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New Photoremovable Protecting Groups for Carboxylic Acids with High Photolytic Efficiencies at Near-UV Irradiation. Application to the Photocontrolled Release of L-Glutamate

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Experimental Procedures

4-Ethyl-1,2-dimethoxybenzene: 4-Bromoveratrole (11.9 g, 54.8 mmol) was dissolved in dry THF (50 mL) under argon and cooled at -78°C. *n*-butyllithium (1.6 M in hexane, 37.68 cm^3, 60.3 mmol) was added dropwise using a syringe. After 1 h at -78°C (formation of a white precipitate) iodoethane (5.3 cm^3, 65.8 mmol) was added dropwise. The reaction mixture was allowed to reach room temperature over 1.5 h, and was quenched by a saturated $NaHCO_3$ aqueous solution (150 cm^3). The aqueous layer was extracted with EtOAc (150 cm^3 x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with 70:30 heptane/EtOAc, to give the 4-Ethyl-1,2-dimethoxybenzene (6.9 g, 41.5 mmol, 76%) as a yellow liquid. NMR (200 MHz, $CDCl_3$) d_H = 1.23 (3 H, t, $^3J_{HH}$ 7.3, CH_3), 2.59 (2 H, q, $^3J_{HH}$ 7.3, CH_2), 3.58 (3 H, s, OCH_3), 3.87 (3 H, s, OCH_3), 6.65-6.85 (3 H, m, H_{ar}).

2-Ethyl-4,5-dimethoxy-1-nitrobenzene 2: NO_2BF_4 (620 mg, 4.67 mmol) was dissolved in dry acetonitrile (15 cm^3) under argon and cooled at -30°C. 2-picoline (0.45 cm^3, 4.67 mmol) was added dropwise using a syringe. After 0.5 h at -30°C 4-Ethyl-

1,2-dimethoxybenzene (706 mg, 4.25 mmol) dissolved in dry acetonitrile (15 cm^3) was added dropwise. The reaction was slowly allowed to reach room temperature overnight. The reaction mixture was concentrated *in vacuo* and the resulting residue dissolved by EtOAC (150 cm^3) and washed by a saturated $NaHCO_3$ aqueous solution (150 cm^3). The aqueous layer was extracted with EtOAc (150 cm^3 x 2). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with 50:50 heptane/EtOAc, to give the 2-Ethyl-4,5-dimethoxy-1-nitrobenzene **2** (693 mg, 3.31 mmol, 78%) as a yellow liquid. NMR (300 MHz, $CDCl_3$) d_H = 1.28 (3 H, t, $^3J_{HH}$ 7.3, CH_3), 2.95 (2 H, q, $^3J_{HH}$ 7.3, CH_2), 3.92 (3 H, s, OCH_3), 3.96 (3 H, s, OCH_3), 6.73 (1 H, s, H_{ar}), 7.58 (1 H, s, H_{ar}).

2-(4,5-Dimethoxy-2-nitrophenyl)propan-1-ol 3: 2-Ethyl-4,5-dimethoxy-1-nitrobenzene **2** (200 mg, 0.89 mmol) was dissolved in dry DMSO (10 cm^3). Paraformaldehyde (100 mg, 3.3 mmol) and Triton B (0.1 cm^3) were added. The reaction mixture was diluted by H_2O after 2 h at 90°C, the product was extracted with EtOAc (3100 x cm^3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with 50:50 heptane/EtOAc, to give the 2-(4,5-dimethoxy-2-nitrophenyl)propan-1-ol **3** (693 mg, 3.31 mmol, 78%) as a yellow solid. (300 MHz, $CDCl_3$) d_H = 1.31 (3 H, d, $^3J_{HH}$ 7.1, CH_3), 3.69-3.84 (3 H, m, CH and CH_2), 3.91 (3 H, s, OCH_3), 3.96 (3 H, s, OCH_3), 6.88 (1 H, s, H_{ar}), 7.43 (1 H, s, H_{ar}).

3-(3,4-dimethoxyphenyl)butan-2-ol (*threo*): 4-Bromoveratrole (6.1 g, 28.1 mmol) was dissolved in dry THF (40 mL) under argon and cooled at -78°C. *n*-butyllithium (1.6 M in hexane, 19.3 cm^3, 30.9 mmol) was added dropwise using a syringe. After 1 h at -78°C (formation of a white precipitate) *cis*-2,3 epoxybutane (2.45 cm^3, 28.1 mmol) and $BF_3.Et_2O$ (3.56 cm^3, 28.1 mmol) were added dropwise. After 10 min at -78°C, the reaction was quenched by a saturated $NaHCO_3$ aqueous solution (150 cm^3). The aqueous layer was extracted with EtOAc (3 x 250 cm^3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with 90:10 to 60:40 heptane/EtOAc, to give the *threo* stereoisomers of 3-(3,4-dimethoxyphenyl)-butan-2-ol (218 mg, 1.04 mmol, 4%) as a white solid. NMR (300 MHz, $CDCl_3$) d_H = 1.22 (3 H, d, $^3J_{HH}$ 6.8, CH_3), 1.26 (3 H, d, $^3J_{HH}$ 7.1, CH_3), 2.62 (1 H, q, $^3J_{HH}$ 7.1, CH_2), 3.65-3.80 (1 H, m), 3.88 (3 H, s, OCH_3), 3.90 (3 H, s, OCH_3), 6.74-6.87 (3 H, m, H_{ar}).

3-(3,4-dimethoxyphenyl)butan-2-ol (*erythro*): Same protocol as for the synthesis of the *threo* isomers by using the ***t**rans*-2,3 epoxybutane instead of the *cis*-2,3 epoxybutane. The residue was purified by flash chromatography eluting with 90:10 to 60:40 heptane/EtOAc, to give the *erythro* stereoisomers of 3-(3,4-dimethoxyphenyl)butan-2-ol (945 mg, 4.49 mmol, 16%) as a white solid. NMR (200 MHz, $CDCl_3$) d_H = 1.13 (3 H, d, $^3J_{HH}$ 6.2, CH_3), 1.34 (3 H, d, $^3J_{HH}$ 6.1, CH_3), 2.67-2.77 (1 H, m), 3.86-3.92 (1 H, m), 3.88 (3 H, s, OCH_3), 3.92 (3 H, s, OCH_3), 6.77-6.89 (3 H, m, H_{ar}); (50 MHz, $CDCl_3$) d_C = 16.34 (s, CH_3CH), 21.32 (s, CH_3CHOH), 47.02 (s, CH benzylic), 56.31 (s, 2 OCH_3), 72.79 (s, CHOH), 111.62 (s, C_{ar2} and C_{ar5}), 120.05 (s, C_{ar6}), 141.92 (s, C_{ar1}), 144.53 (s, C_{ar3}), 149.27 (s, C_{ar4}).

2-(4,5-Dimethoxy-2-nitrophenyl)propyl benzoate I 2-(4,5-dimethoxy-2-nitrophenyl)propan-1-ol **3** (121 mg, 0.5 mmol) was dissolved in dry chloroform (10 cm^3) under argon. 1,3-Dicyclohexylcarbodiimide (DCC; 121 mg, 0.5 mmol), benzoic acid (61 mg, 0.5 mmol) and 4-(*N,N*-dimethylaminopyridine (DMAP) (5mg, cat.) were added. After 5 h at room temperature, the reaction mixture was quenched by a saturated $NaHCO_3$ aqueous solution (100 cm^3). The aqueous layer was extracted with CH_2Cl_2 (150 cm^3 x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with 50:50 heptane/EtOAc to give 2-(4,5-dimethoxy-2-nitrophenyl)propyl benzoate **I (**150 mg, 0.43 mmol, 87%) as a yellow solid. NMR (300 MHz, $CDCl_3$) d_H = 1.43 (3 H, d, $^3J_{HH}$ 7.05, CH_3), 3.91 (3 H, s, OCH_3), 3.92 (3 H, s, OCH_3), 4.10 (1H, m, CH), 5.53 (2H, m, CH_2), 6.90 (1 H, s, H_{ar6}), 7.41-7.55 (4H, m, H_{ar}) 7.96 (2 H, d, $^3J_{HH}$ 7.17, H_{ar}). HPLC retention time on a Analytical SB-C18 Zorbax (4.6 x 250 mm) column using a 30 minutes linear gradient from 0 to 100% acetonitrile in a 0.1% TFA water solution at 1 mL/min: 25.0 min.

3-(4,5-Dimethoxy-2-nitrophenyl)-2-butyl benzoate (DMNPB-benzoate) II : 3-(4,5-dimethoxy-2-nitrophenyl)butan-2-ol **6** (200 mg, 0.78 mmol) was dissolved in dry CH_2Cl_2 (5 cm^3) under argon. Benzoyl chloride (180 cm^3, 1.56 mmol) and pyridine (127 cm^3, 1.56 mmol) were added. After 5 h at room temperature, the reaction mixture was quenched by ice cold water(100 cm^3). The aqueous layer was extracted with CH_2Cl_2 (3 x 150 cm^3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with 60/40 heptane/EtOAc to give DMNPB-benzoate **II** (165 mg, 0.47 mmol, 60%) as a yellow oil. d_H(300 MHz, $CDCl_3$) = 1.38 (3 H, d, $^3J_{HH}$ 6.20,

CH_3), 1.39 (3 H, d, $^3J_{HH}$ 6.90, CH_3), 3.86 (3 H, s, OCH_3), 3.91 (3 H, s, OCH_3), 3.87-4.01 (1H, m, CH), 5.40-5.48 (1 H, m, CH_2), 6.94 (1 H, s, H_{ar6}), 7.34-7.54 (4H, m, H_{ar}) 7.84 (2 H, d, $^3J_{HH}$ 7.17, H_{ar}). HPLC retention time on a Analytical SB-C18 Zorbax (4.6 x 250 mm) column using a 30 minutes linear gradient from 0 to 100% Acetonitrile a 0.1% TFA water solution at 1 mL/min: 25.5 min.

γ-2-(4,5-dimethoxy-2-nitrophenyl)-propyl *N*-BOC-L-glutamate : 2-(4,5-dimethoxy-2-nitrophenyl)propan-1-ol **3** (264 mg, 1 mmol) was dissolved in dry $CHCl_3$ (20 cm^3) under argon. DCC (481 mg, 2.2 mmol), *α-t*-butyl *N*-BOC-L-glutamic acid (669 mg, 2.2 mmol) and DMAP (20 mg, cat.) were added. After 0.5 h at room temperature, the reaction mixture was quenched by a saturated $NaHCO_3$ aqueous solution (50 cm^3). The aqueous layer was extracted with EtOAc (100 cm^3 x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with 40/60 heptane/ EtOAc, to give γ-2-(4,5-dimethoxy-2-nitrophenyl)-propyl *N*-BOC-L-glutamate (502 mg, 0.97 mmol, 97%) as a yellow solid. NMR (300 MHz, $CDCl_3$) d_H = 1.33 (3 H, d, $^3J_{HH}$ 7.23, CH_3), 1.43 (9 H, s, CH_3 *t*Bu), 1.45 (9 H, s , CH_3 Boc), 1.70-2.40 (4H, m), 3.92 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3), 4.00-4.10 (1H, m, CH), 4.23 (2H, m, CH_2), 5.10 (1H, sl, NH), 6.81 (1 H, s, H_{ar6}), 7.43 (1 H, s, H_{ar3}). d_c(75 MHz, $CDCl_3$) = 18.25 (s, CH_3CH), 28.20 (s, CH_3 *t*-Bu), 28.30 (s, CH_2 β), 28.67 (s, CH_3 *t*-Bu), 30.57 (s, CH_2 γ), 33.33 (s, CH_3CH), 53.69 (s, CH_2 α), 56.68 (s, OCH_3), 56.71 (s, OCH_3), 68.69 (s, CH_3CHCH_2), 81.47 (s, C *t*-Bu), 82.57 (s, C Boc), 108.34 (s, C_{ar3}), 109.61 (s, C_{ar6}), 132.54 (s,C_{ar1}), 142.81 (s,C_{ar2}), 147.74 (s,C_{ar4}), 153.19 (s,C_{ar5}), 155.74 (s, CO Boc), 171.62 (s, CO), 173.00 (s, CO *t*Bu).

General procedure for the deprotection of α-*t*-butyl γ-caged-2-butyl *N*-BOC-L-glutamate: α-*t*-butyl γ-3-(4,5-dimethoxy-2-nitrophenyl)-2-butyl *N*-BOC-L-glutamate (157 mg, 0.31 mmol) was dissolved in dry CH_2Cl_2 (4 cm^3), trifluoroacetic acid (TFA; 3 cm^3) was added dropwise at room temperature. The reaction mixture was diluted by H_2O (50 cm^3) after 5 h at room temperature, the product was extracted with EtOAc (3 x 100 cm^3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the γ-3-(4,5-dimethoxy-2-nitrophenyl)-2-butyl-L-glutamate (DMNPB-caged Glu) **IV** (103 mg, 0.27 mmol, 85%) as a yellow solid.

γ-2-(4,5-dimethoxy-2-nitrophenyl)-propyl-L-glutamate **III** NMR (300 MHz, $CDCl_3$) d_H =

1.46 (3 H, d, $^3J_{HH}$ 7.23, CH_3), 1.70-2.70 (4H, m), 3.91 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3), 4.00-4.30 (4H, m, 2 x CH and CH_2), 6.80 (1 H, s, H_{ar6}), 7.42 (1 H, s, H_{ar3}). d_c(50 MHz, MeOD) = 16.98 (s, *C*H_3CH), 25.80 (s, CH_2 β), 29.63 (s, CH_2 γ), 33.37 (s, CH_3*C*H), 52.50 (s, CH_2 α), 55.78 (s, OCH_3), 55.91 (s, OCH_3), 68.75 (s, CH_3CHCH_2), 108.03 (s, C_{ar3}), 109.89 (s, C_{ar6}), 132.10 (s,C_{ar1}), 143.02 (s,C_{ar2}), 147.92 (s,C_{ar4}), 153.40 (s,C_{ar5}), 172.69 (s, CO), 172.80 (s, COOH). HPLC retention time on a Analytical SB-C18 Zorbax (4.6 x 250 mm) column using a 30 min linear gradient from 0 to 100% acetonitrile in a 0.1% TFA water solution at 1 mL/min: 18.1 min.

UV and HPLC analysis of the photolysis of compounds I-IV

A solution (4 mL) of O.2 mM **(I-IV)** in 100 mM phosphate buffer, pH 7.2 was exposed to a 1000 W Hg Lamp from Hanovia focused on the entrance slit of a monochromator at 364 nm (± 0.2 nm). The reactions were monitored by UV and aliquots of samples (100 µL) were injected into Waters 600E HPLC carried out on a Acclaim C18 column (4.6 x 300 nm); elution was performed at a flow rate of 1 mL/min with a linear gradient of acetonitrile in an aqueous solution of TFA (0.1%) from 0 to 100% (*v/v*) over 30 min. The compounds were detected by a Waters 2996 PDA detector operating beetween 200 and 600 nm. Compounds **I-IV** have a retention time of 25.0, 25.5 , 16.8 and 17.0 min respectively, benzoic acid has a retention time of 14.0 min and was quantified for during the photolysis of **I** and **II** and many photolytic by-products were detected presumably due to a photochemical instability of the 1-propylenyl-2-nitro-4,5-dimethoxybenzene derivatives.

Quantification of glutamate release. The glutamic acid formation was quantified by HPLC after formation of a chromophoric derivative formed quantitatively after condensation with o-phtaldialdehyde[1] in a 50 mM Borate buffer pH 9. HPLC retention time on a analytical SB-C18 Zorbax (4.6 x 250 mm) column using a 30 min linear gradient from 0 to 100% Acetonitrile in a 0.1% TFA water solution at 1 mL/min of the glutamate *o*-phtaldialdehyde adduct: 16.7 min.

References

[1] R.F. Chen, C. Scott, E. Trepman, *Biochim. Biophys. Acta* **1979**, *576*, 440-445.