Supporting Information

Engineering a light-regulated $GABA_A$ receptor for optical control of neural inhibition

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A. Supporting Data

B. Synthesis of MAM-6, MAB-6, MAM-0, and MAB-0

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A. Supporting Data

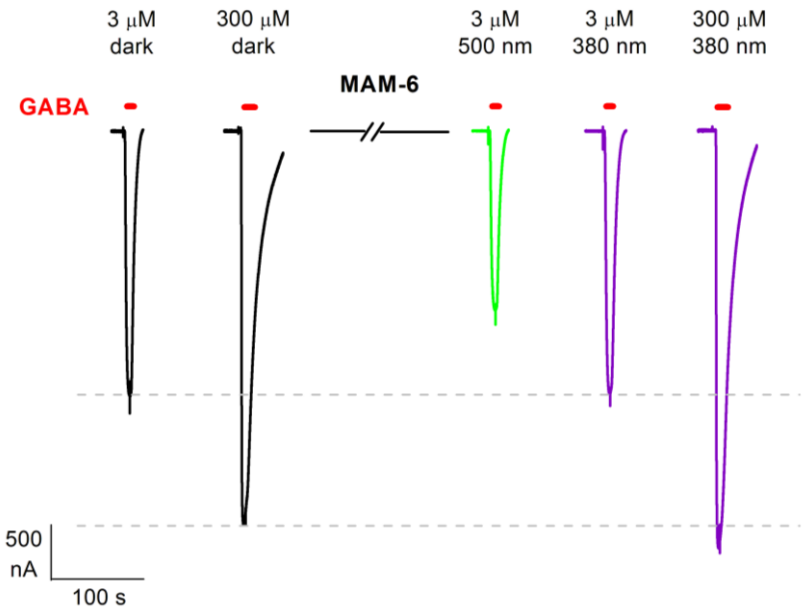


Figure S1. *Trans* MAM-6 inhibits a mutant $GABA_AR$ after conjugation. An oocyte expressing α1(T125C)β2 was tested with 3 μM and 300 μM GABA (black traces on the left). The oocyte was then treated with 50 μM MAM-6 for 10 minutes. After MAM-6 treatment, the current at 3 μM GABA was reduced in 500-nm (green trace) but not in 380-nm light (middle purple trace). The responses evoked by 300 μM GABA before and after MAM-6 attachment indicated that the change in receptor activity was negligible over the course of the experiment. Neither 380-nm nor 500-nm light triggered receptor activation in the absence of externally applied GABA.

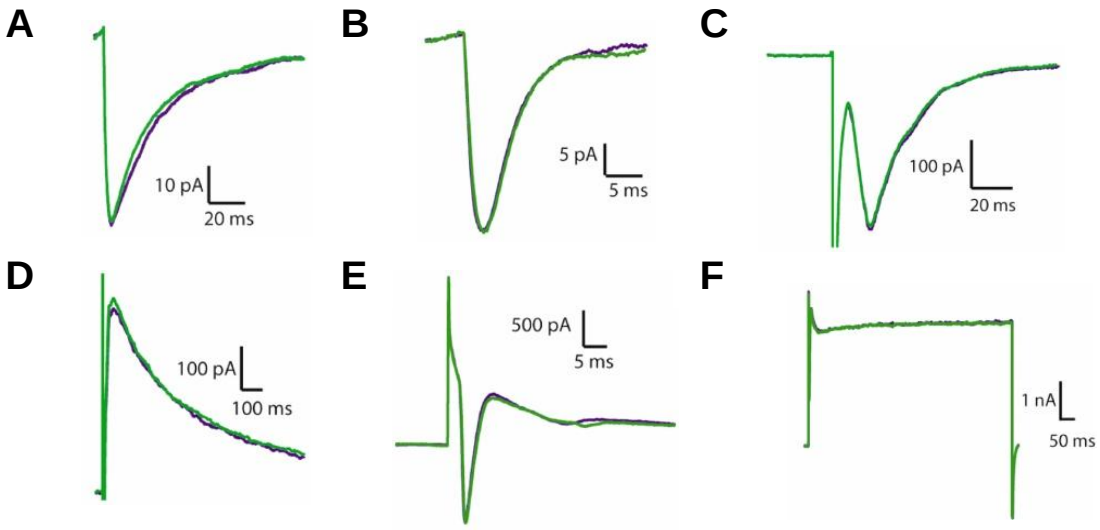


Figure S2. PTL treatment does not confer light sensitivity onto endogenous channels in hippocampal neurons.

(A) MAB-0 treatment does not photosensitize endogenous $GABA_A$Rs in cultured hippocampal neurons. Average mIPSC traces from a representative neuron are shown. I_{500}/I_{380} = 1.00 ± 0.02 (V_{hold} = –60 mV; n = 3, *P* = 0.87).

(B) MAB-0 treatment does not photosensitize endogenous glutamate receptors in cultured hippocampal neurons. Average mEPSC traces from a representative neuron are shown. I_{500}/I_{380} = 0.97 ± 0.10 (V_{hold} = –70 mV; n = 4, *P* = 0.64).

(C) MAB-0 treatment does not photosensitize endogenous AMPARs in a hippocampal slice. Evoked EPSC traces from a CA1 pyramidal neuron are shown. I_{500}/I_{380} = 1.02 ± 0.01 (V_{hold} = –70 mV; n = 4, *P* = 0.2).

(D) MAB-0 treatment does not photosensitize endogenous NMDARs in a hippocampal slice. Evoked EPSC traces from a CA1 pyramidal neuron are shown. Currents were measured 100 ms after the stimulus. I_{500}/I_{380} = 0.95 ± 0.04 (V_{hold} = +40 mV; n = 3, *P* = 0.3).

(E) MAB-0 treatment does not photosensitize endogenous voltage-gated Na^+ channels. Inward currents (elicited by a 200-ms depolarization to –10 mV) from a representative neuron are shown. I_{500}/I_{380} = 0.99 ± 0.05 (V_{hold} = –70 mV; n = 4, *P* = 0.87).

(F) MAB-0 treatment does not photosensitize native voltage-gated K^+ channels. Voltage-gated K^+ currents (elicited by a 200-ms depolarization to +40 mV) from a representative neuron are shown. I_{500}/I_{380} = 0.98 ± 0.05 (V_{hold} = –70 mV; n = 4, *P* = 0.43).

All traces in 380 nm and 500 nm are shown in violet and green colors, respectively.

Table S1. The GABA Sensitivity of Untreated and MAM-6 treated α1-Mutants.

mutation site	MAM-6 conjugated receptor				untreated receptor		comments
	I_{500}/I_{380} (mean ± SEM) [a]	trials	$I_{3\mu M}/I_{3mM}$ (380 nm) (mean ± SEM) [b]	trials	$I_{3\mu M}/I_{3mM}$ (mean ± SEM) [b]	trials	
wild-type	1.08 ± 0.02 (untreated)	9	--	--	0.46 ± 0.03	9	
41	0.62 ± 0.03	10	0.41 ± 0.03	9	0.33 ± 0.03	3	*c*
68	0.47 ± 0.05	8	0.17 ± 0.03	4	0.34 ± 0.03	3	*c,d*
121	0.52 ± 0.03	14	0.34 ± 0.03	14	0.49 ± 0.02	4	*d*
125	**0.60 ± 0.03**	**10**	**0.44 ± 0.07**	**7**	**0.45 ± 0.05**	**4**	***e***
171	0.63 ± 0.03	4	0.20 ± 0.01	4	0.20 ± 0.01	3	*c,d*

a. Current ratio was measured at 3 μM GABA (500 nm vs. 380 nm).

b. The effect of MAM-6 attachment or cysteine substitution on receptor function was evaluated by the change of current ratio at 3 μM vs. 3 mM GABA. As a reference, EC_{50} of the wild-type α1β2 is ~3 μM (first row).

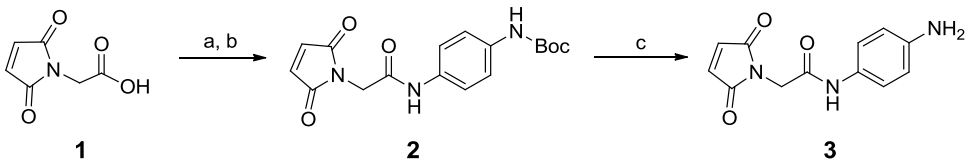
c. The untreated receptor's sensitivity to GABA is lower than that of the wild-type (indicated by $I_{3\mu M}/I_{3mM}$).

d. The receptor's sensitivity to GABA is reduced in both 500-nm and 380-nm lights after MAM-6 attachment.

e. The untreated receptor's sensitivity to GABA is the same as that of the wild-type. The receptor's sensitivity to GABA is unaltered in 380-nm light after MAM-6 attachment. These features make α1(T125C) the best mutant for neurophysiological applications.

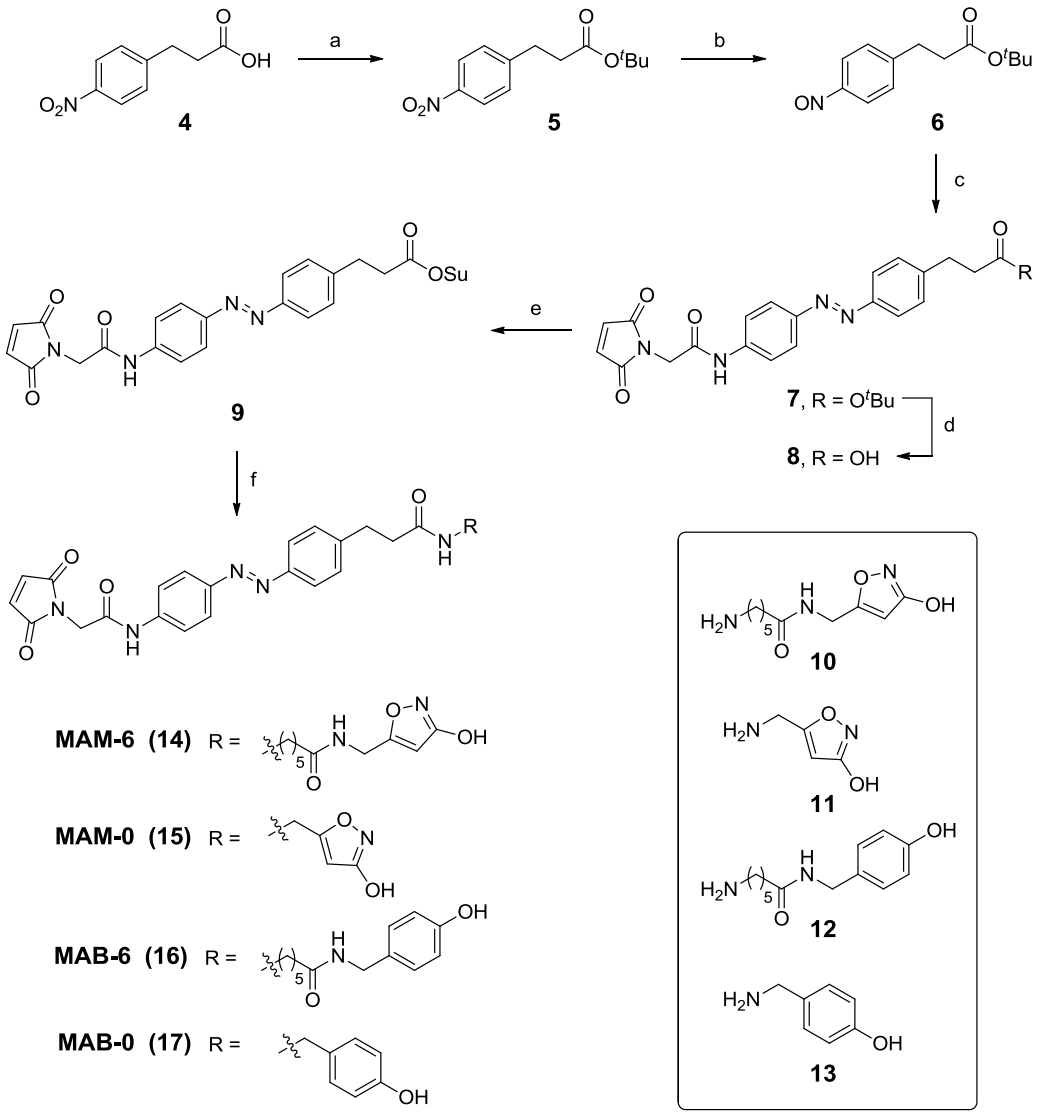
B. Synthesis of MAM-6, MAB-6, MAM-0, and MAB-0

Scheme S1.



Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, rt, 4 h; b) 4-H_2N-Ph-NH-Boc, Et_3N/THF, 81%; c) 10% TFA in CH_2Cl_2, rt, 6 h, 98%.

Scheme S2.

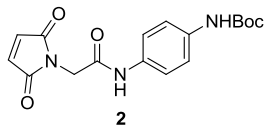


Reagents and conditions: (a) i) ^{t}BuOH/Bu_3N, 2-chloro-1-methylpyridinium iodide,Toluene, reflux for 12 h, 84%; b) i) NH_4Cl/Zn, CH_3O-CH_2CH_2OH, 6 h, rt; ii) $FeCl_3.6H_2O$, 0^{o}C to rt, 2 h; (c) **3**, AcOH, rt, 48 h, 51%; d) 10% TFA in CH_2Cl_2, 97%; e) HOSu/EDC CH_3CN:DMF (3:1) rt, 12 h, 91%; f) (i) **10,** DIPEA/NMP, rt, 24 h, 27%; (ii) **11**,DIPEA/NMP, rt, 24 h, 39%; (iii) **12,** DIPEA/NMP, rt, 24 h, 43%; (iv) **13,** Et_3N/DMF, rt, 12 h, 60%.

▪ General Methods

All reagents and solvents were purchased through Fisher Scientific. All reactions involving air- and moisture-sensitive reagents were performed under an argon atmosphere using syringe-septum cap techniques. DMF and CH_2Cl_2 were dried by storing over molecular sieves (4 Å). THF was distilled from sodium/benzophenone. Analytical thin-layer chromatography (TLC) was carried out using Merck Silica gel 60 F254 aluminum sheets. Compounds were detected as single spots on TLC plates and visualized

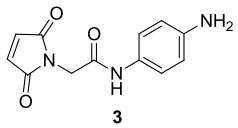
using UV light (254 or 366 nm) and $KMnO_4$ or ninhydrin. Merck silica gel (35–70 mesh) was used for flash chromatography. ^{1}H NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the standard. Chemical shifts are reported in parts per million (ppm). When peak multiplicities are given, the following abbreviations are used: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. ^{13}C NMR spectra were proton decoupled and recorded on a 100 MHz spectrometer using carbon signal of the deuterated solvent as the internal standard. Mass spectra were measured either on a Waters ZQ device for LRMS or at the Notre Dame Mass Spectrometry facility microTOF for HRMS.



2

tert-butyl-4-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido)phenylcarbamate (2): Maleimide **1** (2.0 g, 13 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL), treated with oxalyl chloride (4.8 g, 39 mmol), and DMF (1 drop) at ambient temperature under nitrogen. After 4 h of stirring at room temperature, the solvent was evaporated under reduced pressure, the residue dissolved in anhydrous THF, and added drop wise over a period of 30 min to a stirred suspension of tert-butyl-4-aminophenylcarbamate (2.48 g, 12 mmol) and triethylamine (1.2 g, 12 mmol) at 0 ^{o}C. After completion of addition, the reaction mixture was brought to ambient temperature and stirred for an additional 4 h. The solvent was evaporated under reduced pressure and dissolved in EtOAc (50 mL), washed with aq. $NaHCO_3$, and brine. The extract was dried with anhydrous Na_2SO_4, evaporated, and recrystallized from ethanol to obtain compound **2** (3.4 g, 81%).

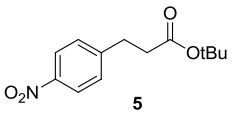
mp: 185-187 ^{o}C.
IR (film) *vmax* 3327.68, 2983.05, 1706.99, 1684.45, 1549.21, 1512.59, 1428.06, 1301.28, 1225.21, 1157.60, 1058.99, 830.96, 695.3.
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H), 7.92 (s, 1H), 7.37 (d, 2H, J = 9.11 Hz), 7.28 (d, 2H, J = 7.33 Hz), 6.72 (s, 2H), 4.24 (s, 2H), 1.42 (s, 9H).
LRMS (ESI) *m/z* calcd for $C_{17}H_{20}N_3O_5$ $[M+H]^+$: 346.4, found 346.8.
HRMS (ESI) *m/z* calcd for $C_{17}H_{19}N_3NaO_5$ $[M+Na]^+$: 368.1217, found 368.1208.



3

***N*-(4-aminophenyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (3):** tert-Butyl-4-(2-(2,5-dioxo-2H-pyrrol-1(5H)-yl)acetamido)phenylcarbamate **2** (3.0 g, 8.6 mmol) was dissolved in 5% TFA in CH_2Cl_2 (50 mL) and stirred at room temperature for 2 h. The reaction mixture was evaporated and dried under reduced pressure to obtain compound **3** (2.0 g, 98%)**.**

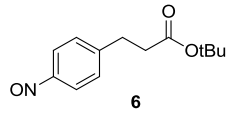
IR (film) *vmax* 3192.09, 3062.14, 2994.35, 1593.22, 1721.07, 1692.90, 1667.54, 1554.85, 1506.95, 1422.43, 1312.55, 1180.13, 1118.15, 825.14, 692.73.
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.92 (bs, 2H), 7.5 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.48 Hz), 6.92 (s, 2H), 4.24 (s, 2H).
LRMS (ESI) *m/z* calcd for $C_{12}H_{12}N_3O_3$ $[M+H]^+$: 246.2, found 246.1.
HRMS (FAB+) *m/z* calcd for $C_{12}H_{12}N_3O_3$ $[M+H]^+$: 246.0873, found 246.0879.



5

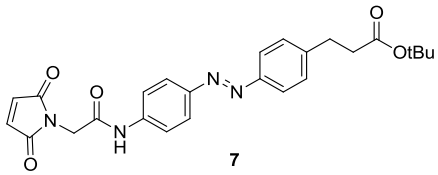
***tert*-butyl-3-(4-nitrophenyl)propanoate (5):** 3-(4-Nitrophenyl)propanoic acid **4** (5.0 g, 25.6 mmol), tert-butanol (1.88 g, 25.6 mmol), and tributylamine (14.8 mL, 62.2 mmol) were dissolved in toluene (100 mL) and added to a suspension of 2-chloro-1-methylpyridinium iodide (7.84 g, 30.6 mmol) in toluene (50 mL). The reaction mixture was refluxed overnight. After removal of the solvent, the dark residue was purified by flash column chromatography (EtOAc/hexane 1:3) to obtain tert-butyl-3-(4-nitrophenyl)propanoate **5** (4.20 g, 84 %) as light yellow solid.

mp: 50 ^{o}C (consistent with the previously reported value; ref. 8)
IR (film) *vmax* 2926.55, 2971.75, 1712.62, 1605.56, 1591.47, 1512.59, 1543.58, 1368.90, 1343.54, 1290.01, 1146.33, 844.86, 689.9.
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, 2H, J = 8.75 Hz), 7.36 (d, 2H, J = 8.80 Hz), 3.00 (t, 2H, J = 7.53 Hz), 2.58 (t, 2H, J = 7.57 Hz), 1.40 (s, 9H).
LRMS (ESI) *m/z* calcd for $C_{13}H_{18}NO_4$ $[M+H]^+$: 252.3, found 252.4.



6

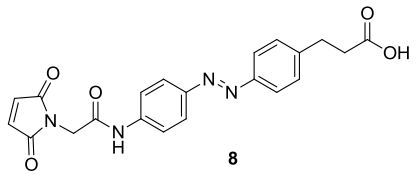
***tert*-butyl-3-(4-nitrosophenyl)propanoate (6):** Tert-butyl-3-(4-nitrophenyl)propanoate (4.18 g, 17.2 mmol) was dissolved in 2-methoxyethanol (200 mL) and to this mixture was added a solution of NH_4Cl (1.36 g, 25.42 mmol) in H_2O (60 mL). The reaction mixture was thoroughly degassed by bubbling a stream of argon through the reaction mixture for approximately 30 min. Zn dust (3.7 g, 56.6 mmol) was added to the solution very slowly. The reaction mixture was stirred for 6 h at room temperature, filtered, and slowly added to a solution of $FeCl_3.6H_2O$ (9.28 g, 36.3 mmol) in a 2:1 mixture of H_2O and EtOH at -10 ℃. After 1 h the mixture was allowed to warm to room temperature and stirred for another hour. The mixture was extracted with EtOAc (3 x 100 mL) and dried with anhydrous Na_2SO_4. The solvent was evaporated to obtain an oil that was purified by flash chromatography (EtOAc /hexane 1:3) to afford compound **6** (3.34 g) as a dark green oil that was used without further purification.



7

***tert*-butyl-3-{4-[(E)-2-{4-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido]phenyl}diazen-1-yl]phenyl}propanoate (7):** A solution of compound **6** (3.0 g, 13.2 mmol) in glacial acetic acid (125 mL) was added to a solution of compound **3** (3.36 g, 13.2 mmol) in glacial acetic acid (125 mL) and stirred for 48 h at room temperature. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (EtOAc/hexane 1:1) to obtain compound **7** (3.14 g, 51%) as an orange solid.

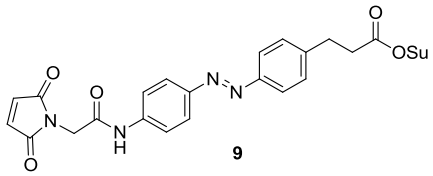
mp: 197-199 ^{o}C.
IR (film) *vmax* 3327.68, 3084.74, 2920.9, 1701.35, 1676.0, 1594.29, 1543.58, 1425.25, 1140.69, 827.96, 695.54.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (br, 1H), 7.88 (d, 2H, *J* = 8.75 Hz), 7.83 (d, 2H, *J* = 8.25 Hz), 7.64 (d, 2H, *J* = 8.69 Hz), 7.35 (d, 2H, *J* = 8.26 Hz), 6.83 (s, 2H), 4.39 (s, 2H), 3.00 (t, 2H, *J* = 7.51 Hz), 2.61 (t, 2H, *J* = 7.57 Hz), 1.44 (s, 9H).
LRMS (ESI) *m/z* calcd for $C_{25}H_{27}N_4O_5$ $[M+H]^+$: 463.5, found 463.9.
HRMS (ESI) *m/z* calcd for $C_{215}H_{26}N_4NaO_5$ $[M+Na]^+$: 485.1795, found 485.1780.



8

3-(4-{4-[2-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-acetylamino]-phenylazo}-phenyl)-propionic acid (8): To a solution of compound **7 (**2.74 g, 5.93 mmol) in CH_2Cl_2 (300 mL) was added TFA (30 mL). After stirring for 24 h at room temperature, water (30 mL) was added and the organic layer was separated and washed with water and brine. After drying with $MgSO_4$, the solvent was removed under reduced pressure to obtain compound **8** (2.35 g, 97%) as an orange-brown solid.

mp: 240-242 ^{o}C.
IR (film) *vmax* 3333. 33, 3084.74, 2932.20, 1706.99, 1681.63, 1597.11, 1543.58, 1433.70, 1306.92, 1244.93, 1205.49, 1154.96, 844.86, 695.54.
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.25 (s, 1H), 11.22 (s, 1H), 7.88-7.83 (m, 6H), 7.44 (d, 2H, *J* = 8.33 Hz), 7.16 (s, 2H), 4.37 (s, 2H), 2.91 (t, 2H, *J* = 7.46 Hz), 2.60 (t, 2H, *J* = 7.58 Hz).
LRMS (ESI) *m/z* calcd for $C_{21}H_{19}N_4O_5$ $[M+H]^+$: 407.4, found 407.5.
HRMS (FAB+) *m/z* calcd for $C_{21}H_{19}N_4O_5$ $[M+H]^+$: 407.1350, found 407.1355.

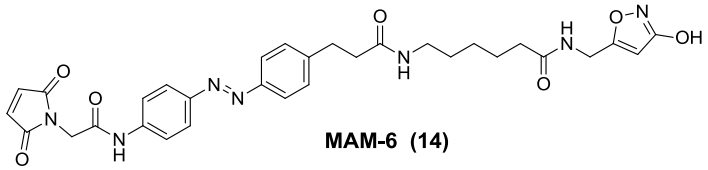


9

3-(4-{4-[2-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-acetylamino]-phenylazo}-phenyl)-propionic acid 2,5-dioxo-pyrrolidin-1-yl ester (9): To a mixture of compound **8** (0.540 g, 1.33 mmol) and N-hydroxysuccinimide (0.148 g, 1.33 mmol) in dry CH_3CN:DMF (3:1, 20 mL), EDC-HCl (0.318 g, 1.66 mmol) was added. The reaction mixture was stirred under a nitrogen atmosphere at room temperature overnight. The organic solvents were removed under reduced pressure and the remaining residue washed with water to obtain an orange solid. The crude solid was recrystallized from acetone to afford compound **9** as an orange solid (610 mg, 91%).

mp**:** 248-250 ^{o}C.
IR (film) *vmax* 3327.68, 3090.39, 2926.55, 2841.80, 1774.60, 1698.53, 1676.0, 1594.29, 1540.78, 1540.76, 1422.43, 1301.28, 1205.49, 1149.14, 2, 1064.68, 833.59, 642.01.
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 7.90 (d, 2H, *J* = 8.93), 7.86-7.83 (m, 4H), 7.54 (d, 2H, *J* = 8.28 Hz), 7.00 (s, 2H), 4.42 (s, 2H), 3.15 (t, 2H, *J* = 7.12), 3.05 (t, 2H, *J* = 6.96 Hz), 2.88 (s, 4H).

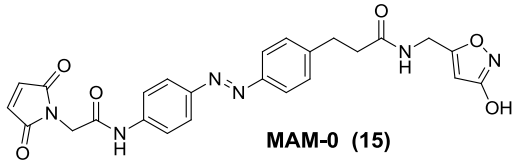
^{13}C NMR (100 MHz, DMSO-d_6,) δ 170.3, 169.5, 168.1, 165.1, 151.4, 148.6, 143.1, 141.4, 134.7, 129.3, 123.6, 122.7, 119.6, 40.4, 31.8, 25.4.
LRMS (ESI) *m/z* calcd for $C_{25}H_{22}N_5O_7$ $[M+H]^+$: 504.5, found 504.2.



MAM-6 (14)

MAM-6 (14): Compound **9** (39.9 mg, 73 µmol) and DIPEA (12 µL, 73 µmol) were added to a solution of tethered muscimol **10** (16 mg, 73 µmol) that was prepared as described (ref. 1), in N-methylpyrrolidinone (2 mL). The reaction mixture was stirred at room temperature overnight and quenched with TFA (6 µL, 73 µmol). The crude product was purified by silica gel chromatography (CH_2Cl_2:MeOH 98:2 to CH_2Cl_2:MeOH:AcOH:H_2O - 94:6:0.1:0.1) to afford an orange solid (12.8 mg, 27%).

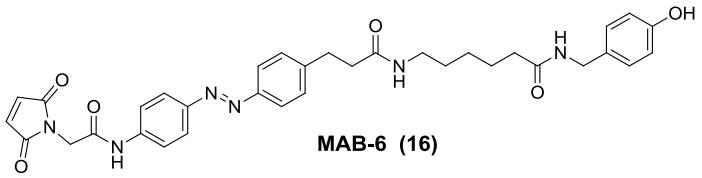
mp: 235-237 ^{o}C.
IR (film) *vmax* 3305.08, 2847.45, 2920.9, 1709.8, 1673.18, 1628.1, 1535.13, 1428.05, 1250.57, 1151.96, 847.0 695.54.
UV (CH_3CN) λ_{max}, nm: 357.
^{1}H-NMR (400 MHz, $CDCl_3$+DMSO-d_6) δ 7.88 (d, 2H, *J* = 8 Hz), 7.81 (d, 2H, *J* = 8 Hz), 7.73 (d, 2H, *J* = 8 Hz), 7.36 (d, 2H, *J* = 8 Hz), 6.87 (s, 2H), 5.76 (s, 1H), 4.40 (s, 2H), 4.32 (s, 2H), 3.14 (t, 2H, *J* = 7 Hz), 3.02 (t, 2H, *J* = 7 Hz), 2.88 (s, 1H), 2.52 (t, 2H, *J* = 7 Hz), 2.17 (t, 2H, *J* = 7.01 Hz), 1.58 (m, 2H), 1.42 (m, 2H), 1.24 (m, 2H).
^{13}C-NMR (100 MHz, $CDCl_3$+DMSO-d_6) δ 173.1, 171.7, 170.7,170.5, 170.4, 165.2, 150.9, 148.3,144.9, 141.3, 134.8, 129.2, 123.7, 122.7, 119.7, 93.6, 39.0, 37.4, 35.7, 35.4, 31.6, 29.5, 29.2, 26.5, 25.3.
HRMS (ESI) *m/z* calcd for $C_{31}H_{34}N_7O_7$ $[M+H]^+$: 616.2514, found 616.2441.



MAM-0 (15)

MAM-0 (15): Compound **9** (34 mg, 67 µmol) and DIPEA (11 µL, 67 µmol) were added to a solution of muscimol (7.7 mg, 67 µmol) in N-methylpyrrolidinone (1.9 mL). The reaction mixture was stirred at room temperature overnight and quenched with TFA (5.1 µL, 67 µmol). The crude product was purified by silica gel chromatography (CH_2Cl_2: MeOH 98:2 to CH_2Cl_2: MeOH:AcOH:H_2O - 94:6:0.1:0.1) to afford an orange solid (13 mg, 39%).

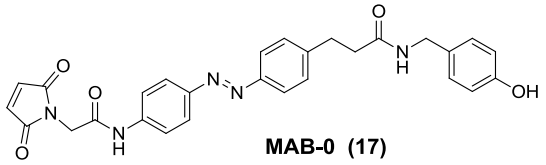
mp: 248-250 ^{o}C.
IR (film) *vmax* 3271.18, 2920.9, 2847.45, 1712.6, 1678.8, 1622.4, 1532.31, 1312.55, 1151.9.
UV (CH_3CN) λ_{max}, nm: 354.
^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6) δ 10.20 (s, 1H), 8.25 (t, 1H, *J* = 5.53 Hz), 7.76 (d, 2H, *J* = 8.86 Hz), 7.71-7.65 (q, 4H), 7.25 (d, 2H, *J* = 8.36 Hz), 6.78 (s, 2H), 5.61 (s, 1H), 4.30 (s, 2H), 4.24 (d, 2H, *J* = 5.59 Hz), 2.93 (t, 2H, *J* = 7.90 Hz), 2.47 (t, 2H, *J* = 7.70 Hz).
^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) δ 171.5, 171.0, 169.6, 170.2, 165.3, 151.2, 148.8, 144.6, 141.4, 134.0, 128.5, 123.9, 123.0, 119.9, 94.0, 36.6, 34.8, 30.8, 24.8.
HRMS (ESI) *m/z* calcd for $C_{25}H_{23}N_6O_6$ $[M+H]^+$: 503.1674, found 503.1670.



MAB-6 (16)

MAB-6 (16): Compound **9** (0.075 g, 0.14 mmol) and DIPEA (24 µL, 0.14 mmol) were added to a solution of tethered 4-hydroxybenzylamine **12** (35 mg, 0.14 mmol) in N-methylpyrrolidinone (3.0 mL). The reaction mixture was stirred at room temperature overnight and quenched with TFA (11 µL) and diluted with EtOAc (50 mL). To the mixture was added water (40 mL) and extracted with EtOAc (3 x 50 mL), dried (Na_2SO_4), and evaporated. The crude residue was purified by silica gel chromatography (CH_2Cl_2:MeOH 98:2 to CH_2Cl_2:MeOH:AcOH:H_2O 90:10:0.1:0.1) to afford an orange solid (37.6 mg, 43%).

IR (film) *vmax* 3305.08, 2932.20, 2847.45, 2451.97, 2401.12, 1706.99, 1670.58, 1622.46, 1543.58, 1509.77, 1428.06, 1247.75, 833.59, 695.54.
UV (CH_3CN) λ_{max}, nm: 355.
^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (d, 2H, *J* = 8.89 Hz), 7.75 (d, 2H, *J* = 8.35 Hz), 7.67 (d, 2H, *J* = 8.87 Hz), 7.37 (t, 1H, *J* = 5.65 Hz), 7.30 (d, 2H, *J* = 8.37 Hz), 7.04 (d, 2H, *J* = 8.49 Hz), 6.83 (s, 2H), 6.72 (d, 2H, *J* = 8.51 Hz), 4.32 (s, 2H), 4.21 (s, 2H), 3.09 (t, 2H, *J* = 6.87 Hz), 2.96 (t, 2H, *J* = 7.31 Hz), 2.47 (t, 2H, *J* = 7.82 Hz), 2.10 (t, 2H, *J* = 7.35 Hz), 1.50-1.58 (m, 2H), 1.36-1.43 (m, 2H), 1.15-1.21 (m, 2H).
^{13}C NMR (100 MHz, $CHCl_3$+CD_3OD) δ 172.3, 172.0, 170.5, 167.9, 155.9, 150.4, 143.5, 137.3, 133.9, 128.9, 128.6, 124.0, 123.2, 123.0, 122.5, 120.0, 114.7, 42.6, 40.1, 39.3, 36.6, 35.7, 31.2, 29.1, 25.3, 25.1.
LRMS (ESI) *m/z* calcd for $C_{34}H_{37}N_6O_6$ $[M+H]^+$: 625.7, found 625.3.
HRMS (ESI) *m/z* calcd for $C_{34}H_{36}N_6O_6$ $[M–H]^-$: 623.2624, found 623.2618.



MAB-0 (17)

MAB-0 (17): Compound **9** (0.05 g, 0.1 mmol) and 4-hydroxybenzylamine (12.2 mg, 0.1 mmol) were dissolved in anhydrous DMF (1 mL) and treated with triethylamine (42 µL, 0.3 mmol). The reaction mixture was stirred overnight at room temperature. The organic solvent was evaporated and the crude residue was purified by silica gel chromatography (CH_2Cl_2:MeOH 98:2 to CH_2Cl_2:MeOH:AcOH:H_2O 90:10:0.1:0.1) to afford an orange solid (7.6 mg, 60%).

IR (film) *vmax* 3305.08, 3033.89, 2920.9, 1704.17, 1630.92, 1597.11, 1540.76, 1419.61, 1160.41, 844.86, 689.91.
UV (CH_3CN) λ_{max}, nm: 357.
^{1}H NMR (400 MHz, CD_3OD) δ 7.84 (d, 2H, *J* = 9.09 Hz), 7.75 (d, 2H, *J* = 8.34 Hz), 7.68 (d, 2H, *J* = 8.34 Hz), 7.30 (d, 2H, *J* = 8.34 Hz), 6.97 (d, 2H, *J* = 8.59 Hz), 6.69 (d, 2H, *J* = 8.34 Hz), 4.36 (s, 2H), 4.22 (d, 2H, *J* = 4.04 Hz), 2.99 (d, 2H, *J* = 7.33 Hz), 2.51 (d, 2H, *J* = 7.58 Hz).
^{13}C NMR (100 MHz, $CHCl_3$+CD_3OD) δ 172.51, 170.28, 167.94, 155.80, 150.92, 143.79, 137.08, 134.23, 128.79, 128.71, 124.28, 123.37, 123.26, 122.50, 119.98, 114.94, 42.6, 40.03, 37.39, 31.29.
HRMS (ESI) *m/z* calcd for $C_{26}H_{26}N_5O_5$ $[M+H]^+$: 512.1928, found 512.1856.

C. Supporting Materials and Methods

C.1. Plasmid and virus preparation

- Wild-type and mutant cDNAs

The cDNAs of wild-type rat $GABA_AR$ α1 (in pGH19), β2 (in pGH19), and γ2S (in pUNIV) were obtained from Professor Cynthia Czajkowski (University of Wisconsin). The genes of α and β subunits were sub-cloned into vector pCDNA3.1 for expression in HEK293T cells. Mutants α1(K70C) and α1(D123C) (in pGH19) were obtained from Professor Cynthia Czajkowski. Other cysteine mutants were prepared by site-directed mutagenesis in the wild-type α1 clones (in pGH19 and pCDNA3.1 for expression in oocytes and HEK293T cells, respectively). Mutations were confirmed by sequencing.

- Construction of pAAV-hSyn-eGFP-2A-myc-α1(T125C)

This bi-cistronic construct was designed to express eGFP and $GABA_AR$ α1(T125C) in a 1:1 ratio using the 2A protein sequence (GSGATNFSLLKQAGDVEENPGP)[2] and to restrict gene expression in neurons using a human synapsin promoter.[3] The vector also contains terminal repeats (TR) and woodchuck hepatitis post-transcriptional regulatory element (WPRE) for packaging into the adeno-associated virus (AAV). A *myc* epitope tag (EQKLISEEDL) was inserted into the N-terminus of α1(T125C) as previously described.[4] The 2A-myc-α1(T125C) fragment was made with primers 5'-TCGATCGGGCCCATGAAGAAAAGTCGGGG-3' and 5'-TCGATCAAGCTTCTATTGATGGGGTGTGGG-3'. The PCR product was digested with ApaI and HindIII to obtain the insert fragment. The eGFP-2A fragment was made via PCR with primers 5'-TCGATCGCTAGCGCTACCGGTCGCCACCATGGTGAGCAAGGGCGAGG-3' and 5'-TCGATCGGGCCCTGGGTTCTCCTCCACGTCTCCAGCCTGCTTGAGCAGGGAGAAGTTTGTAG CGCCAGATCCCTTGTACAGCTCGTCCATGC-3' followed by digestion with ApaI and NheI. The pAAV vector backbone was digested with NheI and HindIII and then ligated with the 2A-myc-α1(T125C) and eGFP-2A fragments. The final construct was confirmed by sequencing.

- Preparation of Adeno Associated Virus (AAV)

AAV9 encoding GFP-2A-myc-α1(T125C) was produced from transfected HEK293T cells as described previously.[5] After ultracentrifugation, the interphase between the 54% and 40% iodixanol fraction, and the lower three-quarters of the 40% iodixanol fraction were extracted and diluted with an equal volume of phosphate-buffered saline (PBS) plus 0.001% Tween 20. This fraction was then buffer exchanged and concentrated with Amicon Ultra-15 Centrifugal Filter Units to a final volume of ~100 µL. Virus was then titered for DNase-resistant vector genomes by Real-Time qPCR relative to WPRE standards. The resulting titer was 7.5×10^{12} vg·mL^{-1}.

C.2. Electrophysiology

- Two-electrode voltage-clamp

Each oocyte was placed in a 0.2-mL perfusion chamber, impaled with two glass microelectrodes (1–2.5 MΩ resistance) filled with 3 M KCl, and voltage clamped (at –80 mV) with an OC-725C amplifier (Warner Instruments) in OR1 solution containing (in mM): 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES; pH 7.4. Drug perfusion, data acquisition, and analysis were carried out with Clampex software through a Digidata 1200 interface (Molecular Devices). Light switching was carried out by a Lambda DG-4 (Sutter Instruments) using 383 nm and 497 nm filters.

- Whole-cell voltage-clamp (HEK293T cells)

Recordings were carried out at room temperature using pipettes with 2.5–4 MΩ resistance. Cells were held at –70 mV. Pipettes were pulled from filamented borosilicate pipettes (G150TF-3, Sutter Instruments). The extracellular solution contained (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 10 HEPES, 2 $CaCl_2$, and 10 glucose; pH 7.4. The intracellular solution contained (in mM): 140 CsCl, 4 NaCl, 10 HEPES, 2 $MgCl_2$, 2 Mg-ATP, and 10 EGTA; pH 7.2. Signals were amplified using a Patch Clamp PC-501A amplifier (Warner Instruments), low-pass filtered at 2 kHz, digitized at 10 kHz by a Digidata 1322A converter (Molecular Devices), and acquired with software Clampex 10 (Molecular Devices). Illumination for PTL photoisomerization was provided by a Lambda-LS xenon lamp (Sutter Instruments) with 379 ± 17 nm and 500 ± 8 nm band pass filters.

The dose-response curves in Figure 2c were fitted by Hill Equation: $I/I_{max} = 1/(1 + (EC_{50}/[GABA])^n)$, where I represents the current amplitude elicited by the given [GABA], I_{max} represents the saturating response, EC_{50} is the [GABA] producing the half maximal response, and n is the Hill coefficient. MAB-0 conjugation did not alter the Hill coefficient when the conjugated receptor was in the "uninhibited" state (0.94 ± 0.22 in 380 nm; 1.04 ± 0.18 for the wild-type; 4 and 3 cells, respectively). However, we observed a reduced Hill coefficient for the conjugated receptor in 500-nm light (0.65 ± 0.07; 4 cells), possibly due to the heterogeneity in receptor antagonism caused by incomplete PTL conjugation.

- Whole-cell voltage-clamp (dissociated hippocampal neurons)

Recordings were performed at room temperature using the same pipette and instrument settings as those for HEK293T recordings. To record miniature IPSCs, cells were held at –60 mV. The extracellular solution contained (in mM): 138 NaCl, 1.5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 5 HEPES, 10 Glucose, 0.025 DNQX, 0.05 APV, and 0.0005 TTX; pH 7.4. The internal solution contained (in mM): 140 CsCl, 4 NaCl, 2 $MgCl_2$, 10 HEPES, 10 EGTA, and 2 Mg-ATP; pH 7.2. To record miniature EPSCs, cells were held at –70 mV. The extracellular solution contained (in mM): 138 NaCl, 1.5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 5 HEPES, 10 Glucose, 0.001 TTX, and 0.02 bicuculline; pH 7.4. The intracellular solution contained (in mM): 8 NaCl, 135 K-gluconate, 4 $MgCl_2$, 10 HEPES, 2 Mg-ATP, and 1 EGTA; pH 7.4. All miniature events were analyzed using the MiniAnalysis software (Synaptosoft). The event detection threshold was set at 5 fold of the root-mean-square noise level, which typically was 2–5 pA. The detected events were further visually verified prior to statistical analysis. For each light condition, a total of 50–380 events were pooled for analysis.

The extracellular solution for recording voltage-gated currents contained (in mM): 138 NaCl, 1.5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 5 HEPES, 10 Glucose, 0.02 biccuculine, 0.025 DNQX, and 0.05 APV; pH 7.4. The intracellular solution contained (in mM): 10 NaCl, 135 K-gluconate, 2 $MgCl_2$, 10 HEPES, 1 EGTA, and 2 Mg-ATP; pH 7.4. Cells were held at –70 mV and stepped for 200 ms to –10 mV (Na^+ channels) or +40 mV (K^+ channels).

- Whole-cell recordings (hippocampal slices)

Slices were placed in a recording chamber mounted on an Olympus BX51WI microscope and perfused with ACSF at RT at 1–2 ml/min. ACSF contained (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 10 Glucose, 1.3 $MgCl_2$, 26 $NaHCO_3$ and 2.5 $CaCl_2$ and was equilibrated with 95% O_2 + 5% CO_2. 500-nm (2.8 mW/cm^2) and 380-nm (2.0 mW/cm^2) light was delivered through the microscope optical port by a Polychrome monochromator (Till Photonics) controlled by software pClamp (Molecular Devices).

Whole-cell current clamp recordings (for Figure 4d) were made from GFP positive CA1 pyramidal cells with glass microelectrodes (pipette resistance = 4–7 MΩ) filled with internal solution containing (in mM): 116 K-gluconate, 20 HEPES, 6 KCl, 2 NaCl, 0.5 EGTA, 4 ATP-Mg, 0.3 GTP-Na. Internal solutions were adjusted to ~ 7.2 pH, ~290 mOsm. A glass stimulating electrode filled with ACSF was placed in stratum radiatum 300–500 µm away from the recorded cell. Synaptic responses were evoked by a 0.2 ms, 10–100 µA current pulse delivered via a stimulus isolation unit (AMPI). Signals were amplified (Axopatch, Molecular Devices), digitized (Digidata, Molecular Devices) and recorded (pClamp, Molecular Devices) to computer. Neuron firing was triggered by depolarizing the postsynaptic cell through current injection and delivering 5 presynaptic stimuli at 200 Hz.

For control experiments verifying a lack of MAB-0 effects on native glutamate receptors, postsynaptic responses were recorded in voltage-clamp mode with an internal solution containing (in mM): 108 Cs-gluconate, 20 HEPES, 2.8 NaCl, 5 TEA-Cl, 0.4 EGTA, 4 ATP-Mg and 0.3 GTP-Na. Picrotoxin (100 µM) was included in the ACSF. AMPAR mediated responses were recorded at –70 mV holding potential and quantified as the peak negative evoked current. NMDAR responses were recorded at +40 mV holding potential and quantified as the positive current amplitude 100 ms post-stimulus.

C.3. Molecular Modeling

Docking of *trans* and *cis* MAB-0 was carried out in GLIDE,[6] a docking program implemented in Maestro (Shrödinger Inc.). A dimer of α1 and β2 was prepared from a previously published homology model for the $GABA_AR$.[7] Thr125 of the α1 subunit was replaced by a cysteine residue, and a receptor grid was created based on the mutant dimer. *Trans* MAB-0 was docked in this grid using the Standard Precision algorithm. To mimic MAB-0 tethering, a positional constraint was applied during docking. This constraint enforced at least one of the reactive maleimide carbons (of MAB-0) to locate within 4.5 Å from the side-chain methylene carbon (of α1C125). A maximum of 100 poses were listed. The docking poses were further inspected visually to remove unreasonable results (for instance, poses with maleimide group pointing away from the cysteine sulfhydryl group were excluded).

All of the docking poses for *trans* MAB-0 insert the azobenzene moiety in the α-β interface. The predominant docking mode of *trans* MAB-0 is shown in Figure 2b, in which the terminal phenol group of *trans* MAB-0 reaches deeply into the GABA-binding pocket (surrounded by the "aromatic box" shown in yellow sticks). Docking of *cis* MAB-0 in the same grid was also performed, but the resulting poses were few and diversely oriented, more likely representing non-specific interactions of *cis* MAB-0 with the receptor. The majority of the *cis* poses place the terminal phenol group outside of the aromatic box, consistent with the observed lack of receptor inhibition in 380 nm (Figure 2c).

D. Supporting References

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