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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 (IC_{50} 7.7 μM) at mGluR2. Amongst the *N*-alkylated analogues, compound **20** was found to be a partial agonist (EC_{50} 9.5 μM) and as well as an antagonist (IC_{50} 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 v*ia* 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-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 μM) than at other subtypes.[6]

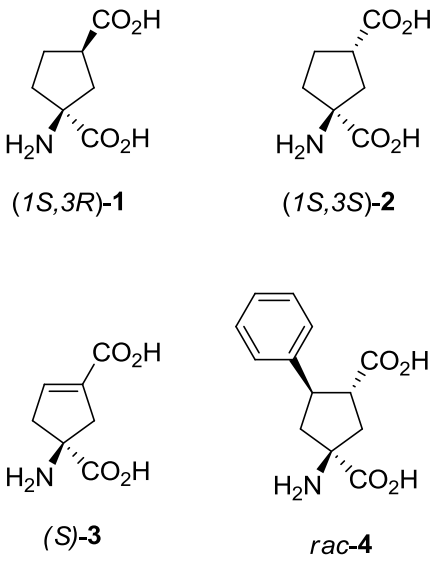
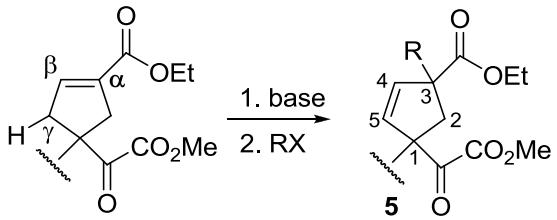


Figure 1. Conformationally restricted glutamate analogues.

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_{50} 18 μM) and mGluR2 (EC_{50} 45 μM) receptors.[7] We also reported the synthesis of the 4-phenyl substituted derivative, *rac*-**4,** and its aryl substituted derivatives. Compound *rac*-**4** is a selective antagonist at mGluR2 with modest activity with an IC_{50} value of 32 μM.[8]

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

The synthesis of the 3-alkylated compounds can be achieved by regioselective deprotonation at the γ-position of the α,β–unsaturated ester moiety of protected derivatives of *rac*-**3** by a strong base to generate the corresponding extended enolate which undergoes regioselective alkylation at the α-position to give 3-alkylated products **5** (Scheme 1).[9]



Scheme 1. Alkylation of α,β–unsaturated ester.

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

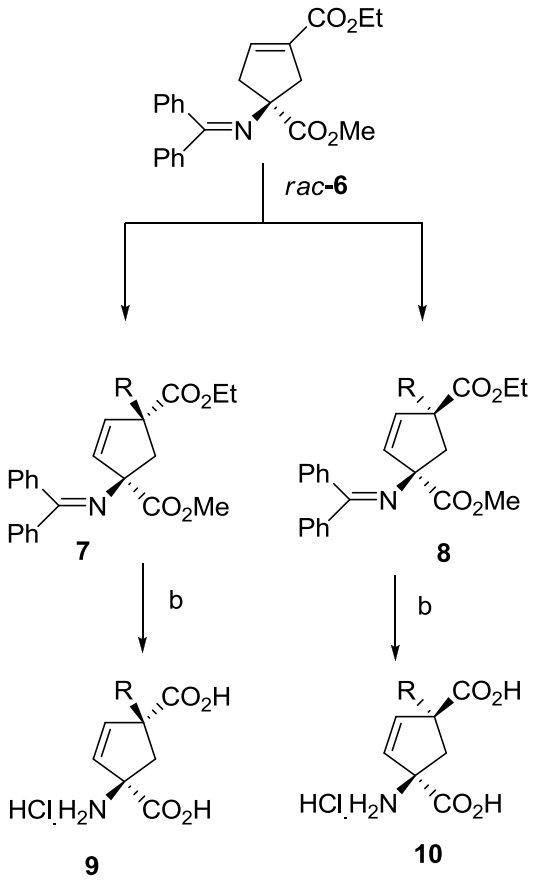
2. Results and discussion

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**[7] with two equivalent of $KN(TMS)_2$ at -78 ^{o}C,

followed by the addition of alkylating agents gave exclusively the α–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 α–alkylation of lithium dienolates derived from carbonyl esters.[10] 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 ^oC; RI or RBr; (b) 10% aq. HCl, reflux.

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

Compounds	**7** (%)	**8** (%)
a (R = Me)	48	6
b (R = CH_2CHCH_2)	45	6
c (R = Bn)	77	11
d [R = $(CH_2)_3Ph$]	36	1.6

^{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 (δ 5.89, d, J = 4.2 Hz) and H-5 resonance (δ 5.71, d, J = 4.2 Hz). The CH_3-3 resonance at δ 1.49 of the major isomer **7a** appeared more downfield than the corresponding resonance (δ 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-2α, H-2α 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).[11] Suitable crystals for X-ray analysis of compounds **8a**, **8b** and **7d** were grown from a mixture of ethyl acetate and hexane.

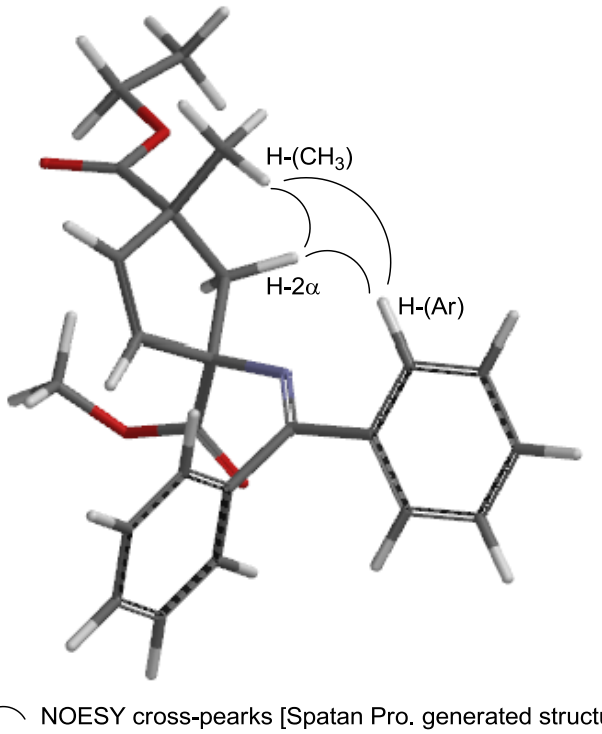


Figure 2. NOESY correlations for compound **7a**.

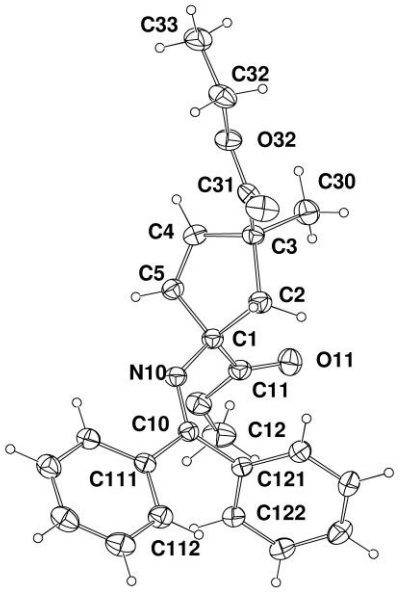


Figure 3. ORTEP diagram of the structure of compound **8a**. Ellipsoids have been drawn at the 20% probability level.

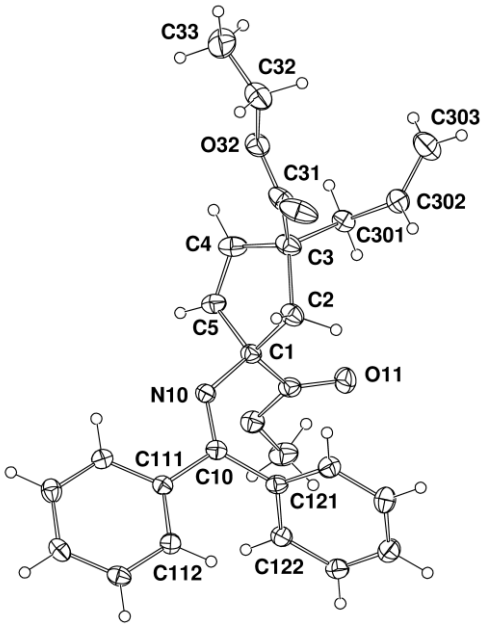


Figure 4. ORTEP diagram of the structure of compound **8b**. Ellipsoids have been drawn at the 30% probability level. Minor components of disordered atoms were omitted for clarity.

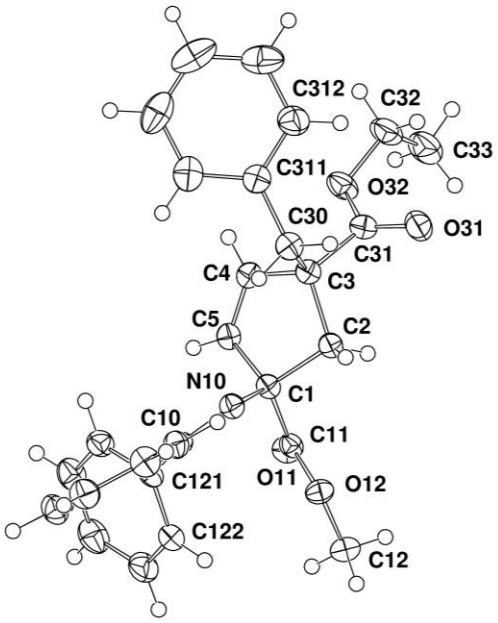


Figure 5. ORTEP diagram of the structure of compound **7c**. Ellipsoids have been drawn at the 30% probability level.

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.

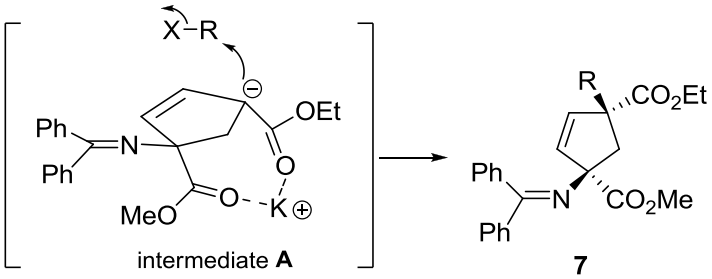


Figure 6. Suggested mechanism leading to the stereochemistry of compound **7**.

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).

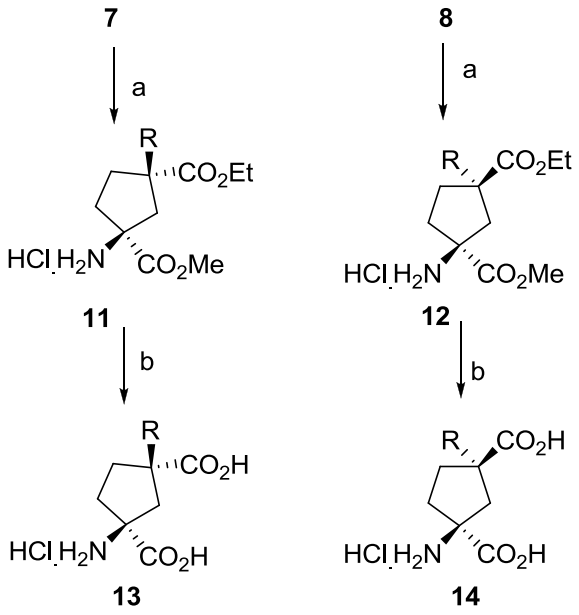
Table 2 Isolated yields (%) of compounds **9** and **10**.

Compounds	**9**	**10**
a (R = Me)	86	80
b (R = CH_2CHCH_2)	100	100
c (R = Bn)	94	98
d [R = $(CH_2)_3Ph$]	63	76

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, H_2, MeOH, RT, (b) 10% aq. HCl, reflux.

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

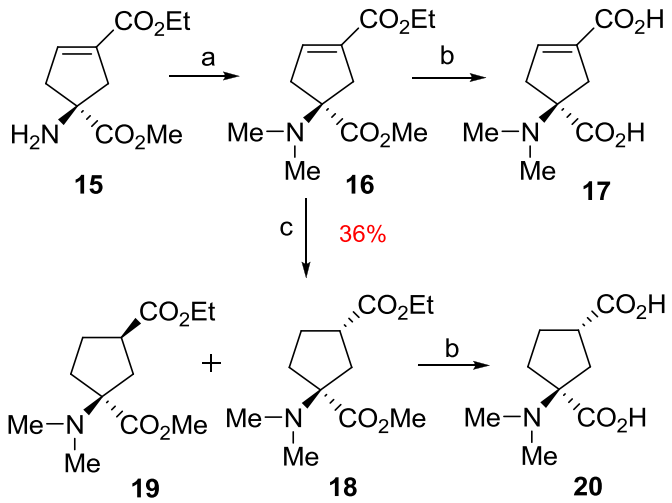
Compounds	**11**	**12**	**13**	**14**
a (R = Me)	83	na[a]	85	na[a]
b (R = Pr)	94	93	99	na[a]
c (R = Bn)	66	70	86	85

[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**[8] with formaldehyde, $NaBH_3CN$, 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, $NaBH_3CN$, acetic acid, CH_3CN (b) 10% aq. HCl, reflux, (c) Mg turnings, dry CH_3OH, 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 δ 2.92 (dq) more downfield than the corresponding resonance (δ 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-3α and H-2α and that for H-2α and H-(CH_3). These confirmed the *cis* orientation of the two carboxyl groups (Fig. 7).

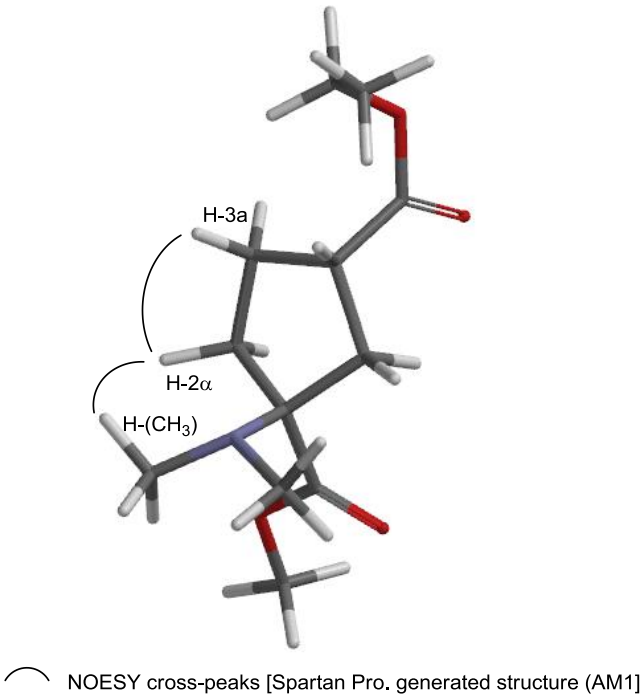


Figure 7. NOESY correlations for compound **18**.

Reduction of α,β–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.[8, 12-14] The *cis* orientation of the two carboxylic groups in **18** is considered to be significant structural features for activities.[6] 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.

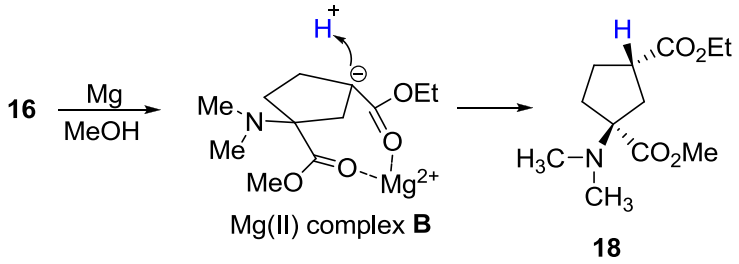
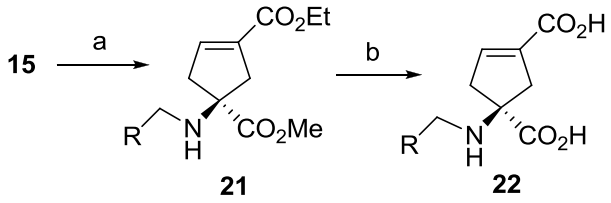


Figure 8. Suggested mechanism leading to the stereochemistry of compound **18**.

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**[8] with various aldehydes in dry THF in the presence of powdered molecular sieves, then followed by the reduction in the presence of $NaBH_3CN$ 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) $NaBH_3CN$, acetic acid, MeOH; (b) 10% aq. HCl, reflux.

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

Compounds	**21**	**22**
a (R = Ph)	80	98
b (R = 2-pyridinyl)	80	100
c (R = 3-pyridinyl)	58	98
d [R = 3-(OH)C_6H_4]	81	92
e [R = 4-(OH)C_6H_4]	69	95

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}S]GTPγS binding assay on membranes from these cells.[5] The results for the agonist and antagonist activities are summarised in Table 5**.**

Table 5 Agonist and antagonist activities of synthesised compounds.

Compounds	mGlu2-ago-AC, EC_{50} ± SD in μM (E_{max}%)	mGlu2-ago-GTP, EC_{50} ± SD in μM (E_{max}%)	mGlu2-antag-GTP, IC_{50} ± SD in μM (E_{max}%)
rac-**3**	73	55 ± 14 (71%)[a]	>300
(S)-**3**	nd[b]	45 ± 10 (38%)[a]	60 (39%)
rac-**9a**	387 (53%)	partial ago[c]	partial anta[c]
rac-**13b**	nd[b]	>1000	7.7 ± 2.1 (71%)
rac-**14c**	nd[b]	nd[b]	200 (67%)
rac-**17**	3(51%)	>100	>100
rac-**20**	nd[b]	9.7 ± 0.5 (34%)	47 (66%)
rac-**22b**	nd[b]	>100	75 ± 11 (100%)
rac-**22c**	nd[b]	>100	>100

[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_{max} of 51% of the maximal glutamate response. However, the agonist activity of **17** was not confirmed in the [^{35}S]GTPγS 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_{50} 9.5 μM and an E_{max} of 34% of the maximal glutamate response. This compound also had inherent partial antagonist activity with an IC_{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).

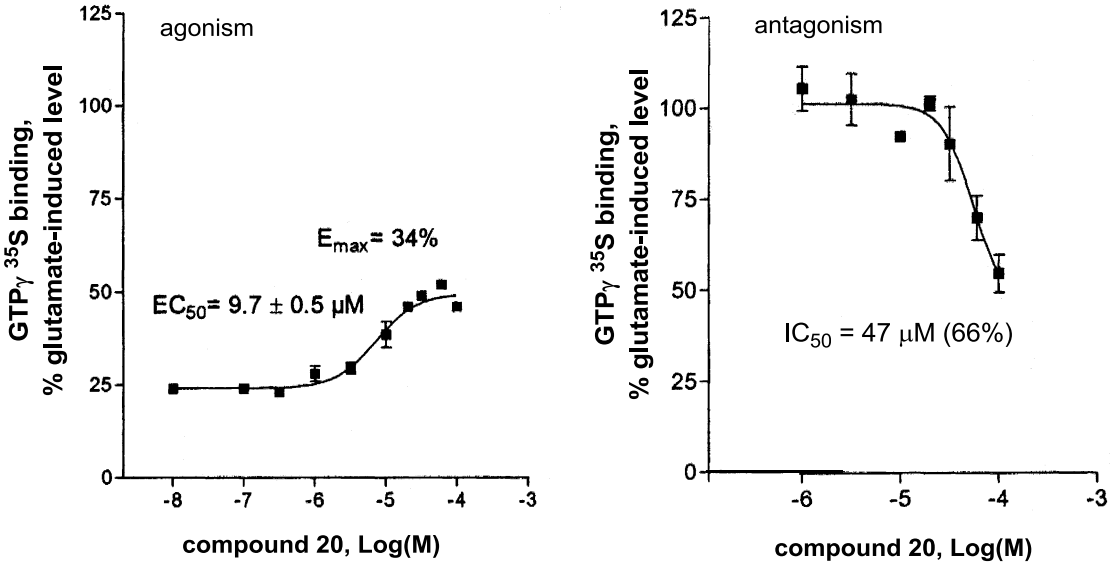


Figure 9. Effect of **20** on human mGluR2: partial agonist and antagonist activity in [^{35}S]GTPγS biding.

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_{50} of 7.7 μM. The concentration-response curve revealed that **13b** was an antagonist with full inhibition of glutamate signalling in the [^{35}S]GTPγS binding assay, obtained from two independent experiments (Fig. 10).

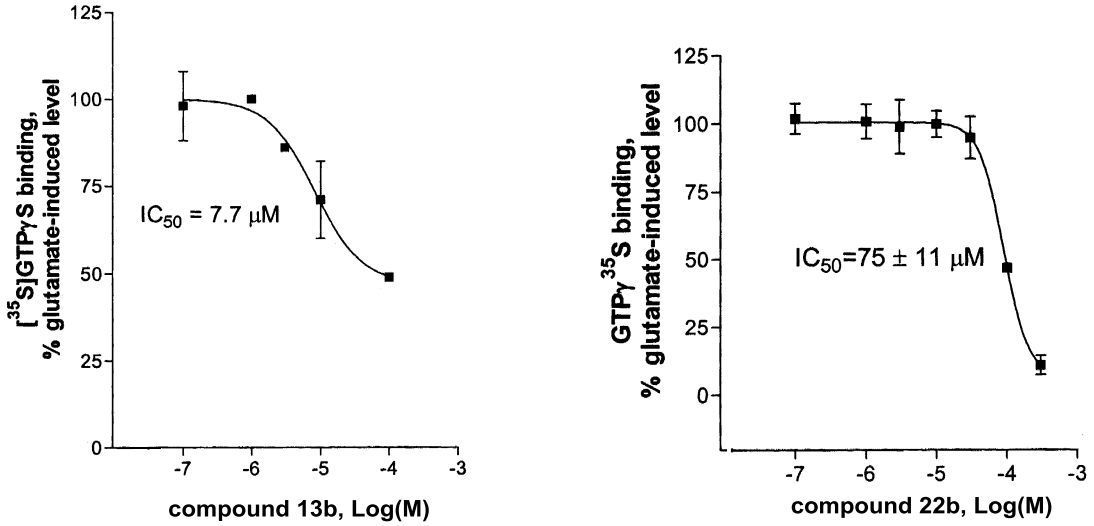


Figure 10. Antagonist activity of **13b** and antagonist activity of **22b** at mGluR2.

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 μM (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 *cis* configuration. This was observed in the compound pair **17** and **20**. The unsaturated analogue **17** showed no activity in the mGluR2 [^{35}S]GTPγS binding assay. Compound **20** was the most active agonist as well as a partial antagonist. This structural feature was also found important in *rac*-**4** and its active analogues and the well-known (*1S,3S*)-ACPA[6]. In summary, our results reveal that the ring flexibility and the *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 (^{1}H NMR) or 75 MHz (^{13}C NMR) in $CDCl_3$ solution. ^{13}C 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

[^{35}S]GTPγS (specific activity 37 MBq/ml) was obtained from Amersham (Little Chalfort, 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 ^{o}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 δ 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 δ 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.

(*1R*,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 δ 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 δ 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. R_f (5% ethyl acetate/ petroleum spirit) 0.36. ^{1}H 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) 4.15-4.05 (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). ^{13}C 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 (CH_2), 78.1 (C), 60.6 (CH_2), 59.3 (C), 51.7 (CH_3), 46.8 (CH_2), 43.9 (CH_2), 14.1 (CH_3). MS (ES^+) *m/z* 418.19 ([MH^+], 100.0%). HRESIMS *m/z* 418.2014 $[MH]^+$, calcd for $C_{26}H_{28}NO_4$ (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%). R_f (5% ethyl acetate/ petroleum spirit) 0.54. ^{1}H 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). ^{13}C 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 (CH_2), 78.1 (C), 60.8 (CH_2), 59.4 (C), 51.9 (CH_3), 45.8 (CH_2), 42.2 (CH_2), 14.3 (CH_3). MS (ES^+) *m/z* 418.19 ([MH^+], 100.0%). HRESIMS *m/z* 418.2013 (MH^+), calcd for $C_{26}H_{28}NO_4$ (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 ^{o}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 $MgSO_4$ 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. 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%). R_f (5% ethyl acetate/ petroleum spirit) 0.35. ^{1}H 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). ^{13}C 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 (CH_2), 60.5 (C), 51.7 (CH_3), 47.4 (CH_2), 45.6 (CH_2), 13.9 (CH_3). MS (ES+) *m/z* 468.0 ([M+H], 100.0%), Anal. calcd for $C_{30}H_{29}NO_4$: 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%). R_f (5% ethyl acetate/ petroleum spirit) 0.65. ^{1}H NMR δ7.82-7.80 (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). ^{13}C 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 (CH_2), 52.0 (CH_3), 48.3 (CH_2), 45.8 (CH_2), 14.0 (CH_3). 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. ^{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). ^{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 (CH_2), 59.2 (C), 51.6 (CH_3), 47.4 (CH_2), 39.1 (CH_2), 35.9 (CH_2), 27.0 (CH_2), 13.9 (CH_3). MS (ES+) *m/z* 496.0 ([MH^+], 100.0%). HRESIMS *m/z* 496.2488 (MH^+), calcd for $C_{32}H_{34}NO_4$ (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. ^{1}H NMR δ 7.56-7.11 (m, 15 H), 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). ^{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 (CH_2), 59.4 (C), 51.8 (CH_3), 46.6 (CH_2), 38.7 (CH_2), 35.9 (CH_2), 27.2 (CH_2), 14.2 (CH_3). MS (ES+) *m/z* 496.0 ([MH^+], 100.0%). HRESIMS *m/z* 496.2488 (MH^+), calcd for $C_{32}H_{34}NO_4$ (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 ^{o}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%).

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

A white solid (0.133 g, 86%). ^{1}H NMR (D_2O) δ 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_2O) δ 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 $C_8H_{12}NO_4$ (MH^+) 186.0761.

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

A white solid (0.124 g, 80%). ^{1}H NMR (D_2O) δ 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_2O) δ 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 ([MH^+], 60%). HRESIMS *m/z* 186.0763 (MH^+), calcd for $C_8H_{12}NO_4$ (MH^+) 186.0761.

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

A pale yellow solid (0.170 g, 100%). ^{1}H NMR (D_2O) δ 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_2O) δ 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.

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

A pale yellow solid (22.1 mg, 100%). ^{1}H NMR (D_2O) δ 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_2O) 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_2O) 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), 2.11 (d, *J* = 14.7 Hz, 1H). ^{13}C NMR (D_2O) δ 174.5 (C), 169.0 (C), 138.8 (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_2O) δ 7.26-7.13 (m, 5H), 6.31 (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_2O) 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_2O) 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_2O) 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.

(*1R*,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_2O) δ 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_2O) δ 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), 37.4 (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 ^{o}C, was slowly added a solution of 1 M aq. HCl (0.5 mL). The reaction mixture was stirred at 0 ^{o}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%).

(*1S*,3S)-3-Ethyl 1-methyl 1-amino-3-methylcyclopentane-1,3-dicarboxylate (11a)**

^{1}H NMR (D_2O) δ 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_2O) δ 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_2O) δ 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), 1.13 – 1.00 (m, 2H), 0.78 (t, *J* = 7.2 Hz, 3H).^{13}C NMR (D_2O) δ 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_3OD) δ 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_2O) δ 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_2O) δ 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_2O) δ 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_2O) δ 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), 2.04-19.4 (m, 2H), 1.16 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (D_2O) 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 ^{o}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%).

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

^{1}H NMR (D_2O) 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_2O) 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 $C_8H_{14}NO_4$ (MH^+) 188.0917.

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

A white solid (77.6 mg, 99%). ^{1}H NMR (D_2O) 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_2O) 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.

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

A white solid (42.0 mg, 86%). ^{1}H NMR (D_2O) 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_2O) 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 (CH_2), 41.3 (CH_2), 31.6 (CH_2), 29.8 (CH_2). MS (ES^+) *m/z* 264 (M+1, 100%). HRMSESI *m/z* 264.1235 (MH^+), calcd for $C_{14}H_{18}NO_4$ (MH^+) 264.1230.

(*1S*,3S)-1-Amino-3-benzylcyclopentane-1,3-dicarboxylic acid (14c)**

A white solid (33.5 mg, 85%). ^1H NMR (D_2O) 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). ^{13}C NMR (D_2O) 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 (CH_2), 39.6 (CH_2), 32.8 (CH_2), 32.0 (CH_2). MS (ES^+) *m/z* 264 (M+1, 100%). HRMSESI *m/z* 264.1233 (MH^+), calcd for $C_{14}H_{18}NO_4$ (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 CH_3CN (30 mL) was added a solution of formaldehyde (40% aq., 11.5 mL, 13.7 mmol) and $NaCNBH_3$ (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 Na_2CO_3 (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 K_2CO_3. 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). ^{13}C NMR δ 173.1 (C), 164.2 (C), 140.0 (CH), 133.9 (C), 74.3 (C), 60.2 (CH_2), 51.7 (CH_3), 40.2 ($2CH_3$), 40.4 (CH_2), 38.8 (CH_2), 14.1 (CH_3). MS (CI) *m/z* 242 (M+1, 100%). HRMSESI *m/z* 242.1392 (MH^+), calcd for $C_{12}H_{20}NO_4$ (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 ^{o}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_2O) 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_2O) 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 (CI) *m/z* 200 (M+1, 100%). HRMSESI *m/z* 200.0923 (MH^+), calcd for $C_9H_{14}NO_4$ (MH^+) 200.0917.

4.3.6 Reduction of compound 16

To a solution of **16** (0.406 g, 1.62 mmol) in dry CH_3OH (30 mL) under a nitrogen atmosphere at 0 ^{o}C, was added Mg turnings (0.393 g, 16.24 mmol). The reaction mixture was left to stir at 0 ^{o}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_2CO_3. The solvent was removed under reduced pressure to give an oil which was purified by PTLC (ethyl aceate/hexane (1:1)) to give compound **18** (0.107 g, 27%) and **19** (35.5 mg, 9%).

(*1S*,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.

(*1S*,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).

(*1S*,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_2O) 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_2O) 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 $C_9H_{16}NO_4$ (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_2O (20 mL) and made basic by adding solid Na_2CO_3. The aqueous solution was extracted with CH_2Cl_2 and the combined extracts were dried over K_2CO_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 δ 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 δ 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%). HRMSESI *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 δ 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 δ 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%). HRMSESI *m/z* 305.1496 (MH^+), calcd for $C_{16}H_{21}N_2O_4$ (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 δ 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 δ 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%). HRMSESI *m/z* 305.1497 (MH^+), calcd for $C_{16}H_{21}N_2O_4$ (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 δ 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 δ 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 (CI) *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 δ 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 δ 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 ^{o}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-(Benzylamino)cyclopent-3-ene-1,3-dicarboxylic acid (22a)**

A white solid (0.102 g, 98%), ^{1}H NMR (D_2O) 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_2O) 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 (CI) *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_2O) δ 8.65 (dd, *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_2O) 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 $C_{13}H_{15}N_2O_4$ (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_2O) 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_2O) 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 $C_{13}H_{15}N_2O_4$ (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_2O) 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_2O) 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 (CI) *m/z* 277.10 (M, 100%). HRMSESI *m/z* 278.1025 (MH^+), calcd for $C_{14}H_{15}NO_5$ (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_2O) δ 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_3OD) δ 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]GTPγS 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 ^{o}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 ^{o}C.

[^{35}S]GTPγS 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 ^{o}C for 30 min. Subsequently, 0.1 nM [^{35}S]GTPγS (approximately 2 × 10^5 DPM) was added in the presence or absence of 30 mM glutamate. The mixture was further incubated for 30 min at 37 ^{o}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_2PO_4/10 mM Na_2HPO_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_{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 (λ=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[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,** $C_{24}H_{25}NO_4$; monoclinic, space group $P2_1/c$, a = 16.9344(19), b = 8.8127(10), c = 16.1275(18) Å, β = 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,** $C_{26}H_{27}NO_4$; monoclinic, space group $P2_1/c$, a = 16.035(4) , b = 9.114(2) , c = 16.769(4) Å, β = 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,** $C_{30}H_{29}NO_4$; triclinic, space group $P\bar{1}$, a = 6.0737(16) , b = 12.605(3) , c = 17.285(5) Å, α = 70.961(4), β = 81.126(4), γ = 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

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References and Notes

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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).

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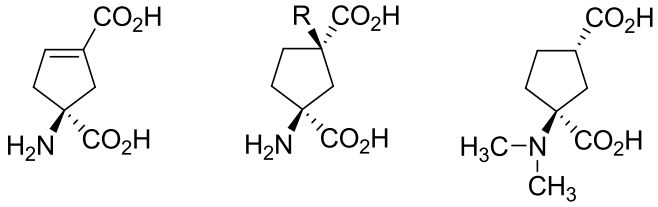
Graphical Abstract

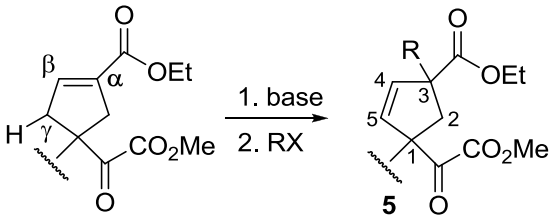
Synthesis and Inhibitory Activities at mGluRs of 3-Alkylated and *N*-Alkylated Cyclopentyl-Glutamate Analogues

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



Table(s)

Schemes 1-5



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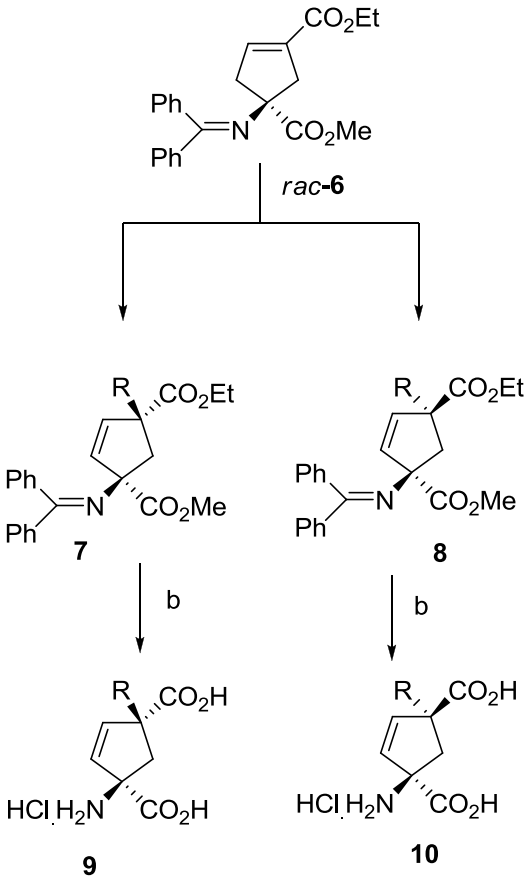

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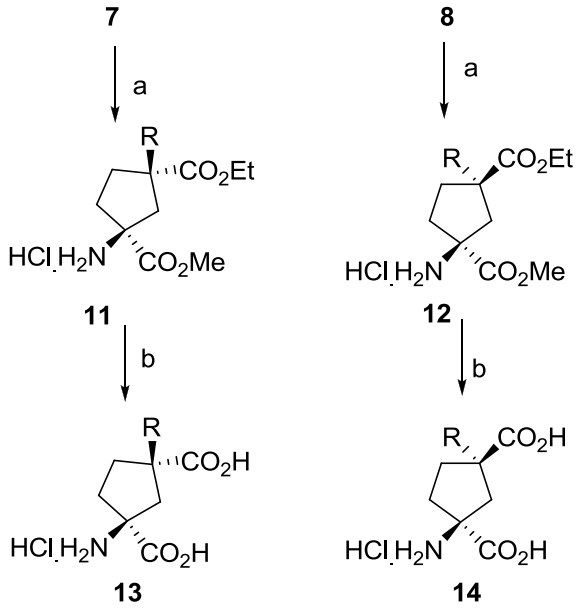
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CO2H
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CO2H
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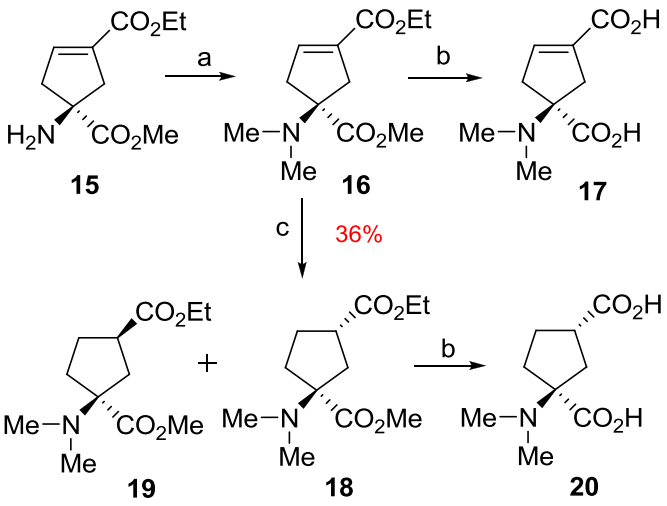
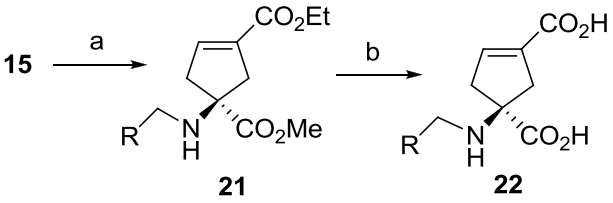
CO2H
14

Scheme 3



Scheme 4



Scheme 5

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 ^oC; 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, $NaBH_3CN$, acetic acid, CH_3CN (b) 10% aq. HCl, reflux, (c) Mg turnings, dry CH_3OH, RT.

Scheme 5. Synthesis of racemic **22**. Reagents and conditions (a) (i) RCHO, THF, powdered molecular sieves (5 Å), RT, (ii) $NaBH_3CN$, 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

Compounds	**7** (%)	**8** (%)
a (R = Me)	48	6
b (R = CH_2CHCH_2)	45	6
c (R = Bn)	77	11
d [R = $(CH_2)_3Ph$]	36	1.6

Table 2

Compounds	**9**	**10**
a (R = Me)	86	80
b (R = CH_2CHCH_2)	100	100
c (R = Bn)	94	98
d [R = $(CH_2)_3Ph$]	63	76

Table 3

Compounds	**11**	**12**	**13**	**14**
a (R = Me)	83	na[a]	85	na[a]
b (R = Pr)	94	93	99	na[a]
c (R = Bn)	66	70	86	85

[a]na : not attempted

Table 4

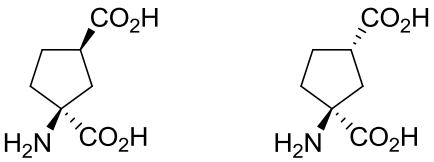
Compounds	**21**	**22**
a (R = Ph)	80	98
b (R = 2-pyridinyl)	80	100
c (R = 3-pyridinyl)	58	98
d [R = 3-(OH)C_6H_4]	81	92
e [R = 4-(OH)C_6H_4]	69	95

Table 5

Compounds	mGlu2-ago-AC, EC_{50} ± SD in μM (E_{max}%)	mGlu2-ago-GTP, EC_{50} ± SD in μM (E_{max}%)	mGlu2-antag-GTP, IC_{50} ± SD in μM (E_{max}%)
rac-**3**	73	55 ± 14 (71%)[a]	>300
(S)-**3**	nd[b]	45 ± 10 (38%)[a]	60 (39%)
rac-**9a**	387 (53%)	partial ago[c]	partial anta[c]
rac-**13b**	nd[b]	>1000	7.7 ± 2.1 (71%)
rac-**14c**	nd[b]	nd[b]	200 (67%)
rac-**17**	3(51%)	>100	>100
rac-**20**	nd[b]	9.7 ± 0.5 (34%)	47 (66%)
rac-**22b**	nd[b]	>100	75 ± 11 (100%)
rac-**22c**	nd[b]	>100	>100

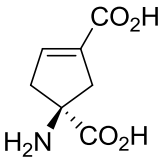
[a]Values from reference 7. [b]nd: not determined. [c]No full dose response.

Figure(s) (use if uploading high quality figure files)

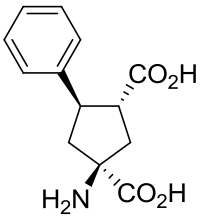


(*1S,3R*)-**1**

(*1S,3S*)-**2**



(S)-**3**



rac-**4**

Fig. 1

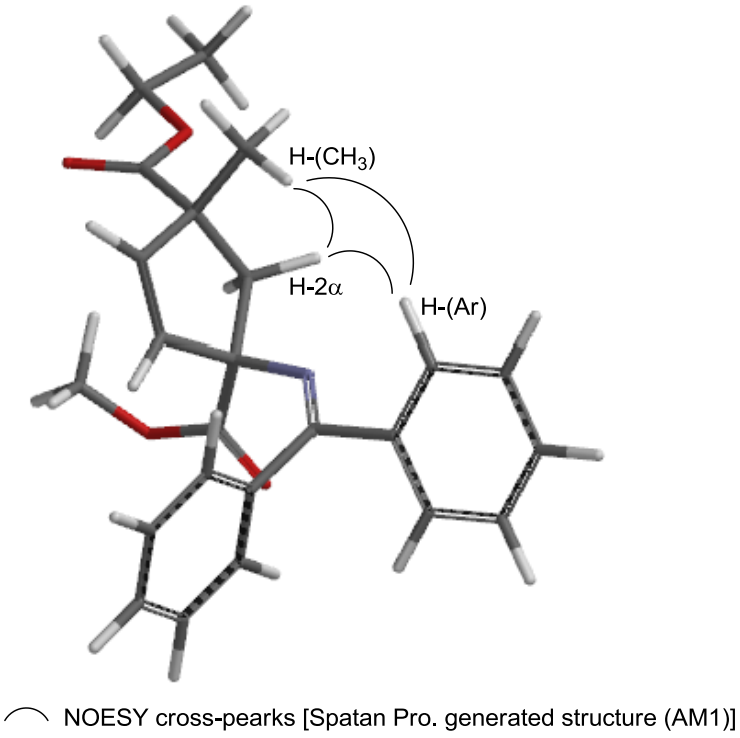
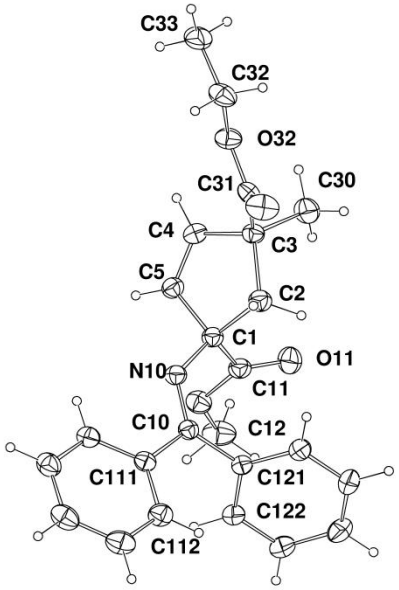


Fig. 2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C31
C30
C4
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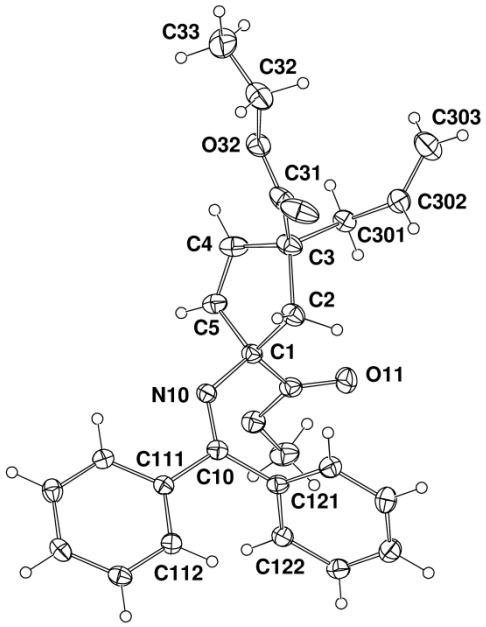
C111
C121
C122
C112

Fig. 3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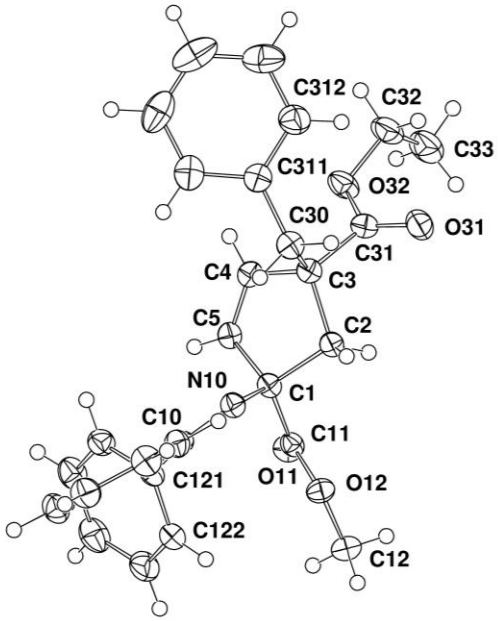
C112
C122

Fig. 4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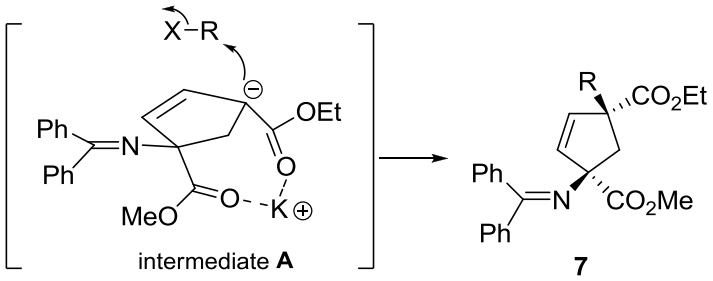
C121
O11
O12
C122
C12

Fig. 5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N
Ph
O
MeO
O
K
intermediate A
R
CO2Et
Ph
N
CO2Me
Ph
7

Fig. 6

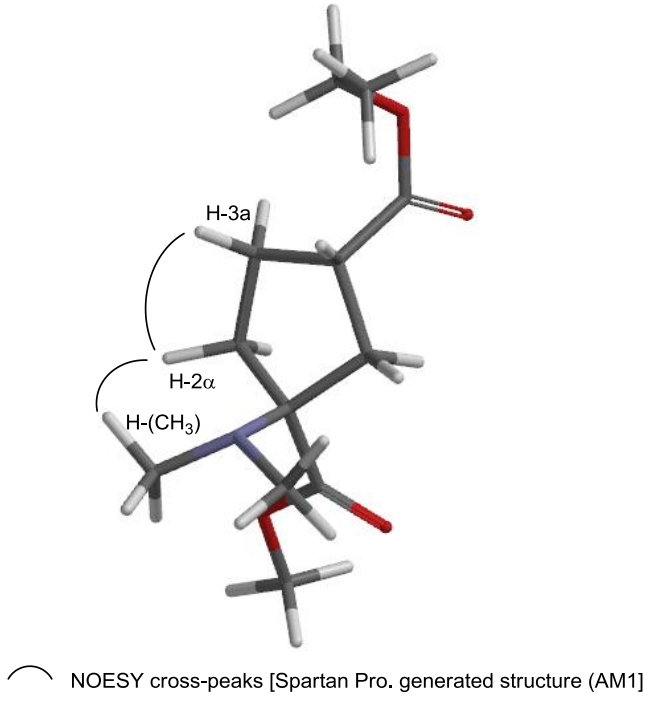
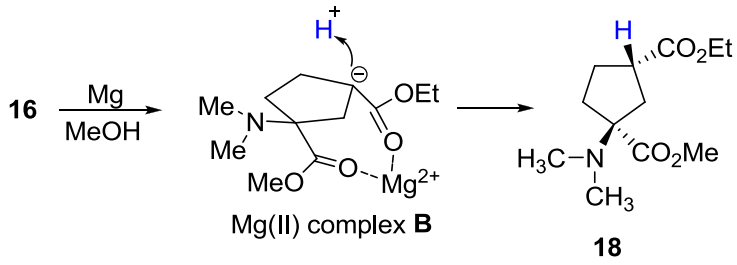

H-3a
H-2α
H-(CH3)
NOESY cross-peaks [Spartan Pro. generated structure (AM1]

Fig. 7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Me
MeO
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CO2Et
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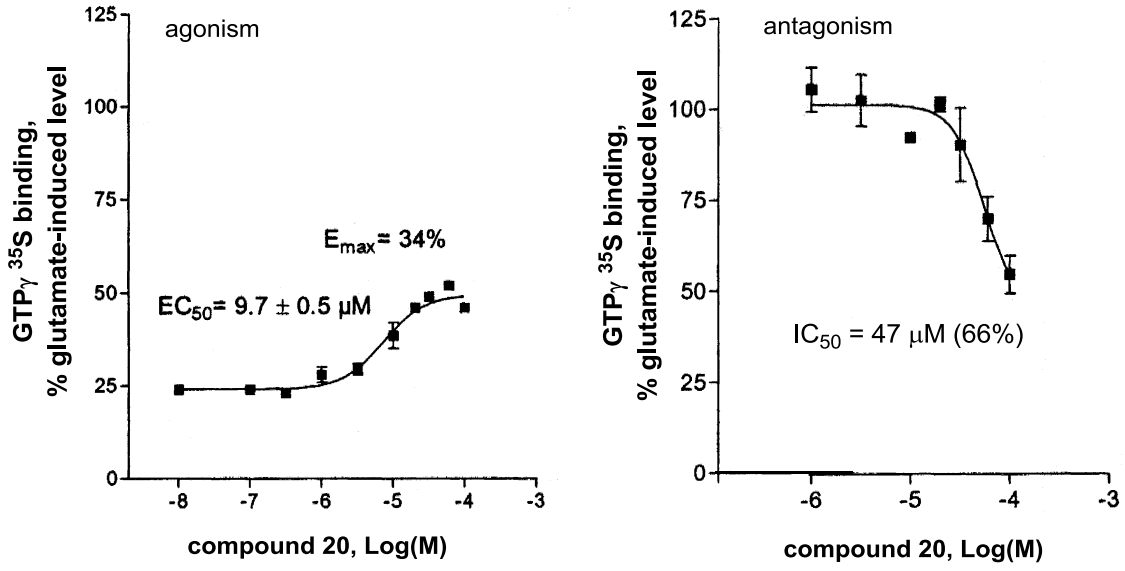
CO2Me
CH3
18

Fig. 8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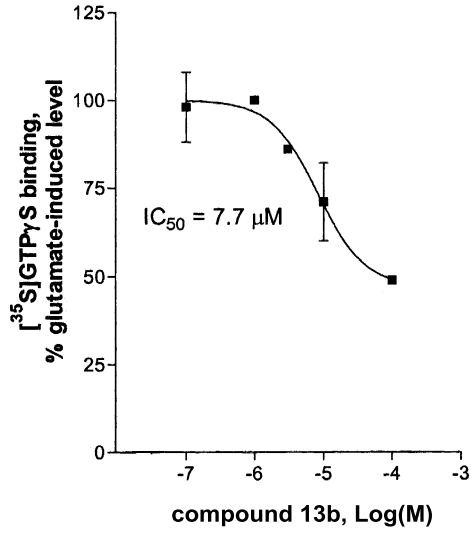
GTPγ 35S binding, % glutamate-induced level
125
100
75
50
25
0
-8
-7
-6
-5
-4
-3
Emax= 34%
EC50= 9.7 ± 0.5 μM
compound 20, Log(M)
antagonism
GTPγ 35S binding, % glutamate-induced level
125
100
75
50
25
0
-6
-5
-4
-3
IC50 = 47 μM (66%)
compound 20, Log(M)

Fig. 9


125
100
75
50
25
0
-7
-6
-5
-4
-3
IC50 = 7.7 μM
[35S]GTPγS binding,
% glutamate-induced level
compound 13b, Log(M)

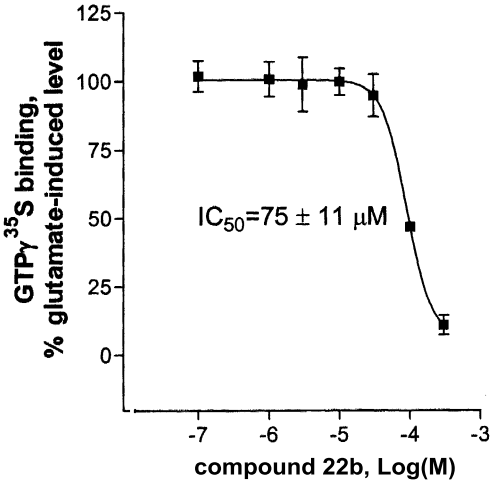

125
100
75
50
25
0
-7
-6
-5
-4
-3
IC50=75 ± 11 μM
GTPγ35S binding,
% glutamate-induced level
compound 22b, Log(M)

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}S]GTPγS biding.

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