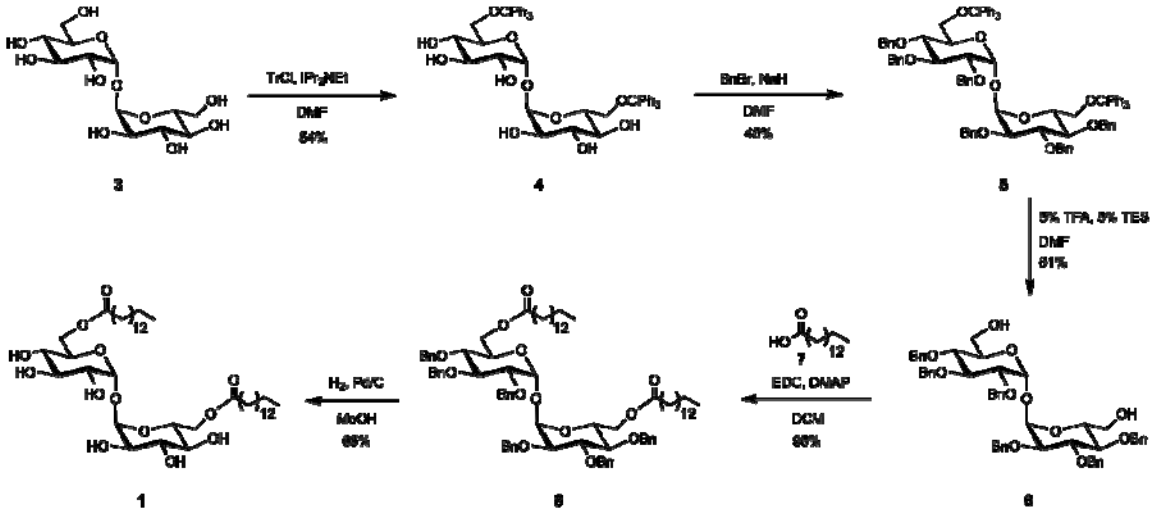
Supporting information for Harland *et al*., "Synthetic Trehalose Glycolipids Confer Desiccation Resistance to Supported Lipid Monolayers"

Detailed explanation of synthetic schemes used to generate synthetic trehalose glycolipids 1 and 2.

All chemical reagents were of analytical grade, obtained from commercial suppliers and used without further purifications. Anhydrous DMF and MeOH were purchased from Aldrich or Acros in sealed bottles. In all cases, magnesium sulfate was used as a drying agent and solvent was removed by reduced pressure with a Buchi Rotovapor R-114 equipped with a Welch self-cleaning dry vacuum. Flash chromatography was performed using Merck 60 Å 230-400 mesh silica or Brockman I Activated Basic Alumina. All 1H and 13C NMR spectra are reported in ppm and referenced to solvent peaks. Spectra were obtain on Bruker AVB-400®, DRX-500®, or AV-500® instruments. Low and high-resolution fast-atom bombardment(FAB) and electrospray ionization (ESI) mass spectra were obtained from the UC Berkeley Mass Spectrometry Laboratory.

Synthesis of trehalose-dipentadecanoyl



6,6'-Di-*O*-trityl-α,α-D-trehalose (4).

A solution of trehalose dehydrate **3** (1.06 g, 3.09 mmol) and dry DMF (20 mL) was stirred with molecular sieves for 1 h at room temperature. Diisopropyl ethylamine (4 mL) was added and the solution was stirred for 20 minutes. Triphenylmethyl chloride (3.45 g, 12.4 mmol) was added in 0.5 g portions over 1 hour. Reaction was stirred overnight at room temperature. DMF was evaporated off and the crude material was purified via column chromatography in 7:1 chloroform:methanol to give compound **4** (1.39 g, 1.68 mmol, 54%). NMR (500MHz, DMSO): δ 3.15 (m, 1H) 3.19 (app d, 1H, *J*=8.5), 3.36 (m, 2H), 3.60 (m, 1H), 4.02 (m, 1H), 4.77 (d, 1H, *J*=6.4), 4.83 (d, 1H, *J*=5.3), 4.89 (d, 1H, *J*=4.9), 5.12 (d, 1H, *J*=3.6), 7.22 (app t, 3H, *J*=7.3), 7.30 (t, 6H, *J*=7.8), 7.42 (app d, 6H, *J*=7.5). ^{13}C NMR (500MHz, DMSO): δ 8.9, 45.8, 63.9, 71.0, 71.5, 72.1, 73.8, 85.9, 93.6, 127.3, 128.2, 128.8, 144.5. HRFABMS (*m/z*): $[M+Na]^+$ calcd for $C_{50}H_{50}O_{11}Na_1$ 849.3245, found 849.3230.

2,3,4,2',3',4'-Hexa-*O*-benzyl-6,6'-di-*O*-trityl-*α*,*α*-D-trehalose (5).

To a solution of compound **4** (0.218 g, 0.264 mmol) in dry DMF (10 mL) was added sodium hydride (0.095 g, 60% dispersion in oil, 2.4 mmol) at room temperature. The solution was stirred for 1 h. Benzyl bromide (0.343 mL, 2.89 mmol) was added dropwise at room temperature. The reaction was stirred for 6 h. The reaction mixture was diluted with dichloromethane and brine and the aqueous layer was extracted 2x with dichloromethane. The combined organic layers were dried with $MgSO_4$. Flash chromatography of the reaction mixture in 6:1 hexanes/ethyl acetate gave compound **5** (0.158 g, 0.0116 mmol 45%). ^{1}H NMR (400MHz, $CDCl_3$): δ 3.10 (dd, 1H, *J*=10.3, 2.3), 3.37 (app. d, 1H, *J*=8.9), 3.77 (dd, 1H, *J*=9.5, 3.6), 3.91(t, 1H, *J*=9.7), 4.01 (t, 1H, *J*=9.3), 4.22 (d, 1H, *J*=9.9), 4.27 (d, 1H, *J*=10.2), 4.70 (m, 2H), 4.77 (d, 1H, *J*=12.0), 4.85 (d, 1H, *J*=10.6), 4.99 (d, 1H, *J*=10.6), 5.52 (d, 1H, *J*=3.6), 6.81 (app. d, 2H, 6.5), 7.09 (app. d, 4H, *J*=4.4), 7.30 (m, 20H), 7.46 (app. d, 6H, *J*=6.7). ^{13}C NMR (400MHz, $CDCl_3$): δ 61.5 (CH_2), 70.7, 72.6, 75.1, 76.0, 77.9, 80.4, 81.9, 86.2, 94.9 (CH), 126.8, 127.0, 127.1, 127.6, 127.7, 127.8, 128.2, 128.5, 128.9, 129.2, 138.0, 138.3, 138.8, 143.9 (aromatic). HRFABMS (*m/z*): $[M+Na]^+$ calcd for $C_{92}H_{86}O_{11}Na_1$ 1389.6062, found 1389.6033.

2,3,4,2',3',4'-Hexa-*O*-benzyl-α,α-D-trehalose (6).

To a solution of compound **5** (0.0601 g, .0440 mmol) in dry chloroform (2 mL) was added triethyl silane (0.050 mL, 0.31 mmol). Trifluoroacetic acid (0.050 mL, 0.67 mmol) was added dropwise to the stirring solution at room temperature. The reaction was stirred for 4 hours. The reaction mixture was diluted with chloroform and washed 2x with brine. The aqueous layer was extracted 2x with chloroform. The combined organic layers were dried with $MgSO_4$. Flash chromatography of the reaction mixture in 4:1 hexanes/ethyl acetate afforded compound **6** (0.024 g, 0.0270 mmol, 61%). ^{1}H NMR (400MHz, $CDCl_3$): δ 3.60, (m, 4H), 4.13 (m, 2H), 4.70 (d, 1H, *J*=11.0), 4.72 (d, 1H, *J*=12.0), 4.77 ((d, 1H, *J*=12.0), 4.94 (app. d, 2H, 12.7), 5.05 (d, 1H, *J*=10.9), 5.18 (d, 1H, *J*=3.5), 7.36 (m, 15H). ^{13}C NMR (400MHz, $CDCl_3$): δ 61.6 (CH_2), 71.3, 73.1, 75.1, 75.6, 79.5, 81.6, 94.1 (CH), 127.6, 127.6, 127.7, 127.9, 128.2, 128.4, 128.5, 138.1, 138.2, 138.8 (aromatic). HRFABMS (*m/z*): $[M+Li]^+$ calcd for $C_{54}H_{58}O_{11}Li_1$ 889.413918, found 889.415300.

2,3,4,2',3',4'-Hexa-*O*-benzyl-6,6'-di-*O*-pentadecanoyl-α,α-D-trehalose (7).

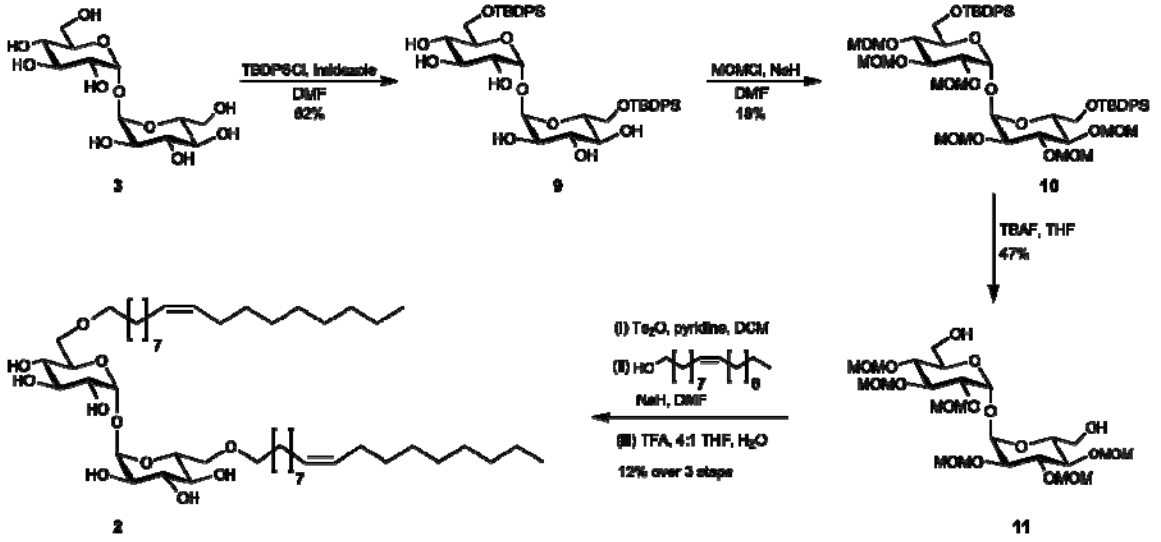
Compound **6** (16.5 mg, 0.0186 mmol) was dissolved in dichloromethane (1.5 mL). Pentadecanoic acid **5** (15.4 mg, 0.0635 mmol) was added to the stirring solution and cooled to 0 ^{o}C. A solution of EDC (50.0 mg, 0.261 mmol) and DMAP (18.3 mg, 0.150 mmol) in DCM (1 mL) was added dropwise to the reaction mixture. Reaction was allowed to warm up to room temperature overnight. The solvent was evaporated off and the residue was purified by flash chromatography in 6:1 hexanes/ethyl acetate to give the product **7** (23.5 mg, 0.0176 mmol, 95%). ^{1}H NMR (400MHz, $CDCl_3$): δ 0.93 (t, 3H, *J*=7.0), 1.31 (m, 24H), 2.28 (t, 2H, *J*=7.9), 3.58 (app. t, 1H, *J*=9.7), 3.61 (dd, 1H, *J*= 9.8, 3.5), 4.10 (m, 2H), 4.20 (dd, 1H, *J*=12.1, 3.6), 4.27 (m, 1H), 4.56 (d, 1H, *J*=10.6), 4.73 (d, 1H, *J*=12.0), 4.76 (d, 1H, *J*=12.0), 4.91 (d, 1H, *J*=10.6), 4.92 (d, 1H, *J*= 10.9), 5.06 (d, 1H, *J*=10.8), 5.22 (d, 1H, *J*=3.5), 7.36 (m, 15H). ^{13}C NMR (400MHz, $CDCl_3$): δ 14.7 (CH_3), 22.7, 24.9, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 29.7, 32.0, 34.2 (CH_2), 62.6, 69.2, 73.0, 75.3, 75.8, 77.6, 79.4, 81.7, 94.07 (CH), 127.5, 127.7, 127.9, 128.0,

128.2, 128.5, 128.5, 137.8, 137.9, 138.6 (aromatic), 173.5 (RCO_2R). HRFABMS (*m/z*): $[M+Na]^+$ calcd for $C_{84}H_{114}O_{13}Na_1$ 1353.8152, found 1353.8144.

6,6'-Di-*O*-pentadecanoyl-α,α-D-trehalose (1).

Compound **7** (20.1 mg, 0.0151 mmol) was dissolved in 100% ethanol (1.5 mL) containing 10% palladium on charcoal (10 mg). The mixture was stirred under H_2 overnight. The catalyst was removed by filtration, the solvent concentrated and the residue purified by flash chromatography in 4:1 chloroform/methanol to give compound **1** (7.7 mg, 0.0097 mmol, 65%). ^{1}H NMR (500MHz, MeOD): δ 0.79 (m, 3H), 1.17 (m, 26H), 1.50 (app. t, 2H, *J*=6.9), 2.23 (app. t, 2H, *J*=7.4), 3.35 (dd, 1H, *J*=9.7, 3.7), 3.66 (t, 1H, *J*=9.3), 3.89 (m, 1H), 4.09 (dd, 1H, *J*=11.9, 5.4), 4.24 (dd, 1H, *J*=11.9, 2.0), 4.93 (d, 1H, *J*=3.7). ^{13}C NMR (500MHz, $CDCl_3$, MeOD): δ 13.3 (CH_3), 22.4, 24.7, 28.9, 29.0, 29.1, 29.2, 29.4, 29.4, 29.4, 31.7, 33.8 (CH_2), 63.0, 70.0, 71.6, 73.1, 93.6 (CH), 174.2 (RCO_2R). HRFABMS (*m/z*): $[M+Na]^+$ calcd for $C_{42}H_{78}O_{13}Na_1$ 813.5335, found 813.5308.

Synthesis of trehalose-dioleoyl



6,6'-di-*O-tert*-butyldiphenylsilyl-α,α-D-trehalose(9)

A solution of trehalose dehydrate **3** (1.09g, 2.88 mmol) in dry DMF (20 mL) was stirred with molecular sieves for 1 h at room temperature. Imidazole (0.753 g, 11.1 mmol) was added and the

solution was stirred for 20 minutes. *tert*-Butylchlorodiphenylsilane (2.34 mL, 9.00 mmol) was added dropwise at room temperature. Reaction was stirred overnight. DMF was evaporated off and compound was purified via column chromatography in 10:1 chloroform:methanol to give protected compound **9** (1.46 g, 1.79 mmol, 62%). ^{1}H NMR (400MHz, DMSO): δ 1.00 (s, 9H), 3.28 (m, 2H), 3.36 (m, 1H), 3.63 (dt, 1H, *J*=13.4, 4.5), 3.82 (m, 3H), 4.64 (d, 1H, *J*=6.4), 4.86 (d, 1H, *J*=4.9), 4.92 (d, 1H, *J*=5.33), 5.06 (d, 1H, *J*=3.64), 7.38 (m, 6H), 7.71 (m, 4H). ^{13}C NMR (400MHz, DMSO): δ 19.5, 27.1, 63.9, 70.4, 72.2, 72.8, 73.7, 93.2, 128.2, 130.1, 133.8, 134.0, 135.6, 135.7. HRFABMS (*m/z*): $[M+Na]^+$ calcd for $C_{44}H_{58}O_{11}Na_1Si_2$ was 841.3410, found 841.3405.

2,3,4,2',3',4'-Hexa-*O*-methoxymethyl-6,6'-di-*O*-*tert*-butyldiphenylsilyl-α,α-D-trehalose (10).

To a solution of compound **9** (1.65 g, 2.01 mmol) in dry dichloromethane (10 mL) was added diisopropylethylamine (4.0 mL, 23 mmol) at 0 ^{o}C. Methoxymethyl chloride (9.96 mmol) was added dropwise. The reaction was allowed to come to room temperature overnight. The reaction mixture was diluted with chloroform and washed 2x with brine. The aqueous layer was extracted 2x with chloroform. The combined organic layers were dried with $MgSO_4$. Flash chromatography of the reaction mixture in 6:1 hexanes/ethyl acetate on Brockman I Activated Basic Alumina afforded compound **10** (0.385 g, 0.355 mmol, 18%). ^{1}H NMR (500MHz, $CDCl_3$): δ 1.04, (s, 9H), 3.10 (s, 3H), 3.28 (s, 3H), 3.41 (s, 3H), 3.56 (dd, 1H, *J*=9.8, 3.6), 3.72 (m, 2H), 3.90 (m, 3H), 4.50 (d, 1H, *J*=6.6), 4.57 (d, 1H, *J*=6.6), 4.80 (d, 1H, *J*=6.1), 4.81 (d, 1H, *J*=5.2), 4.83 (d, 1H, *J*=6.1), 4.86 (d, 1H, *J*=6.2), 5.0 (d, 1H, *J*=3.6), 7.38 (m, 6H), 7.69 (m, 4H). ^{13}C NMR (500MHz, $CDCl_3$): δ 19.3(CH_3), 26.8 (CH_2), 55.4, 56.4, 62.3 (CH_3), 71.6, 76.0, 77.2, 78.4 (CH), 94.3, 96.1, 98.5, 98.6 (CH_2), 127.4, 127.6, 129.6, 133.3, 133.6, 135.6, 135.8 (aromatic). HRFABMS (*m/z*): $[M+Na]^+$ calcd for $C_{56}H_{82}O_{17}Na_1Si_2$ was 1105.4983, found 1105.4969.

2,3,4,2',3',4'-Hexa-*O*-methoxymethyl-α,α-D-trehalose (11).

Compound **10** (78.9 mg, 0.0728 mmol) was dissolved in THF (2mL) room temperature. 1M tetrabutylammonium fluoride (0.2 mL, 0.2 mol) in THF was added dropwise. Reaction was stirred for 6 h. Solvent was evaporated off. Flash chromatography of the reaction mixture in 25:1 chloroform: methanol on Brockman I Activated Basic Alumina afforded compound **4** (20.4 mg, 0.0314 mmol, 47%). ^{1}H NMR (500MHz, $CDCl_3$): δ 3.34 (s, 3H), 3.43 (s, 3H), 3.44 (s, 3H), 3.55 (app t, 1H, *J*=10.0), 3.62 (dd, 1H, *J*=9.8, 3.6), 3.65 (m, 1H), 3.86 (ddd, 1H, *J*=12.5, 6.0, 3.6), 3.96 (m, 2H), 4.65 (d, 1H, *J*=6.2), 4.67 (d, 1H, *J*=6.3), 4.71 (d, 1H, *J*=6.4), 4.77 (d, 1H, *J*=6.3), 4.88 (d, 1H, *J*=6.3), 4.94 (d, 1H, *J*=6.4), 5.13 (d, 1H, *J*=3.5). ^{13}C NMR (500MHz, $CDCl_3$): δ 55.5, 56.2, 56.4 (CH_3), 61.4, 71.2, 76.5, 76.6, 78.4 (CH), 94.6, 96.5, 98.3, 99.1 (CH_2). HRFABMS (*m/z*): $[M+Li]^+$ calcd for $C_{24}H_{46}O_{17}Li_1$ was 613.289505, found 613.290270.

6,6'-Di-*O*-oleyl-*α*,*α*-D-trehalose (1).

To make 6,6' tosyl trehalose, pyridine (0.50 mL, 6.2 mmol) was dissolved in dichloromethane (2 mL) at 0 ^{o}C. *p*-Toluenesulfonic anhydride was added to the stirring solution. To this was added compound **10** (49.4 mg, 0.0841 mmol) dissolved in dry dichloromethane (2 mL). The reaction was run for 30 minutes until all the starting material disappeared. The reaction mixture was diluted with chloroform and washed with water. The aqueous layer was extracted 2x with chloroform. The combined organic layers were dried with $MgSO_4$, the solvent was evaporated off and the residue was dried under vacuum for 0.5 h. Oleyl alcohol (0.257 mL, 0.814 mmol) was dissolved in DMF (1 mL). NaH (0.1 g, 2.5 mmol, *60%* dispersion in mineral oil) was added at rt and stirred for 30 min. To this was added 6,6' tosyl trehalose dissolved in DMF (1 mL) and the stirring solution was heated to 98 ^{o}C and stirred overnight. The reaction mixture was allowed to cool to room temperature, diluted with chloroform and washed with water. The aqueous layer was extracted 2x with chloroform. The combined organic layers were dried with $MgSO_4$, the solvent evaporated off. Flash chromatography of the reaction mixture in 2:1 hexanes/ethyl acetate on Brockman I Activated Basic Alumina afforded the product and oleyl alcohol as an inseparable mixture. This mixture was suspended in 1:1 THF:water (2mL). Trifluoroacetic acid (0.9

mL, 12.1 mmol) was added dropwise at room temperature. Reaction was allowed to run 24 h. The solvent was evaporated off. Flash chromatography of the reaction mixture in 4:1 chloroform/methanol on silica gel afforded **11** (7.95 mg, 0.0094 mmol, 12% over 3 steps). ^{1}H NMR (500MHz, $CDCl_3$/MeOD): δ 0.77 (m, 4H), 1.17 (m, 26H), 1.47 (m, 2H), 1.91 (app d, 4H, *J*=5.3), 3.28 (m, 2H), 3.36 (app d, 1H, *J*=7.4), 3.55 (m, 2H), 3.62 (app d, 1H, *J*=10.1), 3.73 (m, 2H), 5.02 (m, 1H), 5.24 (m, 2H). ^{13}C NMR (500MHz, $CDCl_3$): δ 13.9 (CH_3), 22.5, 25.8, 27.1, 29.2, 29.3, 29.4, 29.5, 29.6, 31.8 (CH_2), 70.2, 70.7, 71.0, 71.5, 72.1, 73.0 (CH), 79.4, 93.4 (CH_2O), 129.7, 129.8 (aromatic). HRFABMS (*m/z*): $[M+Li]^+$ calcd for $C_{48}H_{90}O_{11}Li_1$ was 849.664319, found 849.663920.

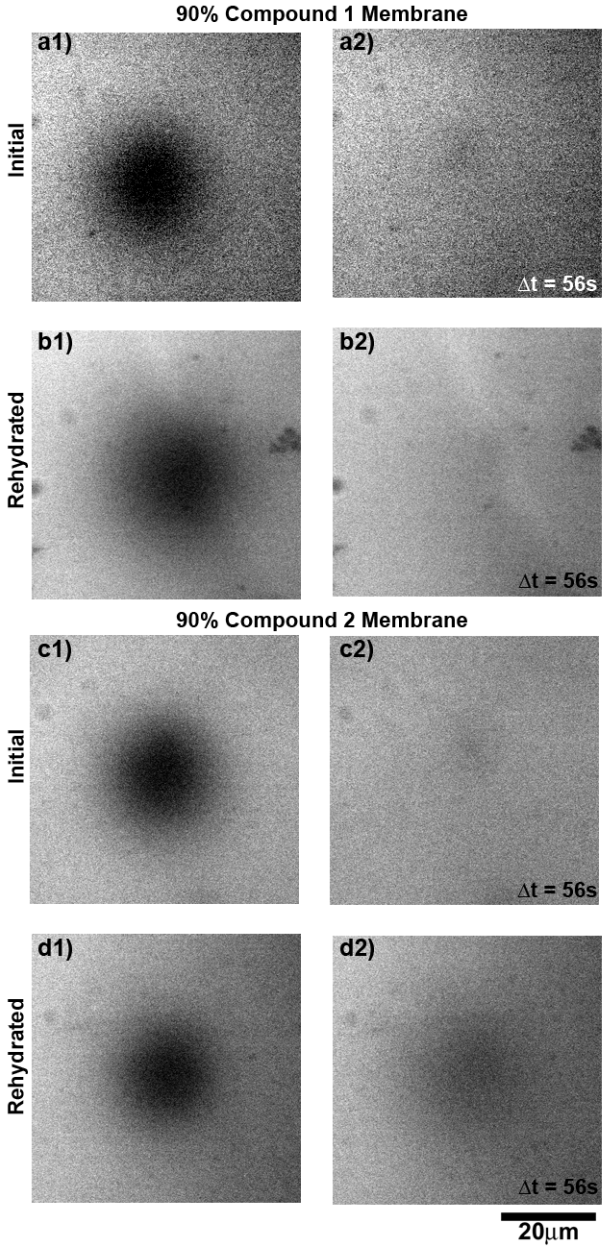


Figure S1. FRAP images of X = 0.90 synthetic glycolipid monolayers before and after a two week dehydration period. Both compound **1** (**a** and **b**) and compound **2** (**c** and **d**) show bright, uniform fields of fluorescence and are able to recover fluorescence after photobleaching of a circular region. Monolayer mobility, and hence integrity, was recovered after the long dehydration indicating the robust nature of the synthetic trehalose glycolipid derived protection.