Supplementary Information

for

Benzothiazole amphiphiles promote the formation of dendritic spines in primary hippocampal neurons

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Chemical Synthesis of BAMs1-3:

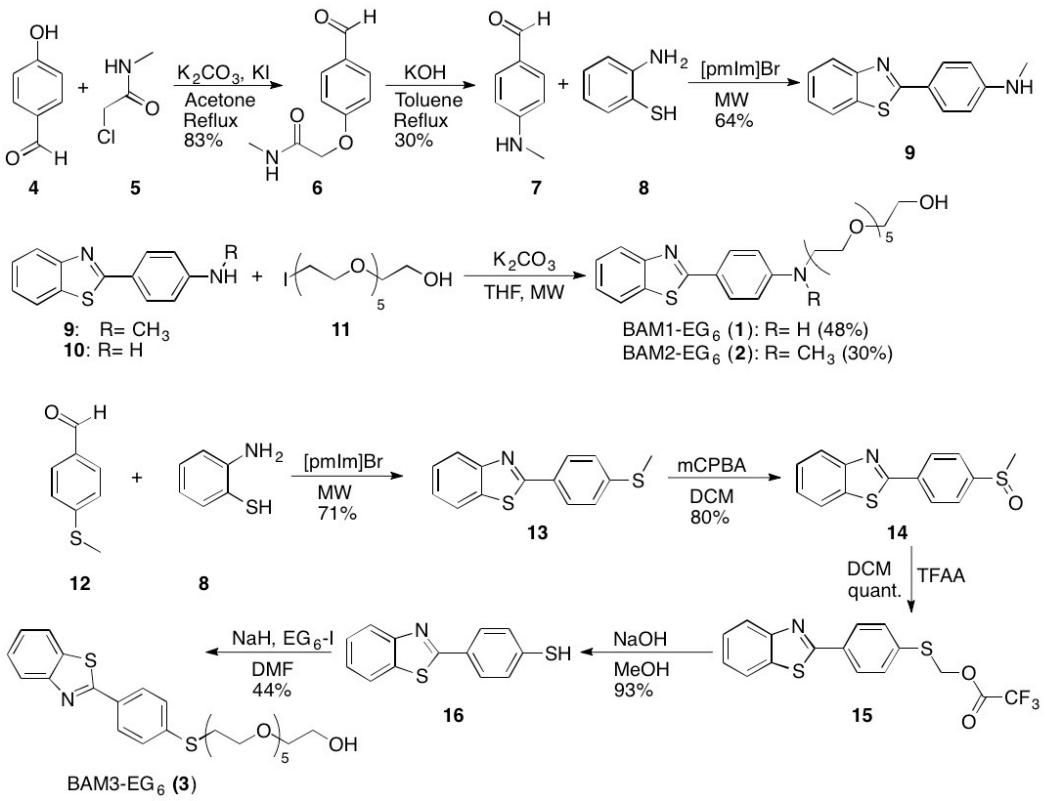

Figure S1. Synthetic scheme for the preparation of BAM**1-3**.

Alkylation of 4-hydroxy benzaldehyde (6):

4-Hydroxy benzaldehyde **4** (2g, 16.5 mmole, 1.1 equiv.) and anhydrous potassium carbonate (K_2CO_3) (4.14g, 29.9 mmole, 2 equiv.) were dissolved in acetone (30 mL) and let stir under nitrogen (N_2) for 30 min. Then 2-chloro-N-methylacetamide **5** (1.61g, 15 mmole, 1 equiv.) and potassium iodide (KI) (249 mg, 1.5 mmole, 0.1 equiv.) were added and let reflux for 24 h. After cooling to room temperature (RT), solids were filtered off and the solvent was removed and replaced with dichloromethane (DCM). Extraction was done with 10% sodium hydroxide (NaOH) followed by column chromatography purification (95% DCM/ methanol (MeOH)) to yield compound **6** as a white solid (2.4 g, 83% yield)

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.91 (s, 1H), 7.88(d, 2H), 7.04 (d, 2H), 6.50 (b, 1H), 4.57 (s, 2H), 2.93 (s, 3 H). ESI-MS *(m/z)*:

194.12 $[M+ H]^+$

Synthesis of 4-N-(methylamino) benzaldehyde (7):

To a round bottom with dry toluene, compound **6** (300 mg, 1.55 mmole, 1 equiv.) and potassium hydroxide (KOH) pellets (174 mg, 3.10 mmole, 2 equiv.) were added and let reflux for 24 h. After cooling to RT, the reaction was put on ice and water was added. The

organic layer was washed 3x with water, dried, and concentrated. Column chromatography (50% ethyl acetate (EtOAc)/Hexanes) yielded compound **7** as a red solid (64 mg, 30% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.72 (s, 1H), 7.71 (d, 2H), 6.61 (d, 2H), 4.41 (b, 1H), 2.91 (s, 3 H). ESI-MS *(m/z)*: 136.19 $[M+ H]^+$

Synthesis of Benzothiazole (9)[(1)]:

A microwave vial was charged with 2-aminothiophenol **7** (45 mg, 0.36 mmole. 1 equiv.), followed by 1-pentyl-3-methylimidazolium bromide ([pmIm]Br)[(2)] (29 mg, 0.18 mmole, 0.5 equiv.) and then 4-(methylamino)benzaldehyde **8** (49mg, 0.36 mmole, 1 equiv.). The mixture was irradiated under MW conditions (150 °C, 4 min). The reaction mixture was extracted with ether/H_2O (4x). The ether was evaporated and the compound was purified by column chromatography (25%DCM/70%Hexanes/5%EtOAc), affording compound **9** as a light orange solid (55 mg, 64% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02 (d, 1H), 7.96 (d, 2H), 7.84 (d, 1H), 7.44 (t, 1 H), 7.32 (t, 1 H), 6.66 (d, 2 H), 2.92 (s, 3 H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 169.05, 154.53, 151.82, 134.73, 129.32 (2C), 126.25, 124.50, 122.68, 122.53, 121.60, 112.24 (2C), 30.54. ESI-MS *(m/z)*: 241.0 $[M+ H]^+$

General protocol for (ethylene glycol)$_6$ (EG_6) addition:

Synthesis of 17-iodo-3,6,9,12,15-pentaoxaheptadecan-1-ol (EG_6-I) was prepared as previously described[(3)]. A microwave vial was charged with EG_6-I (1 equiv.), benzothiazole aniline **9** or **10** (2 equiv.), potassium carbonate (3 equiv.) and tetrahydrofuran (THF). The mixture was irradiated under MW (125 °C, 2 h). The mixture was filtered, concentrated and normal phase column chromatography (4% MeOH/EtOAc) followed by reverse phase column chromatography (3:1 MeOH/H_2O) yielded compound **1** (285 mg, 48% yield) or compound **2** (13 mg, 30% yield).

BAM1-EG_6 (1):

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (d, 1H), 7.92 (d, 2H), 7.84 (d, 1H), 7.43 (t, 1 H), 7.30 (t, 1 H), 6.76 (d, 2 H), 4.97 (b, 1H), 3.73-3.58 (m, 22H), 3.39 (t, 2H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 168.92, 154.51, 151.38, 134.74, 129.13 (2C), 126.24, 124.54, 123.20. 122.55, 121.60, 113.28 (2C), 71.68, 69.81-69.03 (69.81, 69.59, 69.45, 69.30, 69.24, 69.23, 69.03), 68.74, 60.44, 43.86. HR/MS (ESI +): Calcd for $[C_{25}H_{34}N_2O_6S + Na]$ 513.2030 found 513.2029 $[M+Na]^+$

BAM2-EG_6 (2):

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, 1H), 7.93 (d, 2H), 7.84 (d, 1H), 7.42 (t, 1 H), 7.29 (t, 1 H), 6.76 (d, 2 H), 3.72-3.28 (m, 24H), 3.07 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 168.94, 154.64, 151.39, 134.74, 129.17 (2C), 126.19, 124.40, 122.49, 121.57, 121.55, 111.80 (2C), 72.76, 71.0-70.50 (71.00, 70.88, 70.85, 70.80, 70.76, 70.75, 70.71, 70.50), 68.73, 61.93, 52.29, 39.26. HR/MS (ESI-TOFMS +): Calcd for [$C_{26}H_{36}N_2O_6S$ + Na] 527.2191 found 527.2187 [M+ Na]$^+$

2-(4-(methylthio)phenyl)benzo[d]thiazole (13):

2-amino thiophenol **8** (376mg, 3 mmol, 1 equiv.), [pmIm]Br (400 mg, 0.5 equiv), 4-(methylthio)benzaldehyde **12** (457 mg, 3 mmol, 1 equiv.) were added respectively, into a 5 mL microwave tube with a stir bar. The reaction tube was microwaved for 4 min at 130^{o}C. The reaction mixture was dissolved in diethyl ether and extracted with water to remove the ionic liquid solution. The diethyl ether was removed under reduced pressure and the crude solid **13** was purified by recrystallization in a 3:1 mixture of hexanes:EtOAc (547 mg, 71% yield). Analysis of compound **13** matched a previously reported sample.[(4)]

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (d, 1H), 8.01 (d, 2H), 7.90 (d, 1H), 7.49 (t, 1H), 7.38 (t, 1H), 7.33 (d, 2H), 2.55 (s, 3H). ESI-MS *(m/z)*: 258.25 [M+H]$^+$

2-(4-(methylsulfinyl)phenyl)benzo[d]thiazole (14):

2-(4-(methylthio)phenyl)benzo[d]thiazole **13** (300 mg, 1.1 mmol) was dissolved in 6 mL of DCM. *meta*-chloroperoxybenzoic acid (*m*-CPBA) (242 mg, 1.4 mmol) was dissolved in 4 mL of DCM and added dropwise at 0 ^{o}C to the methyl sulfide **13** solution over a period of 20 min. $NaHCO_3$ (80 mg) was added and the solution was let stir. The reaction mixture was monitored by TLC analysis (100% EtOAc) until completion. The white precipitate was filtered away and the DCM was removed under reduced pressure to afford a white solid. The solid was purified by recrystallization in 100% EtOAc to give product **14** (254 mg, 80% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, 2H), 8.11 (d, 1H), 7.94 (d, 1H), 7.78 (d, 2H), 7.53 (t, 1H), 7.44 (t, 1H), 2.79 (s, 3H). ESI-MS *(m/z)*: 274.17 [M+H]$^+$, 296.10 [M+Na]$^+$

((4-(benzo[d]thiazol-2-yl)phenyl)thio)methyl 2,2,2-trifluoroacetate (15):

2-(4-(methylsulfinyl)phenyl)benzo[d]thiazole (**14**) (50 mg, 0.18 mmol) was dissolved in 2 mL of freshly distilled DCM in an oven dried 50 mL round bottom. Trifluoroacetic anhydride (TFAA) (0.15 mL) was added to the reaction flask and the reaction was gently refluxed at 40 ^{o}C for 2 h under N_2. The solvent was removed under reduced pressure to afford the crude product **15** (72 mg, approximately quantitative conversion). The product was taken on to the next step without further purification.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07 (m, 3H), 7.92 (d, 1H), 7.58 (d, 8Hz, 2H), 7.53 (t, 1H), 7.43 (t, 1H), 5.70 (s, 2H)

4-(benzo[d]thiazol-2-yl)benzenethiol (16):

((4-(benzo[d]thiazol-2-yl)phenyl)thio)methyl 2,2,2-trifluoroacetate (**15**) (72mg, 0.19 mmol) was dissolved in 3 mL of MeOH and 0.6 mL of 1M NaOH was added to the reaction flask and refluxed under N_2 for 1 h. The reaction mixture was cooled and the solvent was removed under reduced pressure. 0.6mL of 1M HCl was then added to the crude mixture and the product was extracted into EtOAc by washing the aqueous layer with 3 x 2mL of EtOAc. The organic layer was washed with a saturated NaCl solution and dried over Na_2SO_4. The EtOAc was removed under reduced pressure to afford the crude product **16** (44 mg, 93% crude yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08 (d, 1H), 7.95 (d, 2H), 7.90 (d, 1H), 7.51 (t, 1H), 7.39 (m, 3H), 3.68 (s, 1H). ESI-MS *(m/z)*: 244.28 $[M+H]^+$

17-((4-(benzo[d]thiazol-2-yl)phenyl)thio)-3,6,9,12,15-pentaoxaheptadecan-1-ol (3):

In an oven dried 50 mL round bottom, solid sodium hydride (NaH) (2 mg, 0.074 mmol) was added and the round bottom was tightly capped with a rubber septum. The round bottom was purged with N_2. The crude **4-**(benzo[d]thiazol-2-yl)benzenethiol (**16**) (12mg, 0.05 mmol, 1 equiv.) was dissolved in 1mL of freshly distilled dimethylformamide (DMF) and added dropwise to the round bottom flask containing NaH. The reaction mixture was stirred for 30 min. 17-iodo-3,6,9,12,15-pentaoxaheptadecan-1-ol (EG_6-I, 20 mg, 0.05 mmol, 1 equiv.) was dissolved in 1 mL of freshly distilled DMF in a separate vial and added dropwise into reaction mixture. The reaction was then refluxed under N_2 for 12 h. The reaction mixture was cooled to RT and the solvent was removed under reduced pressure. The product was purified via silica gel flash chromatography (using a gradient of EtOAc:MeOH 0-4%) to afford the desired product **3** as a yellow oil (R_f=0.24, 100% EtOAc). The yellow oil product was purified once more using a reverse-phase preparatory plate (using a 3:1 mixture of MeOH:H_2O as eluent) to give final product **3** (11mg, 44% yield).

BAM3-EG_6 (3):

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04 (d, 1H), 7.99 (d, 2H), 7.89 (d, 1H), 7.48 (t, 1H), 7.41 (d, 2H), 7.37 (t, 1H), 3.74-3.70 (m, 4H), 3.64 (m, 16H), 3.60-3.58 (m, 2H), 3.20, (t, 2H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 167.42, 154.09, 140.57, 134.88, 130.83, 128.01, 127.86, 126.38, 125.18, 123.08, 121.62, 72.50, 70.64-70.30 (70.64, 70.59, 70.55, 70.53, 70.50, 70.30), 69.68, 61.74, 32.08. HR/MS: calcd for $C_{25}H_{33}NO_6S_2$ [M+Na] 530.1641 found [M+Na] 530.1640

Supporting Information References:

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2. Namboodiri, V. V., and Varma, R. S. (2002) Solvent-Free Sonochemical Preparation of Ionic Liquids. *Org. Lett.* **4**, 3161–3163.
3. Prangkio, P., Rao, D. K., Lance, K. D., Rubinshtein, M., Yang, J., and Mayer, M. (2011) Self-assembled, cation-selective ion channels from an oligo(ethylene glycol) derivative of benzothiazole aniline. *Biochim. Biophys. Acta* **1808**, 2877–85.
4. Park, N., Heo, Y., Kumar, M. R., Kim, Y., Song, K. H., and Lee, S. (2012) Synthesis of Benzothiazoles through Copper-Catalyzed One-Pot Three-Component Reactions with Use of Sodium Hydrosulfide as a Sulfur Surrogate. *European J. Org. Chem.* **2012**, 1984–1993.