Experimental section

Chemistry. Thin-layer chromatography (TLC) was performed on silica gel F254 plates (Merck). All compounds were detected using UV-light, $KMnO_4$ or ceric sulphate/ammonium molybdate spraying reagents. Protected and free amino compounds were developed using ninhydrin. The 1,2,5-thiadiazol-3-ol compounds were developed using a solution of $FeCl_3$ in diluted H_2SO_4. ^1H NMR and ^{13}C NMR were recorded on a 300 MHz Varian Gemini spectrometer using $CDCl_3$ or D_2O as solvent with $CDCl_3$ (δ7.25/77.0) or dioxane (δ3.75/67.2), respectively, as internal standards. Chemical shifts are given in ppm (δ) and coupling constants (*J*) are given in Hertz. Column chromatography (CC) was performed using Merck silica gel 60 (0.045–0.063 mm). All solvents and reagents were obtained from Fluka or Aldrich and used as purchased. Analytical HPLC was performed using a XTerra® MS C18 3.5 µm column (150 x 4.6 mm) with 1 mM AcOH or 0.1 % aqueous TFA (25 ºC, 270 nm) as the mobile phase. Preparative HPLC was performed similarly with a XTerra® Prep MS C18 10 µm column (300 x 10 mm). HRMS was performed on a Jeol JMS-HX/HX110A MS instrument at the University of Copenhagen.

cis- & *trans*-2-(2'-Benzyloxyethyl)-1-(2''-*tert*-butoxycarbonylvinyl)-cyclopropane-1-*tert*-butylcarboxylate (10 & 11)

Di-*tert*-butylgluconate (1.43g, 5.93 mmol) was added dropwise to a well-stirred suspension of sodium hydride 60% (589 mg, 14.72 mmol) in dry dimethoxyethane (25 mL) at 25 °C under nitrogen atmosphere (the suspension became pink). The cyclic sulfate **9** (1.7g, 6.58 mmol) was added slowly, and the solution was refluxed for 22 h. (be carefull upon addition, the reaction is exothermic in the beginning!).The reaction mixture was cooled to 25° C, and brine (20 mL) was added followed by Et_2O (50 mL). After the two layers were separated, the aqueous solution was extracted with Et_2O (3 x 100 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed *in vacou* to give a mixture of **10** and **11** as a yellow oil (cis-trans ratio 1:3 by NMR and GC-MS). The crude product was first filtrated through a pad of silica using ethyl acetate-petroleum ether 2:8 and then purified by flash chromatography (EtOAc:heptane 95:5) to give **10** and **11**.

cis-2-(2'-Benzyloxyethyl)-1-(2''-*tert*-butoxycarbonyl-vinyl)-cyclopropane-1-*tert*-butylcarboxylate (10)

(211 mg, 12%). ^1H NMR δ 1.31 (m, 2H, CH_2), 1.43 (s, 9H, *t*But), 1.47 (s, 9H, tBut), 1.60 (m, 1H, CH), 1.84 (dq, 2H, CH_2, *J*=1.2Hz and 6.5Hz), 3.45 (t, 2H, CH_2, *J*=6.3Hz), 4.48 (d, 2H, OCH_2 *J*=2.4Hz), 5.48 (d, 1H,CH *J*=15.8Hz), 7.29 (m, 5H, Ph), 7.42 (d, 1H, CH, *J*=15.8Hz). ^{13}C-NMR δ 23.35, 28.59, 28.68, 28.87, 31.68, 34.65, 69.80, 73.37, 80.59, 82.00, 118.28, 127.92, 127.96, 128.72, 138.67, 148.74, 166.43, 169.80.

Trans-2-(2'-Benzyloxyethyl)-1-(2''-*tert*-butoxycarbonyl-vinyl)-cyclopropane-1-*tert*-butylcarboxylate (11)

(587 mg, 35%) ^1H NMR δ 1.03 (dd, 1H, CH_2, *J*=4.6Hz and 7.1Hz), 1.25 (dd, 1H, CH_2, *J*=6.8Hz and 14.1Hz), 1.43 (s, 9H, *t*But), 1.47 (s, 9H, *t*But), 1.64 (m, 2H, CH_2), 1.83 ((m, 1H, CH), 3.47 (t, 2H, CH_2, *J*=6.5Hz), 4.49 (s, 2H, CH_2), 5.63 (d, 1H,CH *J*=15.8Hz), 7.24 (d, 1H, CH *J*=15.8Hz), 7.31 (m, 5H, Ph). ^{13}C-NMR δ20.62, 28.54, 28.68, 30.53, 31.02, 70.06, 73.48, 80.70, 81.68, 123.37, 127.90, 128.73, 138.64, 142.89, 165.88, 171.99.

cis-2-(2'-Benzoyloxy-ethyl)-cyclopropane-1-carboxylic acid -1-*tert*-butylcarboxylate (12)

Was prepared by the same procedure as above from **10** to give **12** (3.46 g, 55%) yield. ^1H NMR δ 1.44 (s, 9H, *t*But),) 1.83 (td, 1H, *J*=4.2Hz, *J*=9.5Hz) ppm 2.13 (m, 3H, CH_2, CH), 4.39 (m, 2H, CH_2), 7.50 (m, 3H, Ph) 8.05 (dd, 2H, *J*=7.1Hz and 23.0Hz). ^{13}C-NMR δ 24.53, 26.63, 28.310, 28.58, 30.96, 34.19, 64.25, 85.42, 128.70, 129.94, 133.39, 166.82, 169.34, 175.29. HRMS: m/z calcd for $C_{14}H_{13}NO_6^{+\cdot}$: 278.0785 [M+, -*t*Bu]; found: 278.0791.

trans-2-(2'-Benzoyloxy-ethyl)-cyclopropane-1- carboxylic acid- 1-tert-butyl carboxylate (13)

A mixture of carbon tetrachloride (17.5 mL), acetonitrile (17.5 mL) and water (26 mL) was added **11** (3.5g, 8.7 mmol) followed by sodiummetaperiodate (7.6g, 35.5 mmol). To this biphasic solution was added ruthenium trichloride monohydrate (77 mg, 0.34 mmol) and the mixture was stirred vigorously for 12 h at room temperature. A second amount of sodiummetaperiodate (3.7 g, 1.7 mmol) was added and the mixture was stirred for further 5 h to assure of a complete oxidation of the substrate. (Sometimes the presence of the benzylderivative was observed).

Next 30 ml of CH_2C1_2 was added, and the phases were separated. The upper aqueous phase was extracted 3 times with CH_2C1_2. The combined organic extracts were dried (MgS04) and concentrated. The resulting residue was diluted with ether, filtered through a Celite pad, and concentrated. The crude product was purified by flashchromatography (EtOAc:Exane gradient still 60:40) to give compound **13** (1.2 g, 41%) ^1H NMR δ 1.35 (s, 9H, *t*But), 1.51 (dd, 1H, *J*=4.2Hz, *J*=8.2Hz), 1.79 (dd, 1H, *J*=4.1Hz and 9.1Hz) 1.99 (m, 3H, CH_2, CH), 4.24 (m, 2H, CH_2), 7.35 (m, 3H, Ph), 7.84 (d, 1H, *J*=7.2Hz). ^{13}C-NMR δ 24.71, 27.92, 28.56, 31.642, 33.75, 64.18, 85.74, 128.75, 129.93, 133.47, 166.74, 172.40, 173.24. HRMS: m/z calcd for $C_{14}H_{13}NO_6^{+\cdot}$: 278.0785 [M+, -*t*Bu]; found: 278.0798.

***cis*-2-(2'-Benzoyloxy-ethyl)-1-[*N*-(*tert*-butoxycarbonyl)-amino]cyclopropyl-1-*tert*-butylcarboxylate (14)**

Was prepared from 12 by the same procedure as above to give **14** (880 mg 43%) yield. ^{1}H NMR δ 1.40 (s, 9H, *t*But), 1.42 (s, 9H, *t*But), 1.52 (m, 1H, CH), 1.75 (m, 2H, CH_2) 2.11 (m, 2H, CH_2), 4.43 (m, 2H, OCH_2) ppm 5.11 (bs, 1H, NH) 7.48 (m, 3H, Ph) 8.01 (m, 2H, Ph). ^{13}C-NMR 22.59, 22.63, 23.23, 25.40, 25.44, 28.49, 28.82, 32.45, 65.01, 81.70, 128.77, 129.88, 133.33, , 156.60, 165.334, 166.86, 206.85. HRMS: m/z calcd for $C_{18}H_{23}NO_6^{+\cdot}$: 349.1520 [M+, -*t*Bu]; found: 349.1536.

***trans*-2-(2'-Benzoyloxy-ethyl)-1-[*N*-(*tert*-butoxycarbonyl)-amino]cyclopropyl-1-*tert*-butylcarboxylate (15)**

Compound **13** (1.65 g, 4.94 mmol) was dissolved in THF (18 mL), and cooled to 0° C. Dry Et_3N (1.37 mL, 9.88 mmol) and dry ethylchloroformate (0.94 mL, 9.88 mmol) were added and the suspension stirred for 35 min. Next, an aqueous solution of NaN_3 (0.96 g, 14.82 mmol) in H_2O (3.6 mL) was added via syringe to the reaction flask. The reaction mixture was stirred vigorously for 40 min, after which time the ice bath was removed, and the mixture stirred an additional 10 min. The solution was transferred to a separatory funnel with H_2O and EtOAc. The resulting mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in an oven dry RBF (to an oil of approximately 2 mL). (Caution! Do not concentrate the solution to dryness, add toluene and evaporate again to be sure that all the ethyl acetate has been removed.) Toluene (30 mL) was added to the oil via syringe and the solution was heated under reflux at 90° C for 40 min to promote the double Curtius rearrangement (exothermic reaction!). Then *t*-butanol (7 mL, excess) added via syringe to the intermediate isocyanate. The reaction solution was then heated under reflux for 15 hours. After 15 hours, the solution was allowed to cool to 50 °C and the solvents were removed on a rotary evaporator. The resulting oil was dried under vacuum to give a brownish solid that was purified by flash chromatography (EtOAc:Exane gradient still 40:60) afforded **15** (0.830 g, 56%) . ^{1}H NMR δ 1.61 (s, 9H, *t*But), 1.76 (m, 3H, CH_2, CH) 2.22 (q, 2H, CH_2, *J*=6.5Hz) 4.54 (m, 2H, CH_2), 5.30 (bs, 1H, NH) 7.66 (m, 3H, Ph), ppm 8.21 (m, 2H, *J*=8.4Hz, Ph). ^{13}C-NMR 23.23, 26.95, 28.57, 28.84, 29.84, 32.44, 64.85, 81.93, 128.69, 129.93, 133.26, 156.11, 156.15, 166.91, 170.86. HRMS: m/z calcd for $C_{18}H_{23}NO_6^{+\cdot}$: 349.1520 [M+, -*t*Bu]; found: 349.1527.

***cis*-1-*tert*-Butoxycarbonylamino-2-(2'-hydroxy-ethyl)-cyclopropyl-1-*tert*-butylcarboxylate (16)**

To a solution of 14 (1g, 2.59 mmol) in dry methanol (23 mL) dry K_2CO_3 (716 mg, 5.18 mmol) was added. The suspension assumes strong yellow colour and was stired at room temperature for 1h. The suspension was diluted with EtOAc (25 mL) and water (7 mL). The two phase was separated and the water phase was extracted with EtOAc (3 x 25mL) and the organic phase dried on Na_2SO_4. The crude was purified by flash chromatography (EtOAc:Exane gradient still 40:60) giving **16** as a colorless oil (550 mg, 71%) . ^{1}H NMR δ 1.28 (m, 2H, CH_2) 1.44 (s, 9H, *t*But), 1.46 (s, 9H, *t*But) 1.72 (m, 3H, CH_2, CH), 3.82 (m, 2H, CH_2) 4.49 (bs, 1H), 5.35 (bs, 1H). ^{13}C-NMR 22.59, 25.99, 28.54, 28.84, 31.42, 39.14, 62.65, 80.05, 81.52, 156.79, 172.584. HRMS: m/z calcd for $C_{11}H_{19}NO_5^{+\cdot}$: 245.1258 [M+, -*t*Bu]; found: 245.1264.

***trans*-1-*tert*-Butoxycarbonylamino-2-(2'-hydroxy-ethyl)-cyclopropyl-1-*tert*-butylcarboxylate (17)**

To a solution of 15 (800mg, 1.97mmol) in dry methanol (18 ml) dry K_2CO_3 (546 mg, 3.95mmol) was added. The suspension assumes strong yellow colour and was stirred at room temperature for 1h. The suspension was diluted with EtOAc (20 mL) and water (5 mL). The two phase was separated and the water phase was extracted with EtOAc (3 x 20 mL) and the organic phase dried on Na_2SO_4. The crude was purified by flash chromatography (EtOAc:Exane gradient still 40:60) giving **17** as a white solid (350 mg, 60%). ^{1}H NMR δ 1.30 (dd, 1H, *J*=4.6Hz and 9.4Hz), 1.51 (dd, 1H, *J*=4.7Hz and 8.1Hz),1.61 (s, 9H, *t*But) 1.62 (s, 9H, *t*But), 1.85 (m, 2H, CH2), 2.15 (m, 1H, CH) 3.85 (m, 1H, CH_2), 4.81 (bs, 1H,) 5.34 (bs, 1H). ^{13}C-NMR 23.22, 28.57, 28.79, 30.60, 32.44, 38.63, 62.14, 80.77, 81.95, 157.44, 170.709. HRMS: m/z calcd for $C_{11}H_{19}NO_5^{+\cdot}$: 245.1258 [M+, -*t*Bu]; found: 245.1281.

***cis*-1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-(carboxymethyl)-cyclopropane-1-*tert*butylcarboxylate (18)**

The alcohol **16** (0.055 g, 0.182 mmol) was dissolved in 3.5 mL of CCl_4-CH_3CN-H_2O (2:2:3). Sodium periodate (117 mg, 0.549 mmol) was added, followed by $RuCl_3 \cdot H_2O$ (1.5 mg). The reaction mixture was stirred for 2.5 h at 25 °C and then extracted with EtOAc (2 x 5 mL). The combined organic layers were passed through a pad of silica gel and purified on flash chromatography (EtOAc:heptane gradient). The clean franction were concentrated to yield **18** (33 mg, 57 % yield) as a yellow oil. ^{1}H NMR 1.59 (s, 9H, *t*But), 1.61 (s, 9H, *t*But), 1.58-1.25 (overlapping, 2H), 1.81-1.63 (m, 1H), 2.80 (br m,

2H), 5.44 (br s, 1H), 6.67 (br s, 1H). ^{13}C NMR 23.59, 28.48, 28.76, 30.21, 34.08, 39.15, 80.75, 82.14, 157.10, 157.12, 171.97, 176.91, 176.94. HRMS: m/z calcd for $C_{15}H_{26}NO_6^{+\cdot}$: 316.1755 [$M^+$, $+H^+$]; found 316.1762:

trans-1-[_N_-(_tert_-Butoxycarbonyl)amino]-2-(carboxybutyl)-cyclopropane-1-carboxylate (19)

The alcohol **17** (0.055 g, 0.182 mmol) was dissolved in 3.5 mL of CCl_4-CH_3CN-H_2O (2:2:3). Sodium periodate (117 mg, 0.549 mmol) was added, followed by $RuCl_3 \cdot H_2O$ (1.5 mg). The reaction mixture was stirred for 2.5 h at 25 °C and then extracted with EtOAc (2 x 5 mL). The combined organic layers were passed through a pad of silica gel and purified on flash chromatography (EtOAc:Exane gradient still 100:00). The clean franction were concentrated to yield **19** (47 mg 82 %) as a white solid. ^{1}H NMR 1.59 (s, 9H, *t*But), 1.62 (s, 9H, *t*But), 1.51-1.82 (overlapping, 2H, CH_2), 1.86 (m, 1H, CH), 3.00 (ddd, 2H, CH_2, *J*=7.5Hz, 18.2Hz and 27.8Hz), 5.54 (br s, 1H), 6.44 (br s, 1H). ^{13}C NMR 22.64, 25.84, 28.42, 28.78, 33.53, 39.06, 81.48, 82.91, 157.51, 169.94, 175.04. HRMS: m/z calcd for $C_{15}H_{26}NO_6^{+\cdot}$: 316.1755 [$M^+$, $+H^+$]; found 316.1766: .

cis-1-Amino-2-carboxymethyl-cyclopropanecarboxylic acid (3)

To a solution of **18** (0.030 g, 0.09 mmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL), and the solution was stirred for 3 h. The solution was evaporated, and the residue was evaporated from 1 M HCl twice to afford **3** as a white solid (9 mg, 65%). ^{1}H-NMR (D_2O) 1.07 (t, 1H, *J*=7.4Hz, CH_2), 1.59 (dd, 1H, *J*=6.7Hz and 10Hz, CH_2), 1.91 (m, 1H,CH), 2.38 (dd, 1H, *J*=7.8Hz and 17.6Hz, CH_2), 2.52 (dd, 1H, *J*=7.3 Hz, *J*=17.5Hz, CH2). ^{13}C NMR (D_2O) 18.74, 21.56, 32.11, 37.96, 172.54, 175.33. HRMS: m/z calcd for $C_6H_{10}NO_4^{+\cdot}$: 160.0604 [M+, $+H^+$]; found: 160.0605.

trans-1-Amino-2-carboxymethyl-cyclopropanecarboxylic acid (4)

To a solution of **19** (0.047 g, 0.15mmol) in CH_2Cl_2 (4 mL) was added TFA (2 mL), and the solution was stirred for 3 h. The solution was evaporated, and the residue was evaporated from 1 M HCl twice to afford **4** as a white solid (16 mg 75%). ^{1}H-NMR (D_2O) 1.62 (ddd, 2H, CH_2, *J*=6.9Hz, 11.7Hz, and 15.3Hz), 1.99 (m, 1H, CH), 2.68 (dd, 1H, *J*=8.8Hz and 18.1Hz), 2.95 (dd, 1H, *J*=6.2Hz and 18.1Hz). ^{13}C NMR (D_2O) 18.97, 22.66, 31.95, 37.87, 171.42, 176.21. HRMS: m/z calcd for $C_6H_{10}NO_4^{+\cdot}$: 160.0604 [M+, $+H^+$]; found: 160.0610.

cis-2-[[(tert-Butoxycarbonyl)guanidinyl]-ethyl]-1-[_N_-(_tert_-butoxycarbonyl)amino]cyclopropane-1-_tert_-butylcarboxylate (20)

Triphenylphosphine (0.113 g, 0.431 mmol, 1.25 equiv) and 1,3-bis(tert-butoxycarbonyl)guanidine (0.123 g, 0.476 mmol, 1.38 equiv) were added to a solution of the hydroxyethyl derivative **16** (0.104 g, 0.345 mmol) in THF (5 mL) under N_2. This solution was cooled to 0 °C, diisopropil azodicarboxylate (0.08 mL, 0.431 mmol, 1.25 equiv) was slowly added over 30 min, and the reaction mixture was then stirred for 20 h at 25 °C under N_2. The reaction solution was concentrate and the product isoletad by flash chromatography (EtOAc:Exane gradient still 20:80) giving **20** as a clear oil (25 mg, 16 %). ^{13}C NMR 27.86, 27.97, 28.57, 28.87, 30.21, 31.46, 39.18, 44.31, 79.21, 79.95, 81.42, 84.34, 155.24, 156.71, 160.71, 163.96, 172.32. HRMS: m/z calcd for $C_{21}H_{39}N_4O_6^{+\cdot}$: 443.2864 [M+, -Boc, $+H^+$]; found: 443.2896.

trans-2-[[(_tert_-Butoxycarbonyl)guanidinyl]-ethyl]-1-[_N_-(_tert_-butoxycarbonyl)amino]cyclopropane-1-_tert_-butylcarboxylate (21)

Triphenylphosphine (0.66 g, 0.251 mmol, 1.25 equiv) and 1,3-bis(*tert*-butoxycarbonyl)guanidine (0.72 g, 0.277 mmol, 1.38 equiv) were added to a solution of the hydroxyethyl derivative **17** (0.64 g, 0.201 mmol) in THF (3.5 mL) under N_2. This solution was cooled to 0 °C, diisopropil azodicarboxylate (0.05 mL, 0.251 mmol, 1.25 equiv) was slowly added over 30 min, and the reaction mixture was then stirred for 20 h at 25 °C under N_2. The reaction solution was concentrate and the product isoletad by flash chromatography (EtOAc:Exane gradient still 20:80) offering offering **21** as a clear oil (54 mg, 60 %). ^{13}C NMR 23.06, 23.21, 27.12, 28.51, 28.60, 28.76, 28.96, 29.83, 32.43, 40.04, 44.91, 79.46, 80.02, 81.30, 84.34. 155.27, 156.46, 161.47, 163.95, 171.39. HRMS: m/z calcd for $C_{21}H_{39}N_4O_6^{+\cdot}$: 443.2864 [M+, -Boc, $+H^+$]; found: 443.2880.

cis-2-Guanidinyl-ethyl-1-_N_-aminocyclopropane-1-carboxy-lic acid (7)

To a solution of 20 (0.025 g, 0.056 mmol) in CH_2Cl_2 (1 mL) was added TFA (0.5 mL), and the solution was stirred for 3 h. The solution was evaporated, and the residue was evaporated to afford **7** (9 mg, 85%). ^{1}H-NMR (D_2O) 1.21 (dd, 1H, *J*=6.3Hz and 12.6Hz), 1.51 (m, 1H), 1.72 (m, 1H), 1.86 (m, 1H), 2.02 (m, 1H), 3.39 (m, 1H). ^{13}C NMR (D_2O) 18.90, 22.98, 26.32, 38.07, 40.52, 156.95, 162.92, 173.08, 173.14.

HRMS: m/z calcd for $C_7H_{15}N_4O_2^{+\cdot}$: 186.1190 [M+, $+H^+$]; found: 186.1211.

trans-2-guanidinyl-ethyl-1-_N_-aminocyclopropanecarboxylic acid (8)

To a solution of **21** (0.054 g, 0.122 mmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL), and the solution was stirred for 3 h. The solution was evaporated, and the residue was evaporated to afford **8** (18 mg, 80%). ^{1}H-NMR (D_2O) 1.70 (m, 5H), 3.28 (m, 5H). ^{13}C NMR (D_2O) 18.48, 25.42, 25.54, 37.90, 40.64, 156.87, 162.88, 163.35, 171.29, 171.36. HRMS: m/z calcd for $C_7H_{15}N_4O_2^{+\cdot}$: 186.1190 [M+, $+H^+$]; found: 186.1180.

cis-1-_tert_-Butoxycarbonylamino-2-(2'-amino-2'-cyanoethyl)-cyclopropyl-1-_tert_ butylcarboxylate (22)

A Dess Martin periodinane (0.387 g, 0.913 mmol) was added to a solution of **16** (0.250 g, 0.830 mmol) in CH_2Cl_2 (12 mL). After 1 h a thick white suspension had appeared and saturated $NaHCO_3$ (2.30 mL), saturated $Na_2S_2O_3$ (6.9 mL) and Et_2O (9.2 mL) were added. The solution was stirred vigorously until both the layers became clear. The aqueous phase was extracted twice with Et_2O (2 x 15 mL), dried over Na_2SO_4 and evaporated. The crude product was dissolved in in MeOH (1.29 mL) and added to a solution containing potassium cyanide (0.065 g, 1.00 mmol) and ammonium chloride (0.064 g, 1.2 mmol) in 25% aqueous ammonia (0.97 mL). The resulting solution was stirred overnight. The product was extracted with Et_2O, and the organic phase was washed with brine followed by evaporation to efford **22** (0.180 g, 66%). ^{13}C-NMR 22.02, 22.32, 23.70, 24.41, 24.42, 34.36, 34.78, 39.22, 39.26, 43.22, 43.54, 66.29, 80.36, 82.01, 122.32, 156.65, 171.79.

cis-1-_tert_-Butoxycarbonylamino-2-(2'-amino-2-carbamoylethyl)-cyclopropyl-1-tert butylcarboxylate (23)

The compound **22** (0.140 mg, 0.430 mmol) was dissolved in tetrahydrofuran (THF)/EtOH (1:3, 1.95 mL), and to this solution was slowly added a mixture of 35% hydrogen peroxide (0.17 mL) in 1 M NaOH (5.3 mL) at 0 °C. The mixture was stirred for 2 h and then extracted with Et_2O. The organic phase was washed with brine and evaporated to give **23** as an oil (94 mg, 63%). ^{13}C NMR δ 22.63, 24.64, 24.74, 28.48, 30.16, 34.22, 34.28, 39.40, 55.12, 80.07, 81.59, 156.95, 172.30, 172.42, 178.39, 178.56. HRMS: m/z calcd for $C_{12}H_{20}N_3O_5^{-\cdot}$: 286.1408 [$M^-$, -_t_Bu]; found: 286.1410.

cis-1-_tert_-Butoxycarbonylamino-2-(4'-hydroxy-[1,2,5]thiadiazol-3'-ylmethyl)-cyclopropyl-1 _tert_-butylcarboxylate (24)

The aminoamide 23 (0.85 g, 0.248 mmol) was solubilized in pyridine (2 mL) and then cooled to 0 °C. _N_-Thionylaniline (0.08 ml, 0.743 mmol) was added. After stirring for 2 h at room temperature the solvent was removed and the residue was taken up with CH_2Cl_2 (5 ml) and treated with of NH_4Cl (3 ml). The separated organic phase was dried over anhydrous Na_2SO_4 and evaporated off to afford of **24** (15 mg, 16.3%). ^{13}C NMR 23.02, 23.07, 25.99, 26.02, 26.05, 28.35, 28.52, 28.82, 30.22, 39.41, 39.43, 80.56, 81.90, 151.07, 157.06, 157.09, 157.11, 162.60, 172.12, 172.18.

HRMS: m/z calcd for $C_8H_7N_3O_4S^{-\cdot}$: 242.0230 [**see structure above**]; found: 242.0229.

cis-1-Amino-2-(4'-hydroxy-[1,2,5]thiadiazol-3'-ylmethyl)-cyclopropanecarboxylic acid (5)

To a solution of 24 (0.015 g, 0.04 mmol) in CH_2Cl_2 (1 ml) was added TFA (0.5 ml), and the solution was stirred for 2 h. The solution was evaporated, and the residue was evaporated from 1 M HCl twice to afford **5** as a yellow solid (7 mg, 64%). ^{1}H-NMR (D_2O) 1.36 (m, 1H, CH_2), 1.66 (m, 1H, CH2), 2.01 (m, 1H, CH), 2.84 (dd, 1H, _J_=8.5Hz and 16.4Hz), 3.05 (m, 1H). ^{13}C NMR (D_2O) 17.17, 19.05, 23.51, 26.76, 38.59, 57.76, 149.61, 161.60, 172.99. HRMS: m/z calcd for $C_7H_{10}N_3O_3S^+$: 216.0437 [M^+, +H]; found: 216.0437.

tert-Butyl _trans_-(_N_,_N_-bis(_tert_-butoxycarbonyl)amino)-2-ethoxycarbonylcyclopropanecarboxylate (26) and _tert_-butyl _cis_-1-(_N_,_N_-bis(_tert_-butoxycarbonyl)amino)-2-ethoxycarbonylcyclopropanecarboxylate (27).

A solution of ethyl diazoacetate (4.2 g, 37 mmol) in dry CH_2Cl_2 (16 mL) was added via syringe pump over a period of 24 h to a magnetically stirred solution of _tert_-butyl _N_,_N_-bis(_tert_-butoxycarbonyl)-2-aminoacrylate **25** (3.2 g, 9.3 mmol) and dirhodium(II)tetraacetate (42 mg, 95 µmol, 1 %) in dry CH_2Cl_2 (26 mL) kept under argon at reflux. The reaction mixture was then evaporated and column chromatography (toluene/EtOAc 9:1) afforded a 2:1 mixture of 26 and **27** (2.9 g, 73 %). The mixture was submitted to gradient column chromatography (toluene/EtOAc: 98:2 to 9:1) several times using Biotage Flash40™ giving **26** (1.2 g, 30 %) as an oil. ^{1}H NMR ($CDCl_3$) δ

1.28 (3H, t, *J*=7.1 Hz), 1.46 (9H, s), 1.47 (9H, s), 1.53 (s, 9H), 1.78 (dd, 1H, *J*= 5.6Hz and 7.8 Hz), 1.99 (dd, 1H, *J*=5.6 and 9.1 Hz), 2.71 (dd, 1H, *J*=7.8Hz and 9.0 Hz), 4.02 (2H, m); ^{13}C NMR ($CDCl_3$) δ 168.7, 168.6, 151.5, 151.2, 82.4, 82.3, 82.0, 60.9, 45.2, 31.2, 28.0, 27.8, 27.7, 24.6, 14.0. The further eluting fractions yielded **27** (0.6 g, 15 %) as an oil, however still containing ca. 10 % of **26**. ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, *J*=7.2Hz), 1.40 (1H, dd, *J*=6 and 9.9 Hz), 1.45 (9H, s), 1.54 (18H, s) 2.20 (1H, dd, *J*=6.0 and 8.6 Hz), 2.45 (1H, dd, *J*=8.6Hz and 9.9Hz) 4.2 (2H, q, *J*= 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 167.5, 167.1, 151.4, 82.9, 82.0, 61.1, 44.3, 34.7, 28.0, 27.9, 22.7, 14.3.

Compound 26 could be synthesized as a single diastereomer using the following procedure: A solution of ethyl dimethylsulfuranylideneacetate1 (0.400 g , 2.7 mmol) in benzene (1 mL) was added to a magnetically stirred solution of tert-butyl N,N-bis(tert-butoxycarbonyl)-2-aminoacrylate2 (25) (3.2 g, 9.3 mmol) in benzene (2 mL). The reaction mixture was refluxed for 2 h then evaporated. Column chromatography (toluene/EtOAc, 9:1) afforded 26 (0.270 g, 68 %) as an oil.

tert-Butyl _trans_-1-(_N_,_N_-bis(_tert_-butoxycarbonyl)amino)-2-hydroxymethylcyclopropanecarboxylate (28)

A solution of diisobutylaluminium hydride (1 M in toluene, 6.9 mL, 6.9 mmol) was added dropwise at -40 °C to a solution of **26** (1.30 g, 3.0 mmol) in anhydrous Et_2O (77 mL) under nitrogen. The reaction mixture was stirred for 30 minutes, then quenched with saturated aqueous NH_4Cl (35 mL) and warmed to rt. The aqueous phase was extracted with EtOAc (3 x 100 mL) and the combined organic phases dried (Na_2SO_4). Upon evaporation column chromatography (toluene/EtOAc 9:1) gave **28** (0.84 g, 72 %). ^{1}H NMR ($CDCl_3$) δ 0.74 (dd, 1H, *J*=5.8 and 8.2 Hz), 1.45 (9H, s), 1.51 (9H,s), 1.55 (9H, s), 1.74 (1H, dd, *J*=5.8Hz and 9.8 Hz), 2.27 (1H, ddt, *J*=3.4, 8.2Hz and 9.8 Hz), 3.22 (1H, dd, *J*= 9.8Hz and 12.4 Hz), 3.88 (1H, dd, *J*= 3.3Hz and 12.4 Hz); ^{13}C NMR ($CDCl_3$) δ 169.9, 154.8, 152.5, 83.7, 82.8, 81.5, 61.3, 44.3, 32.8, 28.1, 28.0, 27.9, 20.3.

tert-Butyl _trans_-1-(_N_,_N_-bis(_tert_-butoxycarbonyl)amino)-2-formylcyclopropanecarboxylate (29)

Dess Martin periodinane (0.84 g, 2.0 mmol) was added to a solution of **28** (0.70 g, 1.8 mmol) in CH_2Cl_2 (25 mL). After 1 h a thick white suspension had appeared and saturated $NaHCO_3$ (5 mL), saturated $Na_2S_2O_3$ (15 mL) and Et_2O (20 mL) were added. The solution was stirred vigorously until both the layers became clear. The aqueous phase was extracted twice with Et_2O, dried (Na_2SO_4) and evaporated. Column chromatography (toluene/EtOAc, 95:5) gave **29** (0.60 g, 86 %). ^{1}H NMR ($CDCl_3$) δ 1.46 (18 H, s), 1.53 (9H, s), 1.82 (1H, dd, *J*= 6.0Hz and 7.6 Hz), 2.12 (1H, dd, *J*=6.0Hz and 9.1 Hz), 2.78 (1H, ddd, J= 5.3, 7.6Hz and 9.1 Hz), 9.13 (1H, d, *J*= 5.3 Hz); ^{13}C NMR ($CDCl_3$) δ 195.7, 168.1, 151.2, 83.6, 83.2, 82.6, 82.4, 46.0, 38.3, 28.0, 27.9, 27.8, 23.1.

tert-Butyl (_1RS, 2RS_)-1-(_N_,_N_-bis(_tert_-butoxycarbonyl)amino)-2-(_SR_-amino(cyano)methyl) cyclopropanecarboxylate (_SR_-30) _trans_-6a and _tert_-butyl (_1RS,2RS_)-1-(_N_,_N_-bis(_tert_-butoxycarbonyl)amino)-2-(_RS_-amino(cyano)methyl)cyclopropanecarboxylate (_RS_-30)

A solution of **29** (0.6 g, 1.56 mmol) in MeOH (3 mL) was added to a solution, containing KCN (152 mg, 2.34 mmol) and NH_4Cl (152 mg, 1.56 mmol) in aqueous NH_3 (25 %, 2 mL). The reaction mixture was stirred for 48 h. Et_2O was added and the organic phase was washed with brine, dried (Na_2SO_4) and evaporated. Column chromatography (0.5 % MeOH in CH_2Cl_2) yielded ***SR*-30** (0.20 g, 31 %); ^{1}H NMR ($CDCl_3$) δ 1.03 (1H, dd, *J*=6.6Hz and 7.9 Hz), 1.44 (9H, s), 1.50 (9H, s), 1.53 (9H, s), 1.86 (1H, dd, *J*=6.5Hz and 9.7 Hz), 2.29 (1H, td, *J*=8.0Hz and 9.7 Hz) 3.53 (1H, d, *J*=8.3 Hz); ^{13}C NMR ($CDCl_3$) δ 168.7, 153.6, 152.2, 120.0, 83.7, 83.2, 82.2, 44.9, 44.0, 33.7, 28.0, 27.97, 27.95, 21.2. Further elution with the same solvent yielded ***RS*-30** (0.160 g, 25 % yield); ^{1}H NMR ($CDCl_3$) δ 1.19 (1H, dd, *J*= 6.3Hz and 8.1Hz), 1.45 (9H, s), 1.50 (9H, s), 1.56 (9H, s), 1.79 (1H, dd, *J*=6.4Hz and 9.7 Hz), 2.39 (1H, ddd, *J*=6.5Hz, 8.1Hz and 9.7 Hz), 3.71 (1H, d, *J*=6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 169.0, 152.7, 152.1, 120.6, 83.8, 83.0, 82.1, 44.2, 41.6, 32.1, 28.0, 21.0,

The relative stereochemistry is established from the formation of **38** in the hydrolysis of ***RS*-30** to **36**.

tert-Butyl _trans_-1-(_N_,_N_-bis(_tert_-butoxycarbonyl)amino)-2-(amino(carbamoyl)methyl) cyclopropanecarboxylate (30)

A solution of **29** (0.300 g, 0.7792 mmol) in MeOH (1.5 mL) was added to a solution, containing KCN (76 mg, 2.34 mmol) and NH_4Cl (76 mg, 1.4 mmol) in aqueous NH_3 (25 %, 1 mL). The reaction mixture was stirred for 48h. The product was extracted with Et_2O and the combined organic phases were washed with brine, dried (Na_2SO_4) and evaporated to give 0.280 g of a crude diastereomeric mixture (4:5) *trans-tert*-butyl *N*,*N*-bis(*tert*-butoxycarbonyl)-1-amino-2-(amino(cyano)methyl)cyclopropane-carboxylate **30a**. An amount of 220 mg of this mixture was dissolved in THF/EtOH (1:3, 4 mL) and to this solution was slowly added a mixture of aqueous hydrogen peroxide (35 %, 0.13 mL) in 1 M NaOH (3.9 mL) at 0° C. The mixture was stirred for 2 h then extracted with Et_2O. The organic phase was washed with brine and evaporated. Upon column chromatography (CH_2Cl_2/MeOH, 95:5) a 5:2 mixture of the diastereomeres of **31** (145 mg, 55% for two steps) was isolated.

tert-Butyl (_1RS,2RS_)-1-(_N_,_N_-bis(_tert_-butoxycarbonyl)amino)-2-(_RS_-amino(carbamoyl)methyl) cyclopropanecarboxylate (31)

A mixture of aqueous hydrogen peroxide (35 %, 0.13 mL) in 1 M NaOH (3.9 mL) was slowly added to a solution of **30a** (200 mg, 0.49 mmol) in a mixture of THF/EtOH 1:3 (6 mL) at 0 °C. The

mixture was stirred for 2 h then extracted with Et_2O. The organic phase was washed with brine and evaporated. Column chromatography (5 % MeOH in CH_2Cl_2) gave **31** (140 mg, 67 %). ^{1}H NMR ($CDCl_3$) δ 1.41 (1H, dd, *J*=6.3 and 8.0 Hz), 1.45 (9H, s), 1.51 (9H, s), 1.55 (9H, s), 1.72 (1H, dd, *J*= 6.3Hz and 9.8 Hz), 2.08 (1H, dt, *J*=8.1Hz and 9.4 Hz), 2.93 (1H, d, *J*=9.4 Hz) 5.58 (1H, bs), 7.23 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 176.3, 169.9, 154.1, 152.2, 83.1, 82.7, 81.4, 55.3, 43.9, 34.0, 28.0, 27.9, 21.7.

***tert*-Butyl *cis*-1-(*N*,*N*-bis(*tert*-butoxycarbonyl)amino)-2-hydroxymethylcyclopropanecarboxylate (32)**

A solution of diisobutylaluminium hydride (1 M in toluene, 3.4 mL, 3.4 mmol) was added dropwise at -40 °C to a solution of **27** (0.50 g, 1.16 mmol) in anhydrous Et_2O (18 mL) under nitrogen. The mixture was stirred for 4 h, then quenched with saturated aqueous NH_4Cl (1 mL) and warmed to rt. The aqueous phase was extracted with EtOAc (3 x 20 mL) and the combined organic phases dried (Na_2SO_4). Upon evaporation column chromatography (toluene/EtOAc 9:1) gave **32** (0.24 g, 53 %) containing ca. 10 % of **28**. ^{1}H NMR ($CDCl_3$) δ 1.35 (1H, dd, J= 5.1 and 9.3 Hz), 1.46 (9H, s), 1.52 (18H, bs), 1.83 (1H, dd, *J*=5.2, 8.7) 1.90 (1H, dq, *J*=4.3Hz and 8.9 Hz), 3.78 (1H, dd, *J*= 8.7Hz and 11.6 Hz), 3.99 (1H, dd, *J*= 4.2Hz and 11.5 Hz); ^{13}C NMR ($CDCl_3$) δ 169.5, 152.9, 151.5, 83.1, 82.6, 81.8, 59.2, 43.2, 35.7, 28.1, 28.0, 22.8.

***tert*-Butyl *cis*-1-(*N*,*N*-bis(*tert*-butoxycarbonyl)amino)-2-formylcyclopropanecarboxylate (33)**

Dess Martin periodinane (0.37 g, 0.88 mmol) was added to a solution of **32** (0.310 g, 0.80 mmol) in CH_2Cl_2 (12 mL). After 1 h a thick white suspension had appeared and saturated $NaHCO_3$ (1.8 mL), saturated $Na_2S_2O_3$ (5.4 mL) and Et_2O (7.2 mL) were added. The solution was stirred vigorously until both the layers became clear. The aqueous phase was extracted twice with Et_2O, dried (Na_2SO_4) and evaporated. Column chromatography (toluene/EtOAc, 95:5) gave **33** (0.24 g, 78 %) containing ca. 10 % of **29**. ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.50 (18H, s), 1.72 (1H, m, *J*= 6.2 and 9.6 Hz), 2.23 (1H, ddd, *J*= 5.7Hz, 8.4Hz and 9.5 Hz), 2.43 (1H, dd, *J*= 6.2Hz and 8.5 Hz) 9.47 (1H, d, *J*=5.6 Hz); ^{13}C NMR ($CDCl_3$) δ 197.3, 168.0, 151.0, 83.2, 82.9, 46.5, 41.8, 28.0, 27.9, 24.1.

***tert*-Butyl *cis*-1-(*N*,*N*-bis(*tert*-butoxycarbonyl)amino)-2-(amino(carbamoyl)methyl) cyclopropanecarboxylate (35)**

A solution of **33** (0.240 g, 1.56 mmol) in MeOH (2 mL) was added to a solution, containing KCN (61 mg, 2.34 mmol) and NH_4Cl (61 mg, 1.56 mmol) in aqueous NH_3 (25 %, 1 mL). The reaction mixture was stirred for 48h. The product was extracted with Et_2O and the combined organic phases were washed with brine, dried (Na_2SO_4) and evaporated to give 0.170 g of crude *cis-tert*-butyl *N*,*N*-bis(*tert*-butoxycarbonyl)-1-amino-2-(amino(cyano)methyl)cyclopropanecarboxylate **34**. ^{1}H NMR ($CDCl_3$) δ 1.36 (dd, 1H, *J*=6.2Hz and 9.6Hz) 1.42 (9H, s), 1.48 (9H, s), 1.50 (9H, s), 1.79 (1H, dd, *J*=6.3Hz and 8.4 Hz), 2.01 (1H, dt, *J*=8.5Hz and 9.9 Hz) 4.01 (1H, d, *J*=10.1 Hz).

The mixture was redissolved in THF/EtOH (1:3, 4 mL) and to this solution was slowly added a mixture of aqueous hydrogen peroxide (35 %, 0.13 mL) in 1 M NaOH (3.9 mL) at 0° C. The mixture was stirred for 2 h then extracted with Et_2O. The organic phase was washed with brine and evaporated. Column chromatography (CH_2Cl_2/MeOH, 95:5) gave **35** (0.120 g, 45 % for two steps) containing a small amount of **31** that coeluted. ^{1}H NMR ($CDCl_3$) δ 1.42 (1H, dd, *J*= 6.0Hz and 10.0 Hz), 1.47 (9H, s), 1.50 (9H, s), 1.54 (9H, s), 1.79 (1H, dt, *J*= 8.5Hz and 10.0 Hz), 2.04 (1H, dd, *J*= 6.0Hz and 8.4 Hz), 3.61 (1H, d, *J*= 10.3 Hz), 5.55 (1H, bs), 7.56 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 177.0, 169.1, 153.2, 151.7, 82.9, 82.5, 81.5, 53.5, 42.6, 37.7, 27.9, 25.1.

***tert*-Butyl (*1RS,2RS*)-1-(*N*,*N*-bis(*tert*-butoxycarbonyl)amino)-2-(*SR*-amino(carbamoyl)methyl) cyclopropanecarboxylate (31) and *tert*-butyl *trans*-5-amino-2-(*N*,*N*-bis(*tert*-butoxycarbonyl)amino)-5-cyanopent-4-enoate (36)**

A mixture of aqueous hydrogen peroxide (35 %, 0.10 mL) in 1 M NaOH (3.9 mL) was slowly added to a solution of **30b** (160 mg, 0.39 mmol) in THF/EtOH (1:3, 4 mL) at 0 °C The mixture was stirred for 2 h then extracted with Et_2O. The organic phase was washed with brine and evaporated. Column chromatography (CH_2Cl_2/MeOH, 95:5) gave compound **36** (60 mg, 38 %). A 1:3 rotameric ratio can be seen in NMR. Chemical shifts reported for the major rotamer unless stated otherwise. ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.53 (18H, s), 2.85 (td, 1H, *J*=9.2 Hz and 15.1 Hz), 2.97 (1H, ddd, *J*=5.5Hz, 6.8Hz and 15.1Hz), 3.13 (2H, bs), 4.79 (dd, 1H, *J*=5.5, 9.1Hz), 5.38 (dd, 1H, *J*=6.8Hz and 9.2Hz), ^{13}C NMR ($CDCl_3$) δ 168.5, 152.0, 118.2, 116.8, 115.1, 83.2, 81.7, 58.4, 30.0, 28.1, 27.97 (minor rotamer: 168.2, 83.4, 82.0, 57.6, 28.02, 27.91) Further elution yielded **31** (35 mg, 21 %); ^{1}H NMR ($CDCl_3$) δ 1.31 (1H, dd, *J*= 6.0Hz and 8.3 Hz), 1.44 (9H, s), 1.51 (9H, s), 1.53 (9H, s), 1.70 (1H, dd, *J*= 6.0Hz and 10.1 Hz), 2.35 (ddd, 1H, *J*= 6.0Hz, 8.3Hz and 10.1 Hz), 3.23 (d, 1H, *J*=6.0 Hz), 5.50 (1H, bs), 7.39 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 176.2, 169.4, 153.8, 152.2, 83.6, 83.0, 81.7, 51.2, 45.3, 45.0, 33.8, 28.0, 27.9, 27.9, 20.9.

Trans-configuration of **36** established from ^{1}H-NMR chemical shift of the vinylic proton (δ 5.38).[3]

***tert*-Butyl *trans*-1-(*N*,*N*-bis(*tert*-butoxycarbonyl)amino)-2-(4-hydroxy[1,2,5]thiadiazol-3-yl)cyclopropanecarboxylate (37)**

Thionylaniline (134 µL, 164 mg, 1.18 mmol) was added to a solution of **31** (170 mg, 0.40 mmol) in pyridine (4 mL) cooled at 0 °C. After stirring for 2 h at rt the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (5 mL) and treated with saturated NH_4Cl (3 mL). The organic phase was dried (Na_2SO_4) and evaporated. Gradient column chromatography (10 to 20 % Et_2O in heptane) gave **37** as a solid (140 mg, 74 %); ^{1}H NMR

($CDCl_3$) δ 1.25 (9H, s), 1.49 (9H, s), 1.55 (9H, s), 2.10 (1H, dd, *J*=5.6 Hz and 8.1 Hz), 2.19 (1H, dd, *J*= 5.6Hz and 9.4 Hz), 3.42 (1H, dd, *J*= 8.2 and 9.3 Hz); ^{13}C NMR ($CDCl_3$) δ 169.2, 162.7, 151.6, 151.2, 147.3, 82.5, 82.3, 82.0, 46.3, 28.1, 28.0, 27.6, 27.8, 25.7.

1-Amino-2-(4-hydroxy[1,2,5]thiadiazol-3-yl)cyclopropane-carboxylic acid *cis*- and *trans*-6

To a solution of **37** (86 mg, 0.19 mmol) in CH_2Cl_2 (1.5 mL) was added TFA (1.5 mL), and the solution was stirred for 24 h. The solution was evaporated to give 65 mg of the crude product. First one predominant epimer was observed by ^1H NMR, but upon evaporation an epimeric equilibrium mixture between *cis*- and *trans*-**6** was present. ^1H NMR revealed the presence of *cis*- and *trans*-**6** estimated ratio 1:4, which is confirmed by analytical HPLC using either 1 mM AcOH or 0.1 % aqueous TFA as eluent, where *cis*-**6** eluted first and *trans*-**6** eluted second.

cis-**6**. ^1H NMR (D_2O, dioxane as internal reference) 3.10 (1H, dd, *J*=7.9Hz and 10.0Hz) 2.05. (1H, dd, *J*=6.6Hz and 10.1Hz) 1.88 (1H, dd, *J*=6.6Hz, and 7.9Hz)

trans-**6**. ^1H NMR (CD_3CN) δ (major isomer) 1.91 (1H, dd, *J*=6.6 Hz and 7.9 Hz), 2.08 (1H, dd, *J*= 6.5Hz and 10.0 Hz), 3.10 (1H, dd, *J*=7.9, 10.0 Hz); (minor isomer), 1.95 (1H, dd, *J*=6.8Hz and 10.3 Hz), 2.13 (1H, dd, *J*= 6.9and 8.4 Hz), 2.83 (1H, dd, *J*=8.4 Hz and 10.3 Hz)

^{13}C NMR (CD_3CN) δ (*cis*-**6**) 170.8, 163.3, 146.0, 40.5, 23.0, 20.5 (*trans*-**6**) 169.6, 162.4, 145.4, 39.0, 24.3, 18.0.

Computational Details:

Homology models of human mGluR2 (Accession number NP_000830.2) and human mGluR3 (Accession numbuer NP_000831.2) were built using Prime 3.0 (Schrodinger 2011) based on the crystal structure of rat mGluR3 in complex with DCG-IV (PDB code 2E4V) (Muto 2007) as a template. Docking was attempted on both (R,R)- and (S,S)-trans_1 to each model using Glide XP 5.7 (Schrodinger 2011), following model preparation by the protocol recommended for Glide. Only (S,S)-trans_1 could adopt a glutamate-like binding mode. The final (S,S)-trans_1--mGluR complexes in Figure X were obtained by minimizing the poses in the binding sites with receptor heavy atoms frozen, using Macromodel 9.9 (Schrodinger 2011). Prime 3.0, Glide 5.7, Macromodel 9.9. Schrodinger Inc, 101 SW Main Street Suite 1300, Portland OR, USA (2011). Muto, T.; Tsuchiya, D.; Morikawa, K.; Jingami, H. Structures of the extracellular regions of the group II/III metabotropic glutamate receptors. Proc. Natl. Acad. Sci., 104(10) 3759-3764 (2007).

Culturing and transfection of tsA201 Cells

tsA201 cells[4] were cultured in GlutaMAX-I DMEM medium, supplemented with 10% dialyzed fetal bovine serum and penicillin (100 U/ml)/streptomycin (100 mg/ml) at 37 °C in a humidified atmosphere of 5% ambient CO_2. Cells were transfected using PolyFect (Qiagen, West Sussex, UK) with mouse GPRC6A plasmid DNA as previously described.[5,6] To enable efficient coupling of mGPRC6A and mutant receptors to phospholipase C, the receptors were co-expressed with $G\alpha_{qG66D}$ (1:1 transfection ratio).[5,7] For the IP turnover assay, transfected cells were split into poly-D-lysine coated 96-well plates the day before assaying and grown to confluence in inositol-free DMEM medium supplemented with antibiotics, serum and 0.15 MBq/ml *myo*-[2-^{3}H]inositol (GE Healthcare, Buckinghamshire, UK).

Inositol phosphate (IP) turnover assay

The assay was carried out as previously described.[5] In brief, the cells were prewashed for 2×2 hours at 37 °C with buffer containing Hanks' Balanced Salt Solution (HBSS) containing 20 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mg/ml BSA, pH 7.4). The cells were washed and preincubated with buffer or allosteric modulator in 50 µl assay buffer (HBSS containing 20 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 20 mM LiCl, pH 7.4) for 30 min at 37 °C. Following this preincubation, the cells were stimulated with 50 µl of agonist with or without modulator in assay buffer for 30 min at 37 °C. The reactions were stopped by exchanging the buffer with 50 µl 10 mM ice-cold formic acid and incubating the cells at 4 °C for at least 30 min. Yttrium silicate scintillation proximity assay beads (PerkinElmer, Waltham, CA) were used for measuring radioactivity from generated [^{3}H]IP, as previously described.[5,8] Radioactivity was quantified in a Packard TopCount microplate scintillation counter and responses read as counts per minute.

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