

## Photoactivable sphingosine as a tool to study membrane microenvironments in cultured cells.

Massimo Aureli, Simona Prioni, Laura Mauri, Nicoletta Loberto, Riccardo Casellato, Maria Grazia Ciampa, Vanna Chigorno, Alessandro Prinetti, and Sandro Sonnino.

### SUPPLEMENTARY MATERIAL

7-bromo-1-heptanol, **1**, (0.85 mmol), 3,4-dihydropyran (3.8 mmol), and a catalytic amount of *p*-toluenesulfonic acid were dissolved in dry toluene (1.3 ml), and stirred for 1 hr at room temperature<sup>1</sup>. Triethylamine (130  $\mu$ l) was added, and the reaction mixture was dried under vacuum. The obtained compound **2** (98% yield) was dissolved in dry DMSO (6.6 ml) cooled on ice, and lithium acetylide ethylene diamine complex (2.16 mmol) was carefully added. The suspension was allowed to warm to room temperature and stirred for 4 hr. The reaction mixture was then poured into a saturated NaHCO<sub>3</sub> solution (40 ml) and extracted with CHCl<sub>3</sub> (3 $\times$ 14 ml). The combined organic phases were subsequently washed with brine (26 ml), water (26 ml), dried with Na<sub>2</sub>SO<sub>4</sub>, and then concentrated under vacuum. After purification by flash chromatography on a silica gel 60 column (2 $\times$ 15 cm), equilibrated and eluted with hexane/EtOAc (95:5 by vol.), pure **3** was obtained as a colorless oil (90% yield). To a solution of compound **3** (0.77 mmol,) in dry THF (6 ml, -78 °C, under N<sub>2</sub>) and mixed with BuLi (1.6 M in hexane, 0.77 mmol) was added drop wise. After 30 min dry HMPT (1.5 mmol) and then a solution of Garner aldehyde (0.770 mmol) in dry THF (630  $\mu$ l) were added. The reaction mixture was stirred for 2 hr at -78 °C, then allowed to warm to -20 °C, and mixed with a saturated NH<sub>4</sub>Cl (27.5 ml) and EtOAc (12.7 ml). The mixture was for 18 hr at room temperature. After phase separation, the water phase was extracted with EtOAc (5 $\times$ 12.7 ml). The combined organic phases were subsequently washed with brine (12.7 ml), water (12.7 ml), dried with Na<sub>2</sub>SO<sub>4</sub>, and concentrated under vacuum. Purification by flash chromatography on a silica gel 60 column (2 $\times$ 15 cm), equilibrated and eluted with hexane/EtOAc/triethylamine (800:200:1 by vol.) and hexane/EtOAc (800:200 by vol.) yielded pure **4** as a colorless oil (85% yield). Compound **4** (0.657 mmol) in dry THF (23.5 ml) was added drop wise to a blue solution of lithium (35 mmol) in liquid ammonia (18.9 ml), at -78 °C, under The reaction mixture was stirred for 15 hr at -78 °C and then quenched with solid NH<sub>4</sub>Cl (169 mmol). The reaction mixture was allowed to warm at room temperature in order to remove the ammonia. 2 ml

of THF/water (1:1 by vol.) was added drop wise and after phase separation, the water phase was extracted with EtOAc (5x11 ml). The combined organic phases were subsequently washed with brine (11 ml), water (11 ml), dried with Na<sub>2</sub>SO<sub>4</sub>, and concentrated under vacuum. Flash column chromatography (2x15 cm, silica gel 60, hexane/EtOAc 800:200 by vol.) yielded pure compound **5** (85% yield)<sup>2</sup>. To a solution of **5** (0.64 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (39 ml), pyridine (64 μl) and acetic anhydride (67 μl) were added. The reaction mixture was stirred for 19 hr under reflux to obtain compound **6** (92% yield). After concentration under vacuum, compound **6** was dissolved in 27 ml of methanol/acetone (2:1 by vol.) and the reaction mixture was stirred for 1 hr at room temperature. The reaction mixture was then neutralized with saturated NaHCO<sub>3</sub> and 22 ml of CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O (1:1 by vol.) were added. After phase separation, the water phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x11 ml) yielding pure compound **7** (0.635 mmol, 98% yield). To a solution of compound **7** (0.635 mmol) and triethylamine (95 μl) in dry CH<sub>2</sub>Cl<sub>2</sub> (478 μl), methanesulfonyl chloride (0.65 mmol) was added drop wise. After 4 h at 0°, 22 ml of CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O (1:1 by vol.) was added and after phase separation the water phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x11 ml) dried with Na<sub>2</sub>SO<sub>4</sub>, and concentrated under vacuum yielding pure compound **8**. Compound **8** was dissolved in methanol (12 ml), and sodium azide (1.5 mmol) was added. The reaction mixture was stirred for 12 hr at reflux to obtain compound **9**. The reaction mixture was allowed to cool to room temperature and sodium methoxide (0.60 mmol) was added. After 5 hr under stirring at room temperature compound **10** (89% yield) was synthesized. SnCl<sub>2</sub> (0.58 mmol), triethylamine (1.7 mmol), and thiophenol (1.7 mmol) were added to the reaction mixture and stirred for 3 hr at room temperature. Solvent was removed under vacuum and the product purified by column flash chromatography (2x15 cm silica gel 60, methanol/chloroform, 4:1 by vol.)<sup>3</sup> providing pure compound **11** (90% yield). Starting from this point all the reactions were carried out in the dark, only under red safelight. To a solution of compound **11** (0.523 mmol) in anhydrous DMF/methanol (1:2 by vol.)(15 ml) triethylamine (0.523 mmol) and 4-F-3-NO<sub>2</sub>-phenylazide (0.75 mmol) were added, and the reaction mixture was stirred for 36 hr at 60°C<sup>4</sup>. After solvent removal, photoactivable compound was purified on a silica gel 60 column (2x15 cm) equilibrated and eluted with chloroform/methanol (4:1 by vol.), and EtOAc yielding pure compound **12** (91%). The elution profile was characterized by TLC using the solvent system chloroform/methanol, 4:1 by volume. Complete deprotection of

compound **12** was then accomplished with 98% yield by simple treatment with 0.5N HCl in EtOAc for 2 hour. The reaction mixture was dried and the photoactivable sphingosine was purified on a silica gel 60 flash column (2x15 cm) equilibrated and eluted with exane/EtOAc/triethylamine (800:200:1 by vol.).

#### **SUPPLEMENTARY REFERENCE**

- (1) Nieuwenhuizen W. F., S. v. Leeuwen, F. Götz, and M. R. Egmond. 2002. Synthesis of a novel fluorescent ceramide analogue and its use in the characterization of recombinant ceramidase from *Pseudomonas aeruginosa* PA01. *Chemistry and Physics of Lipids* **114**: 181-191.
- (2) Herold P. 1988. Synthesis of D-Erythro- and D-Threo-Sphingosine Derivatives From L-Serine. *Helvetica Chimica Acta* **71**: 354-362.
- (3) Maiti S. N., M. P. Singh, and R. G. Micetich. 1986. Facile conversion of azides to amines. *Tetrahedron Letters* **27**: 1423-1424.
- (4) Mauri L., S. Prioni, N. Loberto, V. Chigorno, A. Prinetti, and S. Sonnino. 2004. Synthesis of radioactive and photoactivable ganglioside derivatives for the study of ganglioside-protein interactions. *Glycoconj J* **20**: 11-23.

#### **SUPPLEMENTARY FIGURES LEGEND**

**Figure 1.** Scheme of reactions to synthesize photoactivable sphingosine.

Supplementary Fig 1

