 100%). HRESIMS $m/z$ 290.1388 (MH$^+$), calcd for C$_{16}$H$_{20}$NO$_4$ (MH$^+$) 290.1387.

4.3.4 Representative procedure for preparation of rac-11 or rac-12
To a stirred solution of compound 7a (0.165 g, 0.422 mmol) in diethyl ether (2 mL) at 0 °C, was slowly added a solution of 1 M aq. HCl (0.5 mL). The reaction mixture was stirred at 0 °C for 2 h and then at RT overnight. The reaction mixture was diluted with water (2 mL). The layers were separated and the aqueous layer was extracted with diethyl ether. Water was removed to give the crude intermediate. The crude intermediate was dissolved in methanol (3 mL). To the resulting solution was added Pd/C (23 mg, 0.042 mmol). The reaction mixture was stirred at RT, under a H$_2$ atmosphere (approx. 1 atm, balloon) for 24 h. The reaction mixture was filtered through a pad of celite and washed with methanol. Methanol was removed to give a yellow oil, which was purified by column chromatography (flash silica, ethyl acetate/hexane (60:40) as eluent) to give 11a as a yellow oil (80.3 mg, 83%).

(IS*,3S*)-3-Ethyl 1-methyl 1-amino-3-methylcyclopentane-1,3-dicarboxylate (11a)
$^1$H NMR (D$_2$O) $\delta$ 4.15-4.06 (m, 2H), 3.78 (s, 3H), 2.95 (d, $J = 15.0$ Hz, 1H), 2.48-2.4 (m, 1H), 2.41-2.33 (m, 1H), 2.01 (ddd, $J = 4.5$, 10.5, 12.0 Hz, 1H), 1.86 (d, $J = 15.0$ Hz, 1H) 1.54 (ddd, $J = 4.5$, 10.5, 12.0 Hz, 1H), 1.39 (s, 3H), 1.19 (t, $J = Hz$, 3H).$^{13}$C NMR (D$_2$O) $\delta$ 175.0 (C), 169.5 (C), 61.1 (C), 59.1 (CH$_2$), 52.3 (C), 50.8 (CH$_3$), 42.2 (CH$_2$), 38.8 (CH$_2$), 31.8 (CH$_2$), 26.6 (CH$_3$), 10.62 (CH$_3$). MS (ES$^+$) $m/z$ 230.1 (M+H, 100%). HRESIMS $m/z$ 230.1389 (MH$^+$), calcd for C$_{11}$H$_{20}$NO$_4$ (MH$^+$) 230.1387.
*(1S*,3S*)-3-Ethyl 1-methyl 1-amino-3-propylcyclopentane-1,3-dicarboxylate (11b)
A yellow oil (97.5 mg, 94%). $^1$H NMR (D$_2$O) $\delta$ 4.14-4.07 (m, 2H), 3.71 (s, 3H), 2.94 (d, $J = 15.0$ Hz, 1H), 2.48-2.40 (m, 1H), 2.31 (ddd, $J = 4.5$, 10.5, 14.4 Hz, H), 2.00 (ddd, $J = 4.5$, 10.5, 14.4 Hz, 1H), 1.86 (d, $J = 15.0$ Hz, 1H) 1.79 – 1.66 (m, 2H), 1.54 (ddd, $J = 4.5$, 10.5, 14.4 Hz, 1H), 1.18 (t, $J = 6.9$ Hz, 3H), 1.00 (m, 2H), 0.78 (t, $J = 7.2$ Hz, 3H). $^{13}$C NMR (D$_2$O) $\delta$ 175.0 (C), 169.5 (C), 61.1 (C), 59.1 (CH$_2$), 52.3 (C), 50.8 (CH$_3$), 42.2 (CH$_2$), 38.8 (CH$_2$), 31.8 (CH$_2$), 30.3 (CH$_2$), 15.8 (CH$_2$), 10.6 (CH$_3$), 10.4 (CH$_3$). MS (ES$^+$) m/z 258.0 (M+H, 100%). HRESIMS m/z 258.1707 (MH$^+$), calcd for C$_{13}$H$_{24}$NO$_4$ (MH$^+$) 258.1700.

*(1S*,3R*)-3-Ethyl 1-methyl 1-amino-3-benzylcyclopentane-1,3-dicarboxylate (11c)
A yellow oil (79.0 mg, 66%). $^1$H NMR (CD$_3$OD) $\delta$ 7.29-7.11 (m, 5H), 4.12-4.04 (m, 2H), 3.78 (s, 3H), 3.13 (d, $J = 13.2$ Hz, 1H), 2.99 (d, $J = 15.0$ Hz, 1H), 2.97 (d, $J = 13.2$ Hz, 1H), 2.42-2.35 (m, 2H), 2.06-2.01 (m, 3H), 1.19 (t, $J = 7.2$ Hz, 3H). $^{13}$C NMR (D$_2$O) $\delta$ 174.3 (C), 169.3 (C), 134.1 (C), 126.5 (2CH), 125.3 (2CH), 123.9 (CH), 61.5 (C), 59.9 (CH$_2$), 52.7 (CH$_3$), 51.0 (C), 51.0 (CH$_2$), 40.0 (CH$_2$), 32.1 (CH$_2$), 32.0 (CH$_2$), 10.4 (CH$_3$). MS (ES$^+$) m/z 306 (M+H, 100%). HRESIMS m/z 306.1705 (MH$^+$), calcd for C$_{17}$H$_{24}$NO$_4$ (MH$^+$) 306.1700.

*(1S*,3R*)-3-ethyl 1-methyl 1-amino-3-propylcyclopentane-1,3-dicarboxylate (12b)
A yellow oil (6.2 mg, 93%). $^1$H NMR (D$_2$O) $\delta$ 4.12-4.09 (m, 2H), 3.71 (s, 3H), 2.94 (d, $J = 15.0$ Hz, 1H), 2.46-2.26 (m, 2H), 2.08–1.95 (m, 1H), 1.85 (d, $J = 15.0$ Hz, 1H), 1.78-1.64 (m, 2H), 1.65–1.55 (m, 1H), 1.21 (t, $J = 6.9$ Hz, 3H), 1.0 (m, 2H), 0.80 (t, $J = 7.2$ Hz, 3H). $^{13}$C NMR (D$_2$O) $\delta$ 174.6 (C), 169.2 (C), 61.5 (C), 59.0 (CH$_2$), 51.5 (C), 50.9 (CH$_3$), 40.4 (CH$_2$), 33.0 (CH$_2$), 32.5 (CH$_2$), 32.2 (CH$_2$), 19.0 (CH$_2$), 10.7 (CH3), 10.4 (CH$_3$). MS (ES$^+$) m/z 258.0 (M+H, 100%). HRESIMS m/z 258.1707 (MH$^+$), calcd for C$_{13}$H$_{24}$NO$_4$ (MH$^+$) 258.1700.

*(1S*,3S*)-3-Ethyl 1-methyl 1-amino-3-benzylcyclopentane-1,3-dicarboxylate (12c)
A yellow oil (43.3 mg, 70%). $^1$H NMR (D$_2$O) $\delta$ 7.23 (br s, 3H), 7.08 (br s, 2H), 4.09-4.07
(m, 2H), 3.8 (s, 3H), 3.11 (d, J = 12.6 Hz, 1H), 2.89 (d, J = 12.6 Hz, 1H), 2.51-2.26 (m, 4H), 2.04-1.94 (m, 2H), 1.16 (t, J = 7.2 Hz, 3H). $^{13}$C NMR (D$_2$O) δ 174.3 (C), 169.3 (C), 134.0 (C), 126.5 (2CH), 125.3 (2CH), 123.8 (CH), 61.4 (C), 59.3 (CH$_2$), 52.9 (CH$_3$), 51.0 (C), 50.9 (CH$_2$), 39.9 (CH$_2$), 32.1 (CH$_2$), 32.0 (CH$_2$), 10.3 (CH$_3$). MS (ES$^+$) m/z 306 (M+H, 100%). HRESIMS m/z 306.1705 (MH$^+$), calcd for C$_{17}$H$_{24}$NO$_4$ (MH$^+$) 306.1700.

4.3.5 Representative procedure hydrolysis of rac-11 and rac-12

Compound 11a (80.0 mg, 0.35 mmol) in 10% aq. HCl (2 mL) was heated at 80 °C for 16 h. After cooling, the reaction mixture was diluted with water (2 mL) and washed with ether (5 mL). Water was removed under reduced pressure to give the HCl salt of 13a as a white solid (84.4 mg, 96%).

(IS*,3S*)-1-Amino-3-methylcyclopentane-1,3-dicarboxylic acid (13a)

$^1$H NMR (D$_2$O) δ 2.81 (d, J = 15.0 Hz, 1H), 2.85-2.16 (m, 2H), 2.03-1.93 (m, 1H), 1.74 (d, J = 15.0 Hz, 1H) 1.66-1.57 (m, 1H), 1.21 (s, 3H). $^{13}$C NMR (D$_2$O) δ 180.0 (C), 173.0 (C), 61.1 (C), 49.8 (C), 43.4 (CH$_2$), 35.0 (CH$_2$), 33.3 (CH$_2$), 25.6 (CH$_3$). MS (ES$^+$) m/z 188 (M+1, 100%). HRMSESI m/z 188.0921 (MH$^+$), calcd for C$_8$H$_{14}$NO$_4$ (MH$^+$) 188.0917.

(IS*,3S*)-1-Amino-3-propylcyclopentane-1,3-dicarboxylic acid (13b)

A white solid (77.6 mg, 99%). $^1$H NMR (D$_2$O) δ 2.91 (d, J = 15.0 Hz, 1H), 2.44-2.36 (m, 1H), 2.29 (dd, J = 8.2, 12.0 Hz, 1H), 1.99 (ddd, J = 4.5, 8.2, 12.0 Hz, 1H), 1.83 (d, J = 15.0 Hz, 1H) 1.78-1.64 (m, 2H), 1.54 (td, J = 4.5, 12.0 Hz, 1H), 1.24-1.03 (m, 2H), 0.78 (t, J = 7.5 Hz, 3H). $^{13}$C NMR (D$_2$O) δ 177.1 (C), 171.0 (C), 61.1 (C), 51.9 (C), 41.9 (CH$_2$), 38.7 (CH$_2$), 31.9 (CH$_2$), 30.3 (CH$_2$), 15.7 (CH$_2$), 10.5 (CH$_3$). MS (ES$^+$) m/z 216 (M+1, 100%). HRMSESI m/z 216.1235 (MH$^+$), calcd for C$_{10}$H$_{18}$NO$_4$ (MH$^+$) 216.1230.

(IS*,3R*)-1-Amino-3-benzylcyclopentane-1,3-dicarboxylic acid (13c)

A white solid (42.0 mg, 86%). $^1$H NMR (D$_2$O) δ 7.26-7.11 (m, 5H), 3.05 (d, J = 13.2 Hz, 1H), 2.90 (d, J = 15 Hz, 1H), 2.87 (d, J = 15 Hz, 1H), 2.33 – 2.20 (m, 2H), 2.03 – 1.94 (m, 2H), 1.90-1.83 (m, 1H). $^{13}$C NMR (D$_2$O) δ 175.9 (C), 170.7 (C), 133.9 (C), 126.3
(2CH), 125.3 (2CH), 124.0 (CH), 61.0 (C), 53.4 (C), 41.7 (CH₂), 41.3 (CH₂), 31.6 (CH₂), 29.8 (CH₂). MS (ES⁺) m/z 264 (M+1, 100%). HRMSESI m/z 264.1235 (MH⁺), calcd for C₁₄H₁₈NO₄ (MH⁺) 264.1230.

(IS*,3S*)-1-Amino-3-benzylcyclopentane-1,3-dicarboxylic acid (14c)
A white solid (33.5 mg, 85%). ¹H NMR (D₂O) δ 7.25-7.12 (m, 5H), 3.14 (d, J = 13.5 Hz, 1H), 2.84 (d, J = 13.5 Hz, 1H), 2.43-2.35 (m, 2H), 2.30-2.20 (m, 2H), 2.09-1.97 (m, 1H), 1.94-1.84 (m, 1H). ¹³C NMR (D₂O) δ 176.8 (C), 171.0 (C), 134.1 (C), 126.4 (2CH), 125.4 (2CH), 123.9 (CH), 61.3 (C), 52.9 (C), 39.7 (CH₂), 39.6 (CH₂), 32.8 (CH₂), 32.0 (CH₂). MS (ES⁺) m/z 264 (M+1, 100%). HRMSESI m/z 264.1233 (MH⁺), calcd for C₁₄H₁₈NO₄ (MH⁺) 264.1230.

Rac-3-Ethyl 1-methyl 1-(dimethylamino)cyclopent-3-ene-1,3-dicarboxylate (16)
To a solution of compound 15 (1.17 g, 5.48 mmol) in CH₃CN (30 mL) was added a solution of formaldehyde (40% aq., 11.5 mL, 13.7 mmol) and NaCNBH₃ (0.92 g, 14.6 mmol). After 20 min of stirring, the pH of the reaction mixture was adjusted to pH = 4, by addition of acetic acid. The reaction mixture was left to stir at RT overnight. The mixture was diluted with a saturated aqueous solution of Na₂CO₃ (30 mL). The resulting solution was extracted twice with ethyl acetate (2 × 30 mL). The combined extracts were washed with a saturated solution of NaCl (30 mL) and dried over K₂CO₃. The solvent was removed under reduced pressure to give a yellow oil, which was purified by column chromatography (flash silica, ethyl acetate/light petroleum (1:1)) to give compound 16 (1.14 g, 86%) as a yellow oil. ¹H NMR δ 6.66 (dd, J = 1.2, 2.4, 4.5 Hz, 1H), 4.19 (q, J = 7.2 Hz, 2H), 3.75 (s, 3H), 3.18 (dd, J = 1.2, 17.4 Hz, 2H), 3.17 (dd, J = 1.2, 17.4 Hz, 1H), 2.76 (ddd, J = 2.4, 4.5, 17.4 Hz, 1H), 2.67 (ddd, J = 2.4, 4.5, 17.4 Hz, 1H), 2.64 (s, 6H), 1.29 (t, J = 7.2 Hz, 3H). ¹³C NMR δ 173.1 (C), 164.2 (C), 140.0 (CH), 133.9 (C), 74.3 (C), 60.2 (CH₂), 51.7 (CH₃), 40.2 (2CH₃), 40.4 (CH₂), 38.8 (CH₂), 14.1 (CH₃). MS (Cl) m/z 242 (M+1, 100%). HRMSESI m/z 242.1392 (MH⁺), calcd for C₁₂H₂₀NO₄ (MH⁺) 242.1387.

Rac-1-(Dimethylamino)cyclopent-3-ene-1,3-dicarboxylic acid (17)
Compound 16 (0.100 g, 0.415 mmol) in 10% aq. HCl (2 mL) was heated at 80 °C for 16 h. After cooling, the reaction mixture was diluted with water (2 mL) and washed with ether (5 mL). The water was removed under reduced pressure to give the HCl salt of 17 as a white solid (95.8 mg, 96%). $^1$H NMR (D$_2$O) δ 6.71-6.69 (br m, 1H), 3.27 (br d, J = 13.8 Hz, 2H), 3.08-3.05 (br m, 1H), 3.02-3.00 (br m, 1H), 2.77 (s, 3H), 2.77 (s, 3H). $^{13}$C NMR (D$_2$O) δ 168.7 (C), 163.4 (C), 137.9 (CH), 129.7 (C), 72.2 (C), 36.1 (2CH$_3$), 36.1 (CH$_2$), 34.3 (CH$_2$). MS (Cl) m/z 200 (M+1, 100%). HRMSESI m/z 200.0923 (MH$^+$), calcd for C$_9$H$_{14}$NO$_4$ (MH$^+$) 200.0917.

4.3.6 Reduction of compound 16

To a solution of 16 (0.406 g, 1.62 mmol) in dry CH$_3$OH (30 mL) under a nitrogen atmosphere at 0 °C, was added Mg turnings (0.393 g, 16.24 mmol). The reaction mixture was left to stir at 0 °C for 2 h then at RT overnight. Methanol was removed under reduced pressure. Water (30 mL) was added to the reaction mixture and the resulting mixture was extracted with ethyl acetate (3 × 40 mL). The combined organic extracted were washed with a saturated aqueous solution of NaCl and dried over K$_2$CO$_3$. The solvent was removed under reduced pressure to give an oil which was purified by PTLC (ethyl acetate/hexane (1:1)) to give compound 18 (0.107 g, 27%) and 19 (35.5 mg, 9%).

(IS*,3S*)-3-Ethyl 1-methyl 1-(dimethylamino)cyclopentane-1,3-dicarboxylate (18)

A yellow solid (0.107 g, 27%), $^1$H NMR δ 4.20 (q, J = 7.2 Hz, 2H), 3.70 (s, 3H), 2.92 (ddd, J = 8.2, 8.2, 16.8 Hz, 1H), 2.47 (dd, J = 8.2, 14.0 Hz, 1H), 2.29 (s, 6H), 2.11 (dd, J = 8.2, 14.0 Hz, 1H), 2.09-1.98 (m, 1H), 1.94-1.71 (m, 3H), 1.29 (t, J = 7.2 Hz, 3H). $^{13}$C NMR δ 175.7 (C), 172.7 (C), 73.7 (C), 60.1 (CH$_2$), 51.6 (CH$_3$), 41.7 (CH), 40.3 (2CH$_3$), 37.6 (CH$_2$), 34.4 (CH$_2$), 27.5 (CH$_2$), 14.1 (CH$_3$). MS (ES+) m/z 244 (M+1, 100%). HRMSESI m/z 244.1547 (MH$^+$), calcd for C$_{12}$H$_{22}$NO$_4$ (MH$^+$) 244.1543.

(IS*,3R*)-3-Ethyl 1-methyl 1-(dimethylamino)cyclopentane-1,3-dicarboxylate (19)

A yellow solid (35.5 mg, 9%), $^1$H NMR δ 4.20 (q, J = 7.2 Hz, 2H), 3.71 (s, 3H), 2.84 (ddd, J = 7.6, 17.2, 17.2 Hz, 1H), 2.50 (dd, J = 7.6, 17.2, 1H), 2.29 (s, 6H), 2.31-2.22 (m,
1H), 2.06-1.78 (m, 4H), 1.29 (t, J = 7.2 Hz, 3H). $^{13}$C NMR δ 175.7 (C), 172.7 (C), 73.7 (C), 60.1 (CH$_2$), 51.6 (CH$_3$), 41.7 (CH), 40.3 (2CH$_3$), 37.6 (CH$_2$), 34.4 (CH$_2$), 27.5 (CH$_2$), 14.1 (CH$_3$).

(IS*,3S*)-1-(Dimethylamino)cyclopentane-1,3-dicarboxylic acid (20)

To a solution of compound 18 (92.4 mg, 0.38 mmol) in THF (1.5 mL) was added 1 M aq. NaOH (1.5 mL) and the resulting mixture was left to stir at RT overnight. THF was removed and the crude product was purified by ion-exchange column chromatography, using Dowex® 1X8 (chloride form, 50-100 mesh) basic exchange resin. The crude product was added as a solution in 1 M aq. NH$_3$, and after washing with demineralized water (120 mL), the product was eluted from the column with 1 M HCl solution to give the HCl salt of 20 as a white solid (87.5 mg, 97%). $^1$H NMR (D$_2$O) δ 3.00 (ddd, J = 8.4, 8.4, 16.4 Hz, 1H), 2.74 (s, 3H), 2.71 (s, 3H), 2.45 (dd, J = 8.4, 15.2 Hz, 1H), 2.32-2.25 (m, 2H), 2.13-1.83 (m, 3H). $^{13}$C NMR (D$_2$O) δ 181.6 (C), 178.0 (C), 70.2 (C), 43.2 (CH), 40.0 (CH$_3$), 39.5 (CH$_3$), 35.2 (CH$_2$), 31.8 (CH$_2$), 28.9 (CH$_2$). MS m/z 202 (M+1, 100%). HRMSESI m/z 202.1077 (MH$^+$), calcd for C$_9$H$_{16}$NO$_4$ (MH$^+$) 202.1077.

4.3.7 General procedure for preparation of compound 21

To a solution of compound 15 (0.470 g, 2.22 mmol) in dry THF (10 mL) was added the appropriate aldehyde (2.22 mmol) and powdered 5Å molecular sieves (2-3 g). The reaction mixture was left to stir at RT. Upon completion, the reaction mixture was filtered through a pad of celite. THF was removed under reduced pressure to give a yellow oil which was diluted with dry methanol (12 mL). To the resulting solution was added NaCNBH$_3$ (0.280 g, 4.44 mmol). The pH of the reaction mixture was adjusted to pH = 4, by the addition of acetic acid. The reaction mixture was left to stir at RT for 20 h. The mixture was diluted with H$_2$O (20 mL) and made basic by adding solid Na$_2$CO$_3$. The aqueous solution was extracted with CH$_2$Cl$_2$ and the combined extracts were dried over K$_2$CO$_3$. The solvent was removed to give a yellow oil which was purified by column chromatography (MeOH/ethyl acetate, 1:10).
**Rac-3-Ethyl 1-methyl 1-(benzylamino)cyclopent-3-ene-1,3-dicarboxylate (21a)**

A yellow oil (0.538 g, 80%), $^1$H NMR $\delta$ 7.34-7.26 (m, 5H), 6.76 (dd, 2.4, 1.8 Hz, 1H), 4.21 (q, $J = 6.9$ Hz, 2H), 3.78 (s, 3H), 3.66 (s, 2H), 3.19 (dd, $J = 2.4$, 14.7 Hz, 1H), 3.14 (dd, $J = 1.8$, 16.5 Hz, 1H), 2.81 (dd, $J = 2.4$, 14.7 Hz, 1H), 2.70 (dd, $J = 1.8$, 16.5 Hz, 1H), 2.0 (br s, 1H, NH), 1.31 (t, $J = 6.9$ Hz, 3H). $^{13}$C NMR $\delta$ 175.9 (C), 164.0 (C), 139.6 (CH), 139.4 (C), 133.4 (C), 128.0 (2CH), 127.8 (2CH), 126.71 (CH), 68.8 (C), 59.9 (CH$_2$), 51.9 (CH$_3$), 48.5 (CH$_2$), 43.2 (CH$_2$), 41.8 (CH$_2$), 13.8 (CH$_3$). MS (ES+) m/z 304 (M+1, 100%). HRMS ESI m/z 304.1545 (MH$^+$), calcd for C$_{17}$H$_{22}$NO$_4$ (MH$^+$) 304.1543.

**Rac-3-Ethyl 1-methyl 1-(pyridin-2-ylmethylamino)cyclopent-3-ene-1,3-dicarboxylate (21b)**

A yellow oil (0.436 g, 80%), $^1$H NMR $\delta$ 8.58-8.50 (m, H), 7.71-7.59 (m, 2H), 7.30-7.18 (m, 2H), 6.68 (br s, 1H), 4.18 (q, $J = 7.2$ Hz, 2H), 3.8 (s, 2H), 3.73 (s, 3H), 3.18 (ddd, $J = 2.1$, 16.5, 16.5 Hz, 2H), 2.82 (dd, $J = 1.5$, 16.5 Hz, H), 2.72 (dd, $J = 1.5$, 16.5 Hz, H), 1.28 (t, $J = 7.2$ Hz, 3H). $^{13}$C NMR $\delta$ 175.7 (C), 164.5 (C), 159.0 (C), 149.2 (CH), 140.0 (CH), 136.7 (CH), 133.9 (C), 122.4 (CH), 122.2 (CH), 68.9 (C), 60.4 (CH$_2$), 52.5 (CH$_3$), 50.0 (CH$_2$), 43.8 (CH$_2$), 42.3 (CH$_2$), 14.4 (CH$_3$). MS (ES+) m/z 305.35 (M+1, 100%). HRMS ESI m/z 305.1496 (MH$^+$), calcd for C$_{16}$H$_{21}$N$_2$O$_4$ (MH$^+$) 305.1496.

**Rac-3-Ethyl 1-methyl 1-(pyridin-3-ylmethylamino)cyclopent-3-ene-1,3-dicarboxylate (21c)**

A yellow oil (0.267 g, 58%), $^1$H NMR $\delta$ 8.49 (d, $J = 1.5$ Hz, 1H), 8.43 (dd, $J = 1.5$, 4.8 Hz, 1H), 7.63 (dd, $J = 1.5$, 7.8 Hz, 1H), 7.19 (dd, $J = 4.8$, 7.8 Hz, 1H), 6.62 (t, 1.5 Hz, 1H), 4.14 (q, $J = 6.9$ Hz, 2H), 3.72 (s, 3H), 3.61 (s, 2H), 3.10 (ddd, $J = 1.5$, 16.5, 16.5 Hz, 2H), 2.72 (dd, $J = 1.5$, 16.5 Hz, 1H), 2.60 (dd, $J = 1.5$, 16.5 Hz, 1H), 2.22 (br s, 1H, NH), 1.23 (t, $J = 6.9$ Hz, 3H). $^{13}$C NMR $\delta$ 175.7 (C), 164.4 (C), 149.7 (CH), 148.5 (CH), 139.8 (CH), 136.0 (CH), 135.5 (C), 133.9 (C), 123.4 (CH), 69.0 (C), 60.4 (CH$_2$), 52.5 (CH$_3$), 46.3 (CH$_2$), 43.7 (CH$_2$), 42.3 (CH$_2$), 14.3 (CH$_3$). MS (ES+) m/z 305 (M+1, 100%). HRMS ESI m/z 305.1497 (MH$^+$), calcd for C$_{16}$H$_{21}$N$_2$O$_4$ (MH$^+$) 305.1496.
Rac-3-Ethyl 1-methyl 1-(3-hydroxybenzylamino)cyclopent-3-ene-1,3-dicarboxylate (21d)

A yellow oil (0.163 g, 81%), $^1$H NMR $\delta$ 7.12 (t, $J = 7.8$ Hz, 1H), 6.80 (d, $J = 7.8$ Hz, 1H), 6.73 (s, 1H), 6.68 (d, $J = 7.8$ Hz, 1H), 6.67 (t, $J = 2.1$ Hz, 1H), 4.17 (q, $J = 6.9$ Hz, 2H), 3.75 (s, 3H), 3.52 (s, 2H), 3.13 (dd, $J = 2.1$, 16.9 Hz, 2H), 2.79 (d, $J = 16.2$ Hz, 1H), 2.69 (dd, $J = 2.1$, 16.9 Hz, 1H), 1.27 (t, $J = 6.9$ Hz, 3H). $^{13}$C NMR $\delta$ 176.1 (C), 165.0 (C), 156.8 (C), 140.8 (C), 140.5 (CH), 133.8 (C), 129.9 (CH), 120.2 (CH), 115.8.0 (CH), 114.9 (CH), 69.2 (C), 60.1 (CH$_2$), 52.8 (CH$_3$), 49.1 (CH$_2$), 43.7 (CH$_2$), 42.2 (CH$_2$), 14.4 (CH$_3$). MS (Cl) $m/z$ 319.36 (M, 100%). HRMSESI $m/z$ 320.1499 (MH$^+$), calcd for C$_{17}$H$_{22}$NO$_5$ (MH$^+$) 320.1498.

Rac-3-Ethyl 1-methyl 1-(4-hydroxybenzylamino)cyclopent-3-ene-1,3-dicarboxylate (21e)

A yellow oil (0.104 g, 69%), $^1$H NMR $\delta$ 7.10 (d, $J = 8.4$ Hz, 2H), 6.68 (t, $J = 2.1$ Hz, 1H), 6.67 (d, $J = 8.4$ Hz, 2H), 4.17 (q, $J = 7.2$ Hz, 2H), 3.75 (s, 3H), 3.52 (s, 2H), 3.13 (ddd, $J = 2.1$, 16.6 Hz, 2H), 2.79 (d, $J = 16.6$ Hz, 1H), 2.69 (dd, $J = 2.1$, 16.6 Hz, 1H), 1.27 (t, $J = 7.2$ Hz, 3H). $^{13}$C NMR $\delta$ 174.1 (C), 164.9 (C), 160.3 (C), 140.5 (CH), 133.8 (C), 130.8 (2CH), 127.6 (C), 116.0 (2CH), 75.0 (C), 68.1 (CH$_2$), 60.9 (CH$_2$), 53.0 (CH$_3$), 44.6 (CH$_2$), 42.6 (CH$_2$), 14.4 (CH$_3$). MS (ES+) $m/z$ 320 (M+1, 100%). HRMSESI $m/z$ 320.1498 (MH$^+$), calcd for C$_{17}$H$_{22}$NO$_5$ (MH$^+$) 320.1498.

4.3.8 Representative procedure for hydrolysis of compound 21

Compound 21a (0.106 g, 0.35 mmol) in 10% aq. HCl (3 mL) was heated at 80 °C for 16 h. After cooling, the reaction mixture was diluted with water (2 mL) and washed with ether (5 mL). The water was removed under reduced pressure to give the HCl salt of 22a as a white solid (102 mg, 98%).

Rac-1-(Benzyllamino)cyclopent-3-ene-1,3-dicarboxylic acid (22a)
A white solid (0.102 g, 98%), $^1$H NMR (D$_2$O) 7.38 (s, 5H), 6.74 (br s, 1H), 4.11 (s, 2H), 3.31 (d, $J = 18.0$ Hz, 2H), 3.04 (d, $J = 18.0$ Hz, 2H). $^{13}$C NMR (D$_2$O) δ 169.7 (C), 163.7 (C), 137.8 (CH), 129.5 (C), 127.4 (C), 126.6 (2CH), 126.5 (CH), 126.1 (2CH), 65.6 (C), 44.5 (CH$_2$), 38.5 (CH$_2$), 36.6 (CH$_2$). MS (Cl) m/z 262 (M+1, 100%). HRMSESI m/z 262.1078 (MH$^+$), calcd for C$_{14}$H$_{16}$NO$_4$ (MH$^+$) 262.1074.

**Rac-1-(Pyridin-2-ylmethylamino)cyclopent-3-ene-1,3-dicarboxylic acid (22b)**

A white solid (0.202 g, 100%), $^1$H NMR (D$_2$O) δ 8.65 (d, $J = 1.5$, 7.8 Hz, 1H), 8.42 (ddd, $J = 1.5$, 7.8, 7.8 Hz, 1H), 7.98 (d, $J = 7.8$ Hz, 1H), 7.88 (ddd, $J = 1.5$, 7.8, 7.8 Hz, 1H), 6.63 (br s, 1H), 4.51 (s, 2H), 3.24 (br d, $J = 17.4$ Hz, 2H), 3.01-2.92 (m, 2H). $^{13}$C NMR (D$_2$O) δ 172.3 (C), 166.6 (C), 147.9 (CH), 144.5 (C), 143.1 (CH), 140.8 (CH), 132.62 (C), 128.8 (CH), 128.2 (CH), 69.9 (C), 43.8 (CH$_2$), 41.8 (CH$_2$), 40.0 (CH$_2$). MS (ES$^+$) m/z 263 (M+1, 100%). HRMSESI m/z 263.1033 (MH$^+$), calcd for C$_{13}$H$_{15}$N$_2$O$_4$ (MH$^+$) 263.1032.

**Rac-1-(Pyridin-3-ylmethylamino)cyclopent-3-ene-1,3-dicarboxylic acid (22c)**

A white solid (0.208 g, 98%), $^1$H NMR (D$_2$O) δ 8.76 (s, 1H), 8.65 (d, $J = 5.7$ Hz, 1H), 8.55 (dd, $J = 1.5$, 8.1 Hz, 1H), 7.93 (dd, $J = 5.7$, 8.1 Hz, 1H), 6.58 (br s, 1H), 4.35 (s, 2H), 3.19 (d, $J = 16.8$ Hz, 2H), 2.96 (d, $J = 17.7$ Hz, 1H), 2.91 (d, $J = 17.7$ Hz, 1H). $^{13}$C NMR (D$_2$O) δ 172.1 (C), 166.4 (C), 148.8 (CH), 142.5 (CH), 142.3 (CH), 140.8 (CH), 132.5 (C), 131.1 (C), 128.0 (CH), 69.4 (C), 43.8 (CH$_2$), 41.6 (CH$_2$), 39.8 (CH$_2$). MS (ES$^+$) m/z 263 (M+1, 100%). HRMSESI m/z 263.1033 (MH$^+$), calcd for C$_{13}$H$_{15}$N$_2$O$_4$ (MH$^+$) 263.1032.

**Rac-1-(3-Hydroxybenzylamino)cyclopent-3-ene-1,3-dicarboxylic acid (22d)**

A yellow solid (0.113 g, 92%), $^1$H NMR (D$_2$O) δ 7.20 (t, $J = 7.5$ Hz, 1H), 6.86 (d, $J = 7.5$ Hz, 1H), 6.80 (d, $J = 7.5$ Hz, 1H), 6.79 (s, 1H), 6.69 (br s, 1H), 3.98 (s, 2H), 3.26 (d, $J = 17.6$ Hz, 2H), 2.96 (d, $J = 16.8$ Hz, 2H). $^{13}$C NMR (D$_2$O) δ 172.4 (C), 166.7 (C), 156.0 (C), 140.9 (CH), 132.6 (C), 132.1 (C), 130.8 (CH), 121.8 (CH), 116.7 (CH), 116.6 (CH) 68.4 (C), 47.4 (CH$_2$), 41.6 (CH$_2$), 39.7 (CH$_2$). MS (Cl) m/z 277.10 (M, 100%). HRMSESI m/z 278.1025 (MH$^+$), calcd for C$_{14}$H$_{16}$NO$_5$ (MH$^+$) 278.1023.
Rac-1-(4-Hydroxybenzylamino)cyclopent-3-ene-1,3-dicarboxylic acid (22e)

A yellow solid (72.0 mg, 95%), $^1$H NMR (D$_2$O) $\delta$ 7.36 (d, $J$ = 8.1 Hz, 2H), 6.64 (d, $J$ = 8.1 Hz, 2H), 6.73 (br s, 1H), 4.13 (s, 2H), 3.36-3.30 (m, 2H), 3.20-3.11 (m, 2H). $^{13}$C NMR (CD$_3$OD) $\delta$ 172.5 (C), 166.5 (C), 159.5 (C), 140.5 (CH), 134.6 (C), 132.8 (2CH), 122.8 (C), 116.7 (2CH), 69.5 (C), 48.1 (CH$_2$), 42.8 (CH$_2$), 41.3 (CH$_2$). MS (CI) m/z 277.10 (M, 100%). HRMSESI m/z 278.1026 (MH$^+$), calcd for C$_{14}$H$_{15}$NO$_5$ (MH$^+$) 278.1023.

4.3.9 Signal transduction at mGlu2 receptors in CHO cells

Human mGluR2 (cloned and expressed in house) were grown in DMEM/Glutamax-I to which 2 mM glutamine, 46 mg/L proline, and 10% dialyzed fetal calf serum were added.

4.3.10 $[^{35}$S]$GTP\gamma$S radioligand binding assay for human mGlu2

Membrane preparation. Cells were grown to confluence. Cells were washed twice with ice-cold PBS without Ca$^{2+}$ and Mg$^{2+}$, scraped off and homogenized in buffer (EDTA mM, Hepes 20 mM). After centrifugation (18,000 rpm, 15 min, 4 °C), the pellet was washed with 0.1 mM EDTA, 20 mM Hepes, and resuspended in the same buffer for protein determination with the Biorad assay. Membrane aliquots were stored at -70 °C.

$[^{35}$S]$GTP\gamma$S radioligand binding. Each incubate contained 10 μg of membrane protein in 250 μL of binding buffer (HEPES 20 mM, NaCl 100 mM, MgCl$_2$ 3 mM, GDP 3 μM, pH 7.4). The incubation was started by addition of an appropriate concentration of agonist and/or antagonist. Compounds were incubated with the membranes at 37 °C for 30 min. Subsequently, 0.1 nM $[^{35}$S]$GTP\gamma$S (approximately 2 x 10$^5$ DPM) was added in the presence or absence of 30 mM glutamate. The mixture was further incubated for 30 min at 37 °C. The reactions were terminated by separating free and bound radioactivity by rapid vacuum filtration using a Packard filtration manifold through GF/B pre-wetted glass fiber filters.

Filters were rapidly washed two times with cold 10 mM NaH$_2$PO$_4$/10 mM Na$_2$HPO$_4$ buffer, pH 7.4. Filters were transferred to vials for subsequent counting in a scintillation counter. Results are expressed as % of glutamate-induced response, the latter being
defined as 100%. Glutamate and amino acids were dissolved and diluted in water. Concentration–response curves were drawn on a logarithmic scale. Sigmoidal curves of best fit were calculated by nonlinear regression analysis using GraphPad software (San Diego, CA). The pIC\textsubscript{50}-value referred to the concentration of a compound producing half the maximum response.

4.3.11 X-ray crystallographic data

Single crystal X-ray diffraction data were collected at 150 K (8b,7c) or 298 K (8a) on a Bruker Smart diffractometer using Mo-Kα radiation (\(\lambda=0.71073\) Å). Following solution by direct methods, the structures were refined against \(F^2\) with full-matrix least-squares using the program SHELXL-97\textsuperscript{15}. All hydrogen atoms were added at calculated positions and refined by use of riding models with isotropic displacement parameters based on those of the parent atoms. Except for the minor components of the disordered atoms in (8b), anisotropic displacement parameters were employed throughout for the non-hydrogen atoms. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 899273-899275. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Crystal data: compound 8a, C\textsubscript{24}H\textsubscript{25}NO\textsubscript{4}; monoclinic, space group \(P2_1/c\), \(a = 16.9344(19)\), \(b = 8.8127(10)\), \(c = 16.1275(18)\) Å, \(\beta = 117.393(2)\)°, \(V = 2137.0(4)\) Å\(^3\), \(Z = 4\), \(D_c = 1.217\) g cm\(^{-3}\). 15529 reflections collected, 5280 independent (\(R_{int} = 0.034\)). Final \(R\) indices, \(R1 = 0.0428\) (for \(I > 2\sigma(I)\)), \(wR2 = 0.1223\) (all reflections), \(S = 0.876\).

Compound 8b, C\textsubscript{26}H\textsubscript{27}NO\textsubscript{4}; monoclinic, space group \(P2_1/c\), \(a = 16.035(4)\), \(b = 9.114(2)\), \(c = 16.769(4)\) Å, \(\beta = 117.079(4)\)°, \(V = 2182.0(9)\) Å\(^3\), \(Z = 4\), \(D_c = 1.271\) g cm\(^{-3}\). 24889 reflections collected, 3750 independent (\(R_{int} = 0.056\)). Final \(R\) indices, \(R1 = 0.0930\) (for \(I > 2\sigma(I)\)), \(wR2 = 0.2330\) (all reflections), \(S = 1.157\). The allyl group was modelled as being disordered over two sets of sites with occupancies refined to 0.773(13) and its complement.

Compound 7c, C\textsubscript{30}H\textsubscript{29}NO\textsubscript{4}; triclinic, space group \(\text{P}\bar{1}\), \(a = 6.0737(16)\), \(b = 12.605(3)\), \(c = 17.285(5)\) Å, \(\alpha = 70.961(4)\), \(\beta = 81.126(4)\), \(\gamma = 84.888(4)\)°, \(V = 1234.9(6)\) Å\(^3\), \(Z = 2\), \(D_c = 1.257\) g cm\(^{-3}\). 14317 reflections collected, 6006 independent (\(R_{int} = 0.029\)). Final \(R\) indices, \(R1 = 0.0475\) (for \(I > 2\sigma(I)\)), \(wR2 = 0.1245\) (all reflections), \(S = 0.947\).
Acknowledgements

We thank Johnson and Johnson Research Pty Limited (Sydney, Australia) for financial support and Prof Susan Pond (Johnson & Johnson Research), Dr. Vic Sipido, and Dr. John Stuart Andrews (Janssen Pharmaceutica, Belgium) for their encouragement and support.

References and Notes

11. CCDC deposition numbers for compounds 7c, 8a and 8b are 899273, 899274 and 899275, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).


**Graphical Abstract**

**Synthesis and Inhibitory Activities at mGluRs of 3-Alkylated and N-Alkylated Cyclopentyl-Glutamate Analogues**

Alison T. Ung,* Stephen G. Pyne, François Bischoff, Anne S. J. Lesage, Brian W. Skelton and Allan H. White
Schemes 1-5

Scheme 1

Scheme 2
Scheme 3
Legends for schemes and tables

**Scheme 1.** Alkylation of α,β-unsaturated ester.

**Scheme 2.** Synthesis of racemic 9 and 10. Reagents and conditions (a) KN(TMS)$_2$, THF, -78 °C; RI or RBr; (b) 10% aq. HCl, reflux.

**Scheme 3.** Synthesis of racemic 13 and 14. Reagents and conditions (a) (i) 1 M HCl, ether, RT, 16 h, (ii) Pd/C, H$_2$, MeOH, RT, (b) 10% aq. HCl, reflux.

**Scheme 4.** Synthesis of racemic 17 and 20. Reagents and conditions (a) HCHO, NaBH$_3$CN, acetic acid, CH$_3$CN (b) 10% aq. HCl, reflux, (c) Mg turnings, dry CH$_3$OH, RT.

**Scheme 5.** Synthesis of racemic 22. Reagents and conditions (a) (i) RCHO, THF, powdered molecular sieves (5 Å), RT, (ii) NaBH$_3$CN, acetic acid, MeOH; (b) 10% aq. HCl, reflux.
Table 1 Isolated yields (%) of compounds 7 and 8.

Table 2 Isolated yields (%) of compounds 9 and 10.

Table 3 Isolated yields (%) of compounds 11, 12, 13 and 14.

Table 4 Isolated yields (%) of compounds 21 and 22.

Table 5 Agonist and antagonist activities of synthesised compounds.
Table 1 Isolated yields (%) of compounds 7 and 8.

Table 2 Isolated yields (%) of compounds 9 and 10.

Table 3 Isolated yields (%) of compounds 11, 12, 13 and 14.

Table 4 Isolated yields (%) of compounds 21 and 22.

Table 5 Agonist and antagonist activities of synthesised compounds.
### Tables 1-5

#### Table 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>7 (%)</th>
<th>8 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (R = Me)</td>
<td>48</td>
<td>6</td>
</tr>
<tr>
<td>b (R = CH_2CHCH_2)</td>
<td>45</td>
<td>6</td>
</tr>
<tr>
<td>c (R = Bn)</td>
<td>77</td>
<td>11</td>
</tr>
<tr>
<td>d [R = (CH_2)_3Ph]</td>
<td>36</td>
<td>1.6</td>
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#### Table 2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (R = Me)</td>
<td>86</td>
<td>80</td>
</tr>
<tr>
<td>b (R = CH_2CHCH_2)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>c (R = Bn)</td>
<td>94</td>
<td>98</td>
</tr>
<tr>
<td>d [R = (CH_2)_3Ph]</td>
<td>63</td>
<td>76</td>
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</tbody>
</table>
Table 3

<table>
<thead>
<tr>
<th>Compounds</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (R = Me)</td>
<td>83</td>
<td>na*</td>
<td>85</td>
<td>na*</td>
</tr>
<tr>
<td>b (R = Pr)</td>
<td>94</td>
<td>93</td>
<td>99</td>
<td>na*</td>
</tr>
<tr>
<td>c (R = Bn)</td>
<td>66</td>
<td>70</td>
<td>86</td>
<td>85</td>
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</table>

*na* : not attempted

Table 4

<table>
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<tr>
<th>Compounds</th>
<th>21</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (R = Ph)</td>
<td>80</td>
<td>98</td>
</tr>
<tr>
<td>b (R = 2-pyridinyl)</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>c (R = 3-pyridinyl)</td>
<td>58</td>
<td>98</td>
</tr>
<tr>
<td>d [R = 3-(OH)C₆H₄]</td>
<td>81</td>
<td>92</td>
</tr>
<tr>
<td>e [R = 4-(OH)C₆H₄]</td>
<td>69</td>
<td>95</td>
</tr>
<tr>
<td>Compounds</td>
<td>mGlu2-ago-AC, EC&lt;sub&gt;50&lt;/sub&gt; ± SD in μM (E&lt;sub&gt;max&lt;/sub&gt;%)</td>
<td>mGlu2-ago-GTP, EC&lt;sub&gt;50&lt;/sub&gt; ± SD in μM (E&lt;sub&gt;max&lt;/sub&gt;%)</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------------------------------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>rac-3</td>
<td>73</td>
<td>55 ± 14 (71%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(S)-3</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45 ± 10 (38%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>rac-9a</td>
<td>387 (53%)</td>
<td>partial ago&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>rac-13b</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>rac-14c</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>rac-17</td>
<td>3(51%)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>rac-20</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.7 ± 0.5 (34%)</td>
</tr>
<tr>
<td>rac-22b</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;100</td>
</tr>
<tr>
<td>rac-22c</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values from reference 7.  
<sup>b</sup>nd: not determined.  
<sup>c</sup>No full dose response.
Figure(s) (use if uploading high quality figure files)

Fig. 1

\[(1S,3R)-1\] \(\text{CO}_2\text{H}\)
\[\text{H}_2\text{N}^+\text{CO}_2\text{H}\]

\[(1S,3S)-2\] \(\text{CO}_2\text{H}\)
\[\text{H}_2\text{N}^+\text{CO}_2\text{H}\]

\[(S)-3\] \(\text{CO}_2\text{H}\)
\[\text{H}_2\text{N}^+\text{CO}_2\text{H}\]

\[\text{rac-4}\] \(\text{CO}_2\text{H}\)
\[\text{H}_2\text{N}^+\text{CO}_2\text{H}\]

---

Fig. 2

\(\text{NOESY cross-peaks [Spatan Pro. generated structure (AM1)]}\)
Fig. 5

Fig. 6
NOESY cross-peaks [Spartan Pro. generated structure (AM1)]

Fig. 7

16 $\xrightarrow{\text{Mg, MeOH}}$ Mg(II) complex B $\xrightarrow{\text{H}^+}$ 18

Fig. 8
Fig. 9

Agonism:
- $E_{\text{max}} = 34\%$
- $EC_{50} = 9.7 \pm 0.5 \mu M$

Antagonism:
- $IC_{50} = 47 \mu M (66\%)$
Fig. 10
Legends for figures 1-10

Figure 1. Conformationally restricted glutamate analogues.

Figure 2. NOESY correlations for compound 7a.

Figure 3. ORTEP diagram of the structure of compound 8a. Ellipsoids have been drawn at the 20% probability level.

Figure 4. ORTEP diagram of the structure of compound 8b. Ellipsoids have been drawn at the 30% probability level. Minor components of disordered atoms were omitted for clarity.

Figure 5. ORTEP diagram of the structure of compound 7c. Ellipsoids have been drawn at the 30% probability level.

Figure 6. Suggested mechanism leading to the stereochemistry of compound 7.

Figure 7. NOESY correlations for compound 18.

Figure 8. Suggested mechanism leading to the stereochemistry of compound 18.

Figure 9. Effect of 20 on human mGluR2: partial agonist and antagonist activity in $[^{35}\text{S}]\text{GTP}_{\gamma}\text{S}$ binding.

Figure 10. Antagonist activity of 13b and antagonist activity of 22b at mGluR2.