Supporting Information

for

A Voltage-Responding Ion Channel Derived by C-Terminal Modification of Gramicidin A

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1) Synthesis of 2.

2) Single-channel recordings in planar lipid bilayer membranes.
1) Synthesis of 2.

Scheme S1. Synthesis of compound 2: a) HOBr, EDC, Et₃N, CH₂Cl₂; b) i: Pd/C, H₂, MeOH; ii: HOBr/HBTU, DIEA, CH₂Cl₂/DMF (3:1), -10 °C; c) TFA, anisole, CH₂Cl₂.
Z-Group cleavage (general procedure 1 GP 1)

The Z-protected polypeptide was dissolved in MeOH (0.003 M). 15 w% Pd/C (5% Degussa E101 NO/W) were added and the mixture was degassed, followed by insertion of H₂ over a balloon (3x). The reaction was warmed to the given temperature and hydrogenated (1 bar) with vigorous stirring until completion. To peptides containing several tryptophane amino acid residues, additional 5 w% Pd/C were added every 1 h of the hydrogenation time until completion. The suspension was filtered off using a pad of Celite® 512 (5 cm), and the Celite layer was rinsed with MeOH. The collected solution was evaporated and dried. In most cases, the desired amines had to be further purified.

HOBt-EDC coupling method (general procedure 2 GP 2)

Both the amine and the acid components were dissolved in CHCl₃ (0.1 M) and cooled to -10°C. 2.5 equiv HOBt and 4.0 equiv DIEA were added to the reaction mixture, followed by the addition of 1.5 equiv EDC after 10 min. The reaction mixture was stirred at -10°C → RT until total coupling.

HOBt-HBTU coupling method (general procedure 3 GP 3)

The compounds were dissolved in a mixture of CH₂Cl₂/DMF (3:1 v/v, 0.01 M) and cooled to -10°C. 3.0 equiv HOBt, 3.0 equiv DIEA were added to the solution followed by 1.5 equiv HBTU after 15 min. The mixture was stirred until total conversion.

Z-Trp-[2-(2'-amidoethoxy)-ethyl]-t-butyl carbamate 4

0.60 g (2.9 mmol) of amine 3[1] were coupled with 1.12 g (3.3 mmol) of Z-Trp-OH according to GP 2: 2.3 mL (13.2 mmol) DIEA, 1.12 g (8.3 mmol) HOBt, 0.96 g (5.0 mmol) EDC. After the aqueous work-up (CHCl₃ as extracting solvent), the crude product was purified by FCC (200 g silica gel, CHCl₃/MeOH 1% → 2% → 3%). 1.25 g (2.4 mmol, 82%) of compound 4 were obtained as a colourless solid. TLC: Rᵣ = 0.43 (CHCl₃/MeOH/25% aq NH₃ 100:10:1); m.p: 58-60 °C (CHCl₃/MeOH); ¹H NMR: (300 MHz, [D₆]DMSO, 298 K) δ = 1.35 (s, 9H, C(CH₃)₃), 2.82-3.97 (m, 1H, Trp -Hβ), 2.98-3.11 (m, 3H, Trp-Hδ, HN-CH₂), 3.12-3.25 (m, 2H, Boc-NH-CH₂), 3.26-3.33 (m, 4H, 2x OCH₂), 4.17-4.31 (m, 1H, Trp-Hα), 4.93 (s, 2H, Z-CH₂), 6.76 (t, 1H, J = 4.9 Hz, Boc-NH), 6.95 (t, 1H, J = 7.2 Hz, Hromatic), 7.05 (t, 1H, J = 7.3 Hz, Hromatic), 7.13 (s, 1H, Hromatic), 7.19-7.42 (m, 7H, Hromatic, H₂C-NH), 7.60 (d, 1H, J = 7.8 Hz, Hromatic), 8.04 (t, 1H, J = 5.0 Hz, Trp-NH), 10.80 (s, 1H, NHindol); ¹³C NMR: (75 MHz, [D₆]DMSO, 298 K) δ =
171.9 (C=O), 155.7 (Z-C=O, Boc-C=O), 137.0 (Z-arom), 136.1 (Trp-C-7a), 128.3, 127.7, 127.5 (Z-arom), 127.3 (Trp-C-3a), 123.8 (Trp-C-2), 120.8 (Trp-C-4), 118.6 (Trp-C-5, Trp-C-6), 111.3 (Trp-C-7), 110.1 (Trp-C-3), 79.2 (C(CH₃)₃), 69.0 (Z-CH₂), 68.7 (OCH₂), 65.3 (OCH₂), 55.6 (Trp-Cα), 39.7 (NCH₂), 36.6 (NCH₂), 28.2 (C(CH₃)₃), 27.9 (Trp-Cβ); IR: (KBr) 3324 m, 3060 w, 2976 w, 2930 w, 1696 s, 1662 s, 1523 m, 1456 w, 1392 w, 1366 w, 1342 w, 1250 w, 1169 w, 1123 w, 1045 w, 861 w, 743 m, 697 w, 667 w, 560 w, 461 w, 425 w; HRMS (ESI): C₂₈H₃₆N₄NaO₆ [M+Na⁺] calcd: 547.2533, found: 547.2523; HPLC: t_R = 10.58 min, Rainin Dynamax C8, A: Bidest. H₂O, B: CH₃CN/i-PrOH 2:1, 60% → 80% B in 25 min, flow 0.7 mL/min and λ = 280 nm; c = 1.05 (CHCl₃), T = 19.5 °C [α]D = -2.2, [α]₅₇₈ = -2.5, [α]₅₄₆ = -2.8, [α]₄₃₆ = -5.3, [α]₃₆₅ = -11.1.

Z-Trp-d-Leu-Trp-[2-(2’-amidoethoxy)-ethyl]-t-butyl carbamate 6

1.00 g (1.9 mmol) of Z-protected compound 4 were deprotected according to GP 1 (40 °C, 3 h) to yield 0.74 g (1.9 mmol) of the corresponding amine (R_f = 0.43). The crude product and 0.89 g (1.9 mmol) of dipeptide 5 were coupled according to GP 3: 1.00 mL (5.7 mmol) DIEA, 0.77 g (5.7 mmol) HOBt, 1.10 g (2.9 mmol) HBTU. After aqueous work-up (CHCl₃ as extracting solvent) the crude product was purified by FCC (200 g silica gel, CHCl₃/acetone 3:1 → 2:1 → 1:1) to give 1.16 g (1.4 mmol, 74%) of tripeptide derivative 6 as colourless solid. TLC: R_f = 0.33 (CHCl₃/Acetone 2:1); m.p: 98-102 ºC. ¹H NMR: (300 MHz, [D₆]DMSO, 298 K) δ = 0.62 (d, 3H, J = 5.9 Hz, D-Leu-Hδ), 0.68 (d, 3H, J = 5.6 Hz, D-Leu-Hδ), 0.96-1.26 (m, 3H, D-Leu-Hβ+γ), 1.35 (s, 9H, C(CH₃)₃), 2.81-2.98 (m, 2H, Trp-Hβ), 2.99-3.26 (m, 6H, Trp-Hβ, 2x HN-CH₂), 3.27-3.41 (m, 4H, 2xOCH₂, overlap with H₂O peak), 4.19 (q, 1H, J = 7.4 Hz, d-Leu-Hα), 4.33 (q, 1H, J = 7.0 Hz, Trp-Hα), 4.39-4.52 (m, 1H, Trp-Hα), 4.91 (s, 2H, Z-CH₂), 6.70-6.83 (m, 1H, Boc-NH), 6.95 (t, 2H, J = 7.3 Hz, H_brom), 6.99-7.08 (m, 2H, H_brom), 7.11 (d, 2H, J = 10.5 Hz, H_brom), 7.16-7.35 (m, 7H, H_brom), 7.40 (d, 1H, J = 7.8 Hz, H_brom), 7.58 (d, 1H, J = 7.8 Hz, H_brom), 7.62 (d, 1H, J = 8.1 Hz, Trp-NH), 7.87-8.01(m, 1H, HN-CH₂), 8.16 (d, 1H, J = 6.7 Hz, d-Leu-NH), 8.21 (d, 1H, J = 8.3 Hz, Trp-NH), 10.78 (s, 1H, NHIndol), 10.82 (s, 1H, NHIndol); ¹³C NMR: (75 MHz, [D₆]DMSO, 298 K) δ = 172.3, 172.1, 171.8 (all C=O), 156.2 (Z-C=O), 156.0 (Boc-C=O), 137.2 (Z-arom), 136.4 (Trp-C-7a), 128.6, 128.0, 127.9, (Z-arom), 127.6, 127.5 (Trp-C-3a), 124.3, 124.1 (Trp-C-2), 121.1, 121.0 (Trp-C-4), 118.9, 118.8 (Trp-C-6), 118.5, 118.4 (Trp-C-5), 111.6
(Trp-C-7), 110.6, 110.2 (Trp-C-3), 79.5 (C(CH₃)₃), 69.3 (Z-CH₂), 68.9 (OCH₂), 65.7 (OCH₂), 55.6, 54.1 (Trp-Cₐ), 51.7 (d-Leu-Cₐ), 40.1 (2x NCH₂), 28.6 (C(CH₃)₃), 28.3 (d-Leu-C₈), 28.0 (Trp-C₈), 24.1 (d-Leu-C₂), 23.1, 21.9 (d-Leu-C₉); HRMS (ESI): C₄₅H₅₇N₇NaO₈ [M+Na⁺] calcd: 846.4166, found: 846.4175; HPLC: tᵣ = 16.23 min, Rainin Dynamax C8, A: Bidest. H₂O, B: CH₃CN/i-PrOH 2:1, 50% → 100% B in 20 min, flow 0.7 mL/min and λ = 280 nm; c = 0.98 CHCl₃, T = 19.5°C [α]D = + 3.1, [α]₅₇₈ = + 3.0, [α]₅₄₆ = + 3.6, [α]₄₃₆ = + 7.5, [α]₃₆₅ = + 12.8.

Z-(Trp-d-Leu)₃-Trp-[2-(2'-amidoethoxy)-ethyl]-t-butyl carbamate 8
1.0 g (1.21 mmol) of tripeptide 6 were deprotected according to GP 1 (40 °C, 4 h) to yield 0.83 g (1.20 mmol) of the corresponding amine (Rᵣ = 0.14, CHCl₃/MeOH/HCOOH 10:1:0.1). The crude amine product and 0.95 g (1.37 mmol) of the tetrapeptide 7[2] were coupled according to GP 3: 0.72 mL (4.11 mmol) DIEA, 0.56 g (4.11 mmol) HOBt, 0.78 g (2.05 mmol) HATU. After aqueous work up (CHCl₃ as extracting solvent) the crude product was purified by FCC (200 g silica gel, CHCl₃/acetone/MeOH 15:10:1 à 15:15:0.1) to give 1.28 g (0.90 mmol, 75%) of heptapeptide-derivative 8 as a colourless solid. TLC: Rᵣ = 0.26 (CHCl₃/MeOH/HCOOH 10:1:0.1);

1H NMR: 300 Hz, [D₆]DMSO, 298 K) = 0.45-0.71 (m, 18H, d-Leu-Hᵈ), 0.81-1.27 (m, 9H, d-Leu-Hᵇ+γ), 1.36 (s, 9H, C(CH₃)₃), 2.77-2.97 (m, 4H, Trp-Hᵇ), 2.99-3.27 (m, 8H, Trp-Hᵇ, 2xHN-C₂H₅), 3.28-3.43 (m, 4H, 2xOCH₂, overlap with H₂O peak), 4.05-4.26 (m, 3H, d-Leu-Hα), 4.27-4.39 (m, 1H, Trp-Hα), 4.40-4.64 (m, 3H, Trp-Hα), 4.88 (dd, 2H, J = 26.4 Hz, J = 12.4 Hz, Z-CH₂), 6.70-6.84 (m, 1H, Boc-NH), 6.87-6.98 (m, 4H, Hₐrom), 6.99-7.06 (m, 4H, Hₐrom), 7.07-7.13 (m, 4H, Hₐrom), 7.14-7.21 (m, 2H, Hₐrom), 7.22-7.41 (m, 7H, Hₐrom), 7.47-7.65 (m, 4H, Hₐrom), 7.90 (d, 1H, J = 7.0 Hz, d-Leu-NH), 7.96-8.09 (m, 2H, Trp-NH, d-Leu-NH), 8.12-8.18 (m, 2H, Trp-NH, d-Leu-NH), 8.21 (d, 1H, J = 7.6 Hz, Trp-NH), 8.26-8.36 (m, 2H, Trp-NH, Hₐ-CH₂), 10.69-10.86 (m, 4H, NHIndol); 13C NMR: (75 MHz, [D₆]DMSO, 298 K) δ = 172.0, 171.7, 171.6, 171.5, 171.3 (all C=O), 155.8 (Z=C=O, Boc-C=O), 136.8 (Zₐrom), 136.1 (Trp-C-7a), 128.9, 128.2, 127.7 (Zₐrom), 127.5, 127.2, 127.1 (Trp-C-3a), 125.3, 123.9, 123.8, 123.7 (Trp-C-2), 120.8, 120.7 (Trp-C-4), 118.4, 118.3 (Trp-C-6), 118.2, 118.1 (Trp-C-5), 111.3, 111.2, 111.1, 111.0 (Trp-C-7), 110.1, 109.8, 109.7, 109.6 (Trp-C-3), 77.6 (C(CH₃)₃), 69.0 (Z-CH₂), 68.6 (OCH₂), 65.3 (OCH₂), 55.7, 54.0, 53.8, 53.7 (Trp-Cₐ), 51.5, 51.4, 51.3 (d-Leu-Cₐ), 40.5, 35.8 (NCH₂), 28.2 (C(CH₃)₃), 27.9, 27.8, 27.7 (d-Leu-C₈), 27.68, 27.6, 27.5, 27.4 (Trp-C₈), 23.9, 23.6 (d-Leu-C₂), 22.8, 22.6, 21.7,
21.5, 21.3 (d-Leu-Cά); HRMS (ESI): C_{79}H_{99}N_{13}Na_{12} [M+Na^+] calcd: 1444.7434, found:1444.7422; HPLC t_{R} = 16.6 min, Rainin Dynamax C8, A: Bidest. H_{2}O, B: CH_{3}CN/iPrOH 2:1, 60% → 100% B in 20 min, flow 0.7 mL/min and \( \lambda = 280 \) nm; c = 1.01 (CHCl_{3}), \( T = 19.5^\circ C \) \([\alpha]_D = -3.1, \quad [\alpha]_{578} = -3.2, \quad [\alpha]_{546} = -3.5, \quad [\alpha]_{436} = -6.4, \quad [\alpha]_{365} = -9.5\)

**Z-Ala-d-Val-Val-d-Val-(Trp-d-Leu)_{3}-Trp-[2-(2'-amidoethoxy)-ethyl]-t-butyl carbamate 10**

450 mg (0.32 mmol) of heptapeptide derivative 8 were deprotected following GP 1 (40 °C, 6 h). The crude product was purified by FCC (40 g silica gel, CHCl_{3}/MeOH/25% aq NH_{3} 100:5:0.5 \( \rightarrow \) 100:10:0.5) to yield 375 mg (0.29 mmol, 91%) of the corresponding amine (\( R_f = 0.05, \) CHCl_{3}/MeOH/25% aq NH_{3} 10:1:0.1). The amine and 167 mg (0.32 mmol) of the tetrapeptide 9 were coupled according to GP 3: 168 µL (0.96 mmol) DIEA, 130 mg (0.96 mmol) HOBt, 182 mg (0.48 mmol) HBTU. After aqueous work-up (CHCl_{3}/i-PrOH as extracting solvent) the crude product was purified by FCC (50 g silica gel, CHCl_{3}/MeOH 100:3) to obtain 380 mg (0.21 mmol, 73%) of undecapeptide derivative 10 as a colourless oil. TLC: \( R_f = 0.16 \) (CHCl_{3}/MeOH/25% aq NH_{3} 10:1:0.1); \(^{1}H NMR: (300 MHz, [D_{6}]DMSO, 298 K) \delta = 0.42-0.62 \) (m, 21H, d-Leu-H_{δ}, Val-H_{β}), 0.65 (d, 3H, J = 6.0 Hz, d-Leu-H_{δ}), 0.70-0.92 (m, 14H, Val-H_{δ}, d-Leu-H_{δ}, Val-H_{δ}), 1.36 (s, 9H, C(CH_{3})_{3}, 1.67-1.86(m, 1H, Val-H_{δ}), 1.89-2.10(m, 2H, Val-H_{β}), 2.77-2.97 (m, 4H, Trp -H_{β}), 2.99-3.27 (m, 8H, Trp-H_{β}, 2x HN-C_{H}2), 3.28-3.44 (m, 4H, 2x OCH_{2}, overlab with H_{2}O peak), 4.03-4.24 (m, 5H, 3x d-Leu-H_{α}, Ala- H_{α}, d-Leu-H_{α}), 4.25-4.38 (m, 2H, Val-H_{α}), 4.39-4.66 (m, 4H, Trp-H_{α}), 4.98 (s, 2H, Z-CH_{2}), 6.69-6.80 (m, 1H, Boc-NH), 6.84-7.14 (m, 12H, H_{arom}), 7.21-7.42 (m, 10H, 9x H_{arom}, 1x NH), 7.46-7.62 (m, 4H, H_{arom}), 7.68-7.83(m, 2H, NH), 7.85-8.07 (m, 5H, NH), 8.14 (d, 1H, J = 7.0 Hz, NH), 8.21 (d, 1H, J = 7.2 Hz, NH), 8.26-8.38 (m, 1H, NH), 10.70 (s, 1H, NH_{Indol}), 10.76 (s, 3H, NH_{Indol}); HRMS (ESI): C_{97}H_{131}N_{17}Na_{16} [M+Na^+] calcd: 1812.9857, found: 1812.9843, HPLC: \( t_{R} = 13.15 \) min, Caltrex, A: H_{2}PO_{4}/NaH_{2}PO_{4} buffer pH 3, B: MeOH, 80% → 100% B in 25 min, flow 0.6 mL/min and \( \lambda = 280 \) nm; c = 0.99 (CHCl_{3}), \( T = 19.5^\circ C \) \([\alpha]_D = +29.1, \quad [\alpha]_{578} = +30.4, \quad [\alpha]_{546} = +34.3, \quad [\alpha]_{436} = +58.4, \quad [\alpha]_{365} = +93.1. \)
HCO-Val-Gly-Ala-d-Leu-Ala-d-Val-Val-d-Val-(Trp-d-Leu)3-Trp-[2-(2'-amidoethoxy)ethyl]-t-butyl carbamate 12

95 mg (53 µmol) of compound 11 were deprotected according to GP 1 (40 °C, 5 h). The crude product was purified by FCC (16 g silica gel, CHCl₃/MeOH/25% aq NH₃ 100:5:0.5 → 100:7:0.5 → 100:10:0.5) to yield 80 mg (48 µmol, 92%) of the corresponding amine (Rᵢ = 0.08, CHCl₃/MeOH/25% aq NH₃ 10:1:0.1). The amine and 20 mg (50 µmol) of the tetrapeptide 11[2] were coupled according to GP 3: 26 µL (150 µmol) DIEA, 20 mg (150 µmol) HOBt, 28 mg (75 µmol) HBTU. After aqueous work-up (CHCl₃/i-PrOH as extracting solvent) the crude product was purified by FCC (7 g silica gel, CHCl₃/MeOH/HCOOH 100:7:3) to give 65 mg (32 µmol, 67%) of gA-derivative 12 as a colourless oil. TLC: Rᵢ = 0.08 (CHCl₃/MeOH 10:1), Rᵢ = 0.34 (CHCl₃/MeOH/HCOOH 10:1:0.5); ¹H NMR: (300 MHz, [D₆]DMSO, 298 K) δ = 0.38-0.69 (m, 24H, D-Leu-Hᵦ, Val-Hᵦ), 0.70-0.93 (m, 24H, D-Leu-Hᵦ, Val-Hᵦ), 0.96-1.29 (m, 15H, Ala-Hᵦ, D-Leu-Hᵦ⁺), 1.35 (s, 9H, C(CH₃)₃), 1.40-1.62 (m, 3H, D-Leu-Hᵦ⁺), 1.72-1.88 (m, 1H, Val-Hᵦ), 1.90-2.08 (m, 3H, Val-Hᵦ), 2.77-2.97 (m, 4H, Trp-Hᵦ), 3.01-3.27 (m, 8H, Trp-Hᵦ, 2x HN-C₄H₂), 3.44-3.58 (m, 4H, OCH₂), 3.63-3.79 (m, 2H, Gly-Hᵦ), 4.03-4.35 (m, 10H, 2x Ala-Hᵦ, 4x D-Leu-Hᵦ, 4x Val-Hᵦ), 4.39-4.62 (m, 4H, Trp-Hᵦ), 6.71-6.83 (m, 1H, Boc-NH), 6.86-7.14 (m, 12H, Harom), 7.21-7.34 (m, 4H, Harom), 7.43-7.63 (m, 4H, Harom), 7.79-7.93 (m, 2H, NH), 7.95-8.10 (m, 8H, NH), 8.16-8.38 (m, 7H, NH), 8.43-8.56 (m, 1H, NH), 10.72 (s, 1H, NHindol), 10.77 (s, 3H, NHindol); HRMS (ESI): C₁₀₆H₁₅₄N₂₁O₁₉ [M+H⁺] calcd: 2025.1730, found: 2025.1663, HPLC: tᵣ = 16.05 min, Caltrex, A: H₃PO₄/NaH₂PO₄ buffer pH 3, B: MeOH, 75% → 100% B in 30 min, flow 0.7 mL/min and λ = 280 nm.

HCO-Val-Gly-Ala-d-Leu-Ala-d-Val-Val-d-Val-(Trp-d-Leu)₃-Trp-[2-(2'-amidoethoxy)ethyl]ammonium trifluoroacetate 2

0.5 mL TFA were added gradually to a mixture of 14 mg (7 µmol) of compound 12 in 1 mL of CH₂Cl₂/Anisole (1:1) to cleave the Boc-protective group (15 min, RT). After the completion of the reaction, the mixture was diluted with 2 mL toluene and evaporated under reduced pressure to dryness. The crude product was purified by gel filtration (10 g sephadex LH-20, CHCl₃/MeOH 1:1) to give 12 mg (6 µmol, 86%) of ammonium salt 2 as a white powder. TLC: Rᵢ = 0.02 (CHCl₃/MeOH/HCOOH 10:1:0.5); ¹H NMR: (400 MHz, [D₆]DMSO, 300 K) δ = 0.43-0.62 (m, 19H, D-Leu-Hᵦ, Val-Hᵦ),
0.65 (d, 3H, J = 6.6 Hz, D-Leu-Hδ), 0.72-0.91 (m, 26H, D-Leu-Hδ, Val-Hγ), 0.93-1.28 (m, 12H, Ala-Hβ, D-Leu-Hβ+γ), 1.40-1.60 (m, 3H, D-Leu-Hβ+γ), 1.72-1.85 (m, 1H, Val-Hβ), 1.92-2.06 (m, 2H, Val-Hβ), 2.82-2.96 (m, 4H, Trp-Hβ), 3.00-3.26 (m, 8H, Trp-Hβ), 3.32-3.41 (m, 7H, 2x OCH₂, NH₃⁺), 3.65-3.77 (m, 2H, Gly-Hα) 4.08-4.35 (m, 10H, 2x Ala-Hα, 4x D-Leu-Hα, 4x Val-Hα), 4.41-4.60 (m, 4H, Trp-Hα), 6.86-6.97 (m, 4H, Harom), 6.98-7.12 (m, 8H, Harom), 7.25-7.32 (m, 4H, Harom), 7.50-7.58 (m, 4H, Harom), 7.67-7.78 (m, 5H, NH), 7.87-8.05 (m, 7H, 6x NH, HCO), 8.06-8.15 (m, 2H, NH), 8.16-8.24 (m, 3H, NH), 8.29 (t, 1H, J = 5.7 Hz, NH), 8.35 (d, 1H, J = 8.0 Hz, NH), 10.70 (s, 1H, NHIndol), 10.73-10.80 (m, 3H, NHIndol); HRMS (ESI): C₁₀₃H₁₄₅F₃N₂₁O₁₉ [M-CF₃COO⁻] calcd: 1925.1227, found: 1925.1206, HPLC: tR = 10.21 min, Caltrex, A: H₃PO₄/NaH₂PO₄ buffer pH 3, B: MeOH, 75% → 100% B in 30 min, flow 0.7 mL/min and λ = 280 nm.


2) Single channel recordings in planar lipid bilayer membranes

Compound 2 was dissolved in methanol to prepare stock solutions. These were added to the bath or pipette solution to final concentration of 10⁻⁶ M so that the final methanol concentration did not exceed 0.1 %. Planar lipid membranes were prepared by painting a solution of DPhPC in n-decane (25mg / mL) over the aperture of a polystyrene cuvette with a diameter of 0.20 mm. 2, dissolved in methanol, was added to both sides of the cuvette (final concentration in the cuvette 2.5 × 10⁻¹⁰ mol/L). Current detection and recording were performed with a patch-clamp amplifier Axopatch 200B, a Digidata A/D converter and pClamp6 software (Axon Instruments, Foster City, CA, USA). The acquisition frequency was 5 kHz. The data were filtered with a digital filter at 50 Hz for further analysis, applying the pClamp 9.2 software.

Heterodimer channels of 2 and gA were formed by addition of gA to the cis-side and of 2 to the trans-side of the cuvette to an already formed membrane (final concentration in the cuvette 2.5 × 10⁻¹⁰ mol/L). As a result the cations entered the gA-monomer at a positive applied voltage and the 2-monomer at a negative applied voltage.