Electronic Supplementary Material (ESI) for ChemComm.

Development of an Acid Ceramidase Activity-Based Probe

Cécile M. J. Ouairy,[a] Maria J. Ferraz,[a,b] Rolf G. Boot,[a,b] Marc P. Baggelaar,[a] Mario van der Stelt,[a] Monique Appelman,[b] Gijsbert A. van der Marel,[a] Bogdan I. Florea,[a] Johannes M. F. G. Aerts*[a,b] and Herman S. Overkleeft*[a]

[a] *Leiden Institute of Chemistry, Leiden University, P. O. Box 9502, 2300 RA Leiden, The Netherlands; Tel: +31715274342; E-mail: h.s.overkleeft@chem.leidenuniv.nl or j.m.f.g.aerts@lic.leidenuniv.nl*

[b] *Department of Medical Biochemistry, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AS Amsterdam, The Netherlands*

Supporting Information

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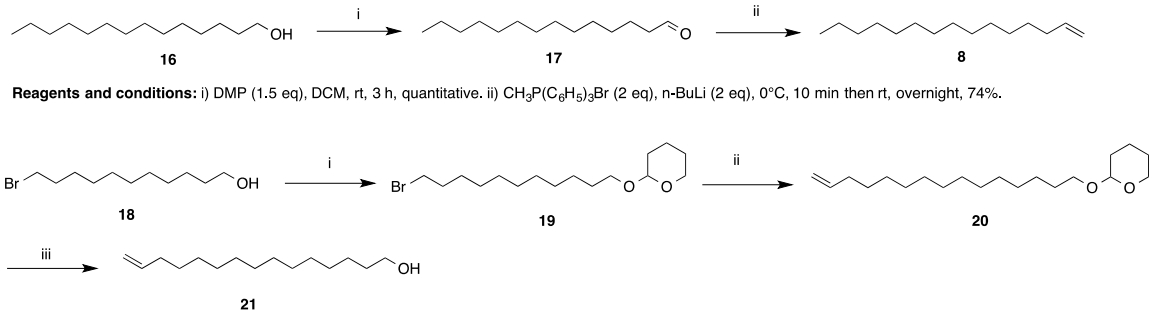
Synthesis of compounds 1, 2, 3, 4, 5, 6.

General Methods

Reactions were carried out in flame-dried glassware, under an argon atmosphere and at room temperature, unless noticed otherwise. Solvents and reagents were obtained commercially and used as received unless noticed otherwise. Reaction grade solvents were stored on 4 Å molecular sieves or 3 Å for MeOH. All solvents were removed by in *vacuo* evaporation at ~ 40 ºC. Reaction progress was monitored by TLC analysis using silica gel coated aluminium sheets (Merck, silica gel 60, F_{254}) with detection by UV absorption (254 nm) and/or by spraying with a solution of $KMnO_4$ (5 g/L) and K_2CO_3 (25 g/L) in H_2O or a solution of $(NH_4)Mo_7O_{24}\cdot 4H_2O$ (10 g/L) in EtOH, followed by charring at ~ 150 ºC. Flash column chromatography was performed on silica gel with a particle size of 40-63 μm and a pore size of 60 Å for all compounds. For reversed-phase HPLC purification, an automated HPLC system equipped with a C18 or C4 semiprep column was used. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 400, 600 or 850 spectrometer. Chemical shifts are given in ppm (δ) relative to the internal standard of the solvent used or to the deuterated solvent signal. For the ^{1}H-NMR spectra: $CDCl_3$ (TMS, 0 ppm or $CHCl_3$, 7.26 ppm), MeOD-d_4 (MeOH, 3.31 ppm), D_2O (H_2O, 4.79 ppm). For the ^{13}C-NMR spectra: $CDCl_3$ (77.16 ppm), MeOD-d_4 (49.0 ppm). All ^{13}C spectra are proton decoupled. HRMS were recorded by direct injection (2 μL of a 2 μM solution in H_2O/ACN/*t*BuOH; 1/1/1) on a mass spectrometer (Thermo Finnigan LTZ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 ºC) with resolution R=60000 at m/z 400 (mass range m/z= 100 – 750) and dioctylpthalate (m/z = 391.28428) as a "lock mass". Infrared spectra were recorded on a PerkinElmer UATR Two and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter (Sodium D-line, λ = 589 nm) at ambient temperature.

Synthesis of probes 1, 2



Supporting Scheme 1. Synthesis of alkenes **8** and **21.**

Tetradecanal 17

DMP (5.94 g, 14.0 mmol, 1.5 eq) was added to a solution of tetradecanol **16** (2.00 g, 9.3 mmol) in DCM (30 mL) and the mixture was stirred for 3 h at RT. A mixture of sat. aq. $NaHCO_3$:$Na_2S_2O_3$ (1N) (1:1) (120 mL) was added and the mixture was extracted with Et_2O (90 mL × 2). The combined organic layers were washed with water (30 mL), brine (90 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the aldehyde **17** as a white solid without any further purification (1.97 g, quantitative). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (t, J = 1.6 Hz, 1 H), 2.42 (m, 2 H), 1.68 – 1.46 (m, 2 H), 1.27 (m, 20 H), 0.88 (t, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.2, 44.1, 32.01, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 22.9, 22.2, 14.3. IR ν□ (cm^{-1}) 2951, 2913, 2849, 1603. These data are in accordance with literature precedence.[1]

Pentadec-1-ene 8

Methyltriphenylphosphonium bromide (2.43 g, 6.80 mmol, 2.0 eq) was suspended in anhydrous THF (10 mL) and *n*-Buli (4.25 mL, 6.8 mmol, 2.0 eq) was added at 0 °C. The reaction was then stirred for 10 min at 0 °C. Tetradecanal **17** (0.72 g, 3.4 mmol) dissolved in anhydrous THF (10

mL) was added to the ylide at 0 °C and the reaction mixture was stirred overnight at RT. Et_2O (100 mL) was then added and the mixture was washed with water (2 × 130 mL) and brine (60 mL). Water layers were extracted with Et_2O (50 mL) and the combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo.* The residue was purified by column chromatography (100% PE) to yield **8** (530 mg, 74%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (m, 1 H), 5.02 - 4.89 (m, 2 H), 2.04 (q, J = 6.8 Hz, 2 H), 1.26 (m, 22 H), 0.88 (t, J = 6.8 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.4, 114.2, 34.0, 32.1, 29.9, 29.84, 29.83, 29.79, 29.7, 29.5, 29.3, 29.1, 22.9, 14.3. IR ν (cm^{-1}) 2924, 2855, 2327, 2342, 910, 658, 1641. These data are in accordance with literature precedence.[2]

2-((11-bromoundecyl)oxy)tetrahydro-2*H*-pyran 19

PPTS (500 mg, 2 mmol, 0.1 eq) was added to a stirred solution of 11-bromoundecanol **18** (5 g, 20 mmol) and 3,4-dihydro-2*H*-pyran (2.7 mL, 30 mmol, 1.5 eq) dissolved in DCM (100 mL) at room temperature under argon. The reaction mixture was stirred for 4 h and it was then treated with sat. aq. $NaHCO_3$ (2 × 100 mL). The organic and aqueous layers were separated and the aqueous layer was extracted with DCM (2 × 60 mL). The combined organic layers were then washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification with column chromatography (PE/EA (95:5)) produced the protected alcohol **19** (6.43 g, 96%) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.59 - 4.57 (m, 1 H), 3.90 – 3.85 (m, 1 H), 3.77 – 3.71 (m, 1 H), 3.53 – 3.48 (m, 1 H), 3.40 (t, J = 7.2 Hz, 2 H), 3.38 – 3.36 (m, 1 H), 1.89 – 1.53 (m, 8 H), 1.41 – 1.29 (m, 16 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 99.0, 67.8, 62.5, 34.2, 33.0, 30.9, 29.9, 29.7, 29.6, 29.5, 28.9, 28.3, 26.4, 25.6, 19.8. IR ν (cm^{-1}) 1134, 1119, 1078, 1032, 1023. These data are in accordance with literature precedence.[3]

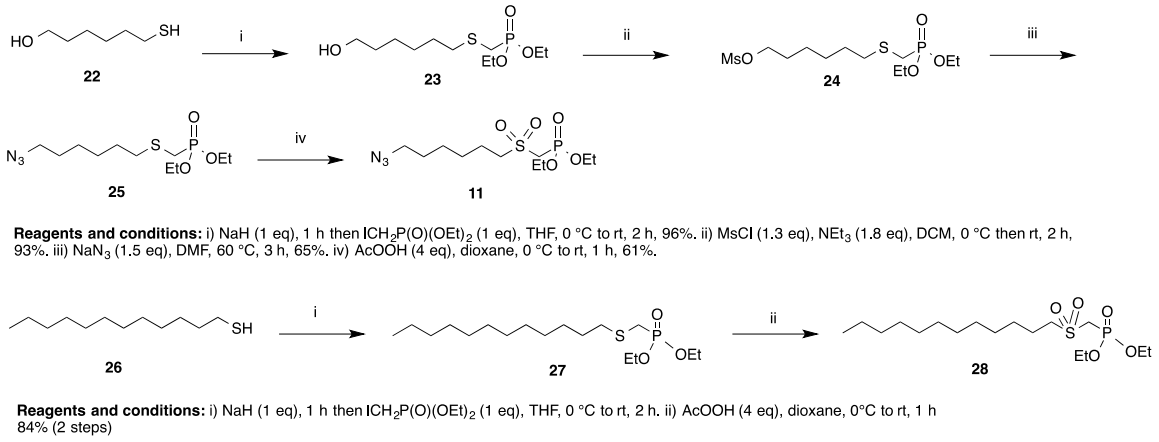
2-(penta-14-en-1-yloxy)tetrahydro-2*H*-pyran 20

To a stirred solution of 2-(11-bromo-undecyloxy)-tetrahydropyran **19** (1 g, 2.98 mmol) in THF (7 mL) was added dropwise 3-butenyl magnesium bromide (12 mL, 5.96 mmol, 2 eq, 0.5 M in THF) and Li_2CuCl_4 (3 mL, 0.3 mmol, 0.1 eq, 0.1 M in THF) at 0 °C under argon. The reaction mixture was stirred for 2 h at this temperature and was then quenched with sat. aq. NH_4Cl (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were then washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude was purified on a silica gel column (PE/EA (10:1)) to give the desired compound **20** (685 mg, 74%) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.85 – 5.76 (m, 1 H), 5.01 (dd, J = 17.0, 2.0 Hz, 1 H), 4.92 (dd, J = 10.0, 0.8 Hz, 1 H), 4.58 (t, J = 2.4 Hz, 1 H), 3.90 – 3.85 (m, 1 H), 3.76 – 3.70 (m, 1 H), 3.53 – 3.47 (m, 1 H), 3.41 – 3.35 (m, 1 H), 2.06 – 2.01 (m, 2 H), 1.86 – 1.85 (m, 1 H), 1.83 – 1.80 (m, 1 H), 1.70 -1.62 (m, 6 H), 1.43 – 1.34 (m, 20 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.4, 114.2, 99.0, 67.8, 62.5, 34.0, 30.9, 30.4, 29.9, 29.8, 29.7, 29.64, 29.63, 29.3, 29.1, 26.4, 25.6, 19.8. IR ν (cm^{-1}) 1136, 1120, 1078, 1022, 906. These data are in accordance with literature precedence.[4]

Pentadec-14-en-1-ol 21

To a stirred solution of alkene **20** (193 mg, 0.62 mmol) in MeOH (2 mL) was added $TsOH.H_2O$ (180 mg, 0.95 mmol, 1.5 eq) at room temperature under argon. The reaction mixture was stirred 6 h at this temperature and the crude was then concentrated *in vacuo* before sat. aq. $NaHCO_3$ was added (20 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were then washed with sat. aq. $NaHCO_3$ (20 mL), brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude was purified with column chromatography (PE/EA (10:1)) to afford the deprotected alcohol **21** as a white solid (119 mg, 84%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.87 – 5.77 (m, 1 H), 5.02 – 4.96 (m, 1 H), 4.95 – 4.91 (m, 1 H), 3.64 (t, J = 6.4 Hz, 2 H), 2.18 – 2.00 (m, 2 H), 1.60 – 1.53 (m, 2 H), 1.45 (sl, 1 H, OH), 1.45 – 1.26 (m, 20 H). ^{13}C NMR

($CDCl_3$, 100 MHz) δ 139.4, 114.2, 63.2, 34.0, 32.9, 32.0, 29.78, 29.75, 29.7, 29.64, 29.57, 29.3, 29.1, 25.9. IR ν (cm^{-1}) 3323, 3228, 1059, 991, 912. These data are in accordance with literature precedence.[5]



Supporting Scheme 2. Synthesis of phosphonates **11** and **28.**

Diethyl (((6-hydroxyhexyl)thio)methyl)phosphonate 23

6-Mercaptohexan-1-ol **22** (1.40 mL, 10.2 mmol) was dissolved in THF (50 mL) at 0 °C. NaH (60% disp. in mineral oil, 0.45 g, 11.3 mmol, 1.1 eq) was added and the solution was stirred for 1 h at 0 °C. Diethyl iodomethylphosphonate (1.7 mL, 10.2 mmol, 1.0 eq) was added and the mixture was stirred for 2 h after which TLC analysis indicated complete consumption of the starting material. The solution was quenched by adding HCl 1N (60 mL). Water layer was extracted with DCM (3 × 60 mL) and the combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure. The title compound **23** was purified by column chromatography (100% EtOAc) and obtained as a colorless oil (2.79 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.27 – 4.03 (m, 4 H), 3.61 (t, J = 6.5 Hz, 2 H), 2.72-2.68 (m, 4 H), 1.88 (s, 1 H, OH), 1.62-1.56 (m, 4 H), 1.43 – 1.36 (m, 4 H), 1.33 (t, J = 6.9 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 62.8, 62.7, 33.5, 32.6, 28.9, 28.4, 25.4 (J = 150 Hz), 25.3, 16.6. IR ν (cm^{-1}) 3512.2-

2993.2, 2920, 2851, 2360, 1024, 633. HRMS calculated for $[C_{11}H_{25}O_4PS + H]^+$ 285.12851, found 285.36147 $[M + H]^+$.

Diethyl (((6-azidohexyl)thio)methyl)phosphonate 25

To a stirred solution of compound **23** (2.79 g, 9.8 mmol) in DCM (50 mL) at 0 °C, was added NEt_3 (2.50 mL, 17.9 mmol, 1.8 eq). MsCl (1.05 mL, 13.6 mmol, 1.4 eq) was added carefully and the reaction mixture was allowed to warm up and stirred at RT for 2 h. The crude was then diluted with DCM (50 mL) and washed with water (50 mL). The aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo* to afford the mesylate **24** as a colorless oil (3.31 g, 93%). This compound was directly used in the next step.

Mesylate **24** (3.31 g, 9.1 mmol) was dissolved in DMF (50 mL) and NaN_3 (0.89 g, 13.7 mmol, 1.5 eq) was added, the reaction mixture was stirred at 60 °C for 3 h. Then water (100 mL) and Et_2O (2 × 100 mL) were added and the phases were separated. Aqueous layer was extracted with Et_2O (2 × 100 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (100% EtOAc) afforded the azide **25** as a colorless oil (1.83 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 - 4.14 (m, 4 H), 3.25 (t, J = 6.8 Hz, 2 H), 2.72-2.65 (m, 4 H), 1.64-1.55 (m, 4 H), 1.44 – 1.37 (m, 4 H), 1.34 (t, J = 6.9 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 62.8, 62.7, 51.5, 33.5, 28.9, 28.8, 28.3, 26.1, 26.0 (d, J = 139 Hz), 16.6. IR ν □(cm^{-1}) 2980, 2932, 2859, 959, 824, 1248, 1163, 1049. HRMS: calculated for $[C_{11}H_{24}N_3O_3PS + H]^+$ 310.13495, found 310.13488 $[M + H]^+$.

Diethyl (((6-azidohexyl)sulfonyl)methyl)phosphonate 11

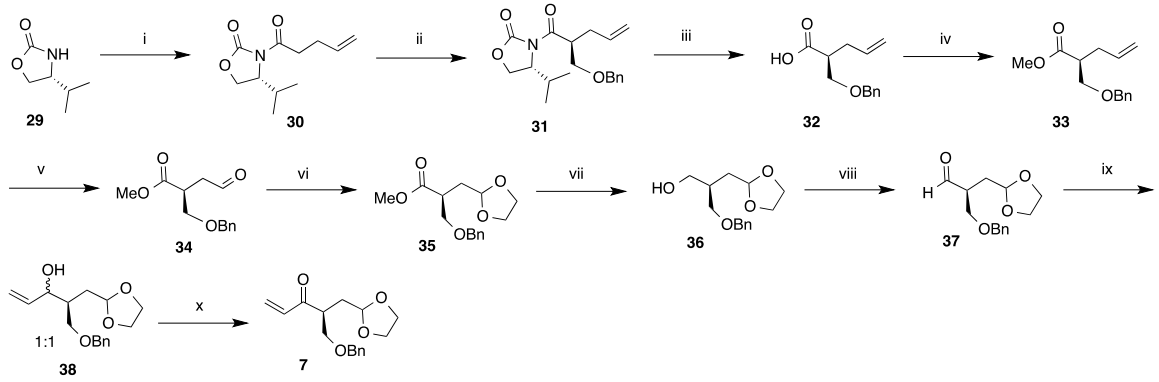
Compound **25** (1.83 g, 5.9 mmol) was dissolved in dioxane (20 mL) at 0 °C and AcOOH (4.30 mL, 25.4 mmol, 4.3 eq) was added. The reaction mixture was allowed to warm up at RT and

stirred for 1 h. Sat. aq. $NaHCO_3$ (50 mL) was added and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography (DCM/EtOH (95:5)) and afforded the sulfone **11** as colorless oil (1.23 g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.26 - 4.18 (m, 4 H), 3.54 (d, J = 16.4 Hz, 2 H), 3.34 (t, J = 8.0 Hz, 2 H), 3.26 (t, J = 6.8 Hz, 2 H), 1.91-1.83 (m, 2 H), 1.62 – 1.58 (m, 2 H), 1.51 – 1.46 (m, 4 H), 1.37 (t, J = 6.9 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.8, 63.7, 54.4, 51.3, 50.6 (d, J = 140 Hz), 28.6, 28.0, 26.3, 21.9, 16.4, 16.4. IR ν □(cm^{-1}) 2917, 2911, 976, 1258, 1144, 1107. HRMS: calculated for $[C_{11}H_{24}N_3O_5PS + Na]^+$ 364.1065, found 364.1067 $[M + Na]^+$.

Diethyl ((dodecylsulfonyl)methyl)phosphonate 28

Dodecanethiol **26** (2 g, 9.88 mmol) was dissolved in THF (30 mL) at 0 °C. NaH (60% disp. in mineral oil, 435 mg, 10.9 mmol, 1.1 eq) was added and the solution was stirred for 1 h at 0 °C. Diethyl iodomethylphosphonate (1.65 mL, 9.89 mmol, 1 eq) was added and the mixture was stirred for 2 h. It was then quenched by the addition of HCl 1N (50 mL). The water layer was extracted with DCM (3 × 50 mL) and the combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and evaporated. The crude was dissolved in dioxane (35 mL), at 0 °C and AcOOH (6.7 mL, 39.5 mmol, 4.0 eq) was added. The reaction was allowed to warm to room temperature and stirred for 1 h. Sat. aq. $NaHCO_3$ (60 mL) was added and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified with column chromatography (PE / EA (60:40)) and the sulfone **28** was obtained as a white solid (3.18 g, 84%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ□ 4.24 (2 × q, J = 7.2 Hz, 4 H), 3.55 (d, J = 16.8 Hz, 2 H), 3.33 (t, J = 7.6 Hz, 2 H), 1.89 – 1.81 (m, 2 H), 1.48-1.42 (m, 2 H), 1.41 (t, J = 7.2 Hz, 6 H), 1.36 – 1.26 (m, 16 H), 0.88 (t, J = 6.4 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ□ 63.8, 63.7, 54.7, 50.1 (d, J = 140 Hz), 32.0, 29.7, 29.6, 29.44, 29.37,

29.2, 28.5, 22.8, 22.0, 16.5, 16.4, 14.2. IR ν□ (cm^{-1}) 1319, 1256, 1161, 1016, 978, 847, 808. HRMS calculated for: $[C_{17}H_{37}O_5PS+ H]^+$ 385.2176, found: 385.2172 $[M + H]^+$.



Reagents and condtions: i) a) *n*BuLi (1.1 eq), -78°C, 30 min, b) pent-4-enoyl chloride (1.1 eq), - 78 °C, 30 min then warm to - 20°C, THF, 57%. ii) $TiCl_4$ (1eq), *i*-Pr_2NEt (1eq), $ClCH_2OBn$ (1.2 eq), DCM, 0°C, 1 h then 0°-10 °C, 1 h 30 min, 61%. iii) LiOH (2 eq), H_2O_2 (30% H_2O), THF/H_2O (1:1), 5 °C, 2 h, 89%. iv) MeOH (1.1 eq), DIC (1.1 eq), cat DMAP (0.04 eq), DCM, rt, 2 h, 73%. v) a) O_3 (excess), - 78 °C, 15 min, b) NEt_3 (2 eq), DCM, rt, 3 h quantitative. vi) PPTS (0.15 eq), ethylene glycol (9 eq), toluene, reflux, overnight, 94%. vii) $LiAlH_4$ (2 eq), THF, 0 °C then rt, overnight, 87%. viii) DMP (1.5 eq), DCM, rt, 3 h, 88%. ix) vinylmagnesium bromide (2 eq), THF, rt, 30 min, 63%. x) DMP (1.5 eq), DCM, rt, overnight, quantitative.

Supporting Scheme 3. Synthesis of α,β-unsaturated ketone **7.**

(*R*)-3-((*S*)-2-((benzyloxy)methyl)pent-4-enoyl)-4-isopropyloxazoli-din-2-one 31

(4*R*)-4-Isopropyloxazolidin-2-one **29** (2.86 g, 22.1 mmol) was dissolved in 20 mL THF at – 78 °C. *n*-BuLi (15.2 mL, 24.4 mmol, 1.1 eq) was then added and the resulting mixture was stirred for 30 min at – 78 °C. Pent-4-enoyl chloride (2.7 mL, 24.4 mmol, 1.1 eq) was then added and the resulting mixture was stirred for 30 min at the same temperature and then warm to – 20 °C. It was then quenched by the addition of water (50 mL) and extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (50 mL), brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give the crude **30** (2.67 g, 57%) as a yellow oil.

The oxazolidinone **30** (2.67 g, 12.6 mmol) was then dissolved in 40 mL DCM and *i*$PrNEt_2$ (2.3 mL, 13.86 mmol, 1.1 eq) followed by $TiCl_4$ (1.0 M in DCM) (14 mL, 14.0 mmol, 1.1 eq) were added dropwise at 0 °C. The resulting mixture was stirred for 1 h. Then $ClCH_2OBn$ (purety 60% by nmr) (3.8 mL, 16.38 mmol, 1.3 eq) was added keeping the internal temperature between 5 °C and 10 °C and the mixture was stirred for 1.5 h. Sat. aq. NH_4Cl (80 mL) was then added and the

organic layer was separated. The aqueous layer was extracted with $CHCl_3$ (2 × 60 mL) and the combined layers were washed with sat. aq. NH_4Cl (100 mL), brine (100 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude was purified with column chromatography (PE/EA (90:10)) to afford the oxazolidinone **31** (2.55 g, 61%) as a colorless oil. $[\alpha]^{20}_D$ = - 45 (c = 0.8 $CHCl_3$).[6] ^1H NMR ($CDCl_3$, 400 MHz) δ 7.34 – 7.25 (m, 5 H), 5.79 – 5.73 (m, 1 H), 5.08 - 5.03 (m, 1 H), 5.02 - 4.99 (m, 1 H), 4.54 – 4.46 (m, 3 H), 4.35 – 4.28 (m, 1 H), 4.24 (t, *J* = 8.6 Hz, 1 H), 4.17 (dd, *J* = 8.6, 3.2 Hz, 1 H), 3.75 (dd, *J* = 9.2, 7.6 Hz, 1 H), 3.64 (dd, *J* = 9.2, 5.2 Hz, 1 H), 2.46 – 2.42 (m, 1 H), 2.36 – 2.29 (m, 2 H), 0.89 (d, *J* = 7.2 Hz, 3 H), 0.79 (d, *J* = 7.2 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.3, 154.0, 138.2, 135.1, 128.4, 127.8, 127.7, 117.3, 73.2, 71.1, 63.3, 58.6, 43.3, 33.2, 28.5, 18.0, 14.7. IR ν (cm^{-1}) 1098, 1699, 1387, 1202, 1098. HRMS calculated for: $[C_{19}H_{25}NO_4 + H]^+$ 354.1676, found: 354.1676 $[M + H]^+$.

(*S*)-2-((benzyloxy)methyl)pent-4-enoic acid 32

To a stirred solution of LiOH (232 mg, 9.69 mmol, 2.1 eq) and H_2O_2 (30% in water) (9 mL) in a mixture of THF (15 mL) and water (15 mL), at 5 °C, under argon, was slowly added a solution of the oxazolidinone **31** (1.53 g, 4.62 mmol) in THF (15 mL) keeping the internal temperature inferior to 10 °C. The reaction mixture was stirred 2 h at this temperature and was then quenched by the addition of $Na_2S_2O_3$ (60 mL) and sat. aq. $NaHCO_3$ (60 mL). The aqueous layer was washed with $CHCl_3$ (60 mL) and acidified with HCl 1N (60 mL). The aqueous layer was then extracted with $CHCl_3$ (3 × 60 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude was purified with column chromatography (PE/EA (50:50)) to afford the carboxylic acid **32** (910 mg, 89%) as a pale yellow solid. $[\alpha]^{20}_D$ = + 1.5 (c = 0.2, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 7.36 – 7.26 (m, 5 H), 5.81 – 5.73 (m, 1 H), 5.07 (dd, *J* = 17.2, 1.6 Hz, 1 H), 5.05 (dd, *J* = 13.6, 1.6 Hz, 1 H), 4.52 (s, 2 H), 3.67 (dd, *J* = 9.2, 7.6 Hz, 1 H), 3.59 (dd, *J* = 9.2, 7.6 Hz, 1 H), 2.83 – 2.77 (m, 1 H), 2.49 – 2.43 (m, 1 H), 2.36 –

2.31 (m, 1 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.9, 137.9, 134.6, 128.6, 127.9, 127.8, 117.7, 73.4, 69.8, 45.5, 32.8. IR ν (cm^{-1}) 1765, 1274, 1207, 1101, 920, 736. HRMS calculated for: $[C_{13}H_{16}O_3 + H]^+$ 221.1178, found: 221.1179 $[M + H]^+$.

(*S*)-methyl 2-((benzyloxy)methyl)pent-4-enoate 33

To a stirred solution of the acid **32** (2.5 g, 11.4 mmol) in DCM (40 mL) was added MeOH (500 μL, 12.3 mmol, 1.1 eq) and DMAP (110 mg, 0.9 mmol, 0.08 eq). DIC (1.9 mL, 12.2 mmol, 1.1 eq) was added to the mixture, which was stirred for 2 h, at room temperature, under argon. Then the urea was removed by filtration and the mixture was treated with HCl (20 mL) and extracted with DCM (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude was purified with column chromatography (PE/EA (95:5)) to afford the ester **33** (1.95 g, 73%) as a colorless oil. $[\alpha]^{20}_D$ = + 236 (c = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.26 (m, 5 H), 5.87 – 5.61 (m, 1 H), 5.13 – 4.96 (m, 2 H), 4.51 (s, 2 H), 3.69 (s, 3 H), 3.66 (dd, *J* = 9.2, 7.6 Hz, 1 H), 3.56 (dd, *J* = 9.2, 5.6 Hz, 1 H), 2.83 – 2.76 (tt, *J* = 7.6, 6.1 Hz, 1 H), 2.44 – 2.27 (m, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 138.2, 134.9, 128.5, 127.7, 117.3, 73.3, 70.3, 51.8, 45.8, 33.2. IR ν (cm^{-1}) 1738, 1454, 1436, 1365, 1196, 1172, 1102, 918, 737, 699, 531. HRMS calculated for: $[C_{14}H_{18}O_3 + H]^+$ 235.1329, found: 235.1327 $[M + H]^+$.

(*S*)-methyl 2-((benzyloxy)methyl)-4-oxobutanoate 34

In a 250 mL two-necked flask fitted with a glass tube to admit ozone, a $CaCl_2$ drying tube and a magnetic stirrer bar, was charged the ester **33** (1.83 g, 7.81 mmol) in anhydrous DCM (120 mL) and MeOH (5 mL). The flask was cooled to – 78 °C and ozone was bubbled through the solution. When the solution turned blue, ozone addition was stopped and the reaction mixture was stirred for 15 min. Argon was then bubbled to remove the excess of ozone. To this solution was then added NEt_3 (1.7 mL, 12.23 mmol, 1.6 eq) and the solution was allowed to warm up and stirred for

3 h. The solvent was evaporated and the crude was then washed with water (40 mL). The aqueous layer was then extracted with DCM (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude **34** (1.84 g, quantitative, colorless oil) was used without any further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1 H), 7.44 – 7.08 (m, 5 H), 4.50 (s, 2 H), 3.70 (s, 3 H), 3.67 (d, *J* = 5.7 Hz, 2 H), 3.23 – 3.21 (m, 1 H), 2.99 (dd, *J* = 18.3, 8.2 Hz, 1 H), 2.73 (dd, *J* = 18.3, 5.4 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.1, 173.0, 137.8, 127.9, 127.7, 73.2, 69.7, 52.3, 42.7, 39.9. IR ν□ (cm^{-1}) 2868, 1725, 1363, 1208, 1104, 1027, 946, 739, 699, 622, 533. HRMS calculated for: $[C_{13}H_{16}O_4 + Na]^+$ 259.0941, found: 259.0940 $[M + Na]^+$.

(*S*)-methyl 2-((1,3-dioxolan-2-yl)methyl)-3-(benzyloxy)propanoate 35

To a stirred solution of the aldehyde **34** (1.9 g, 8.04 mmol) in toluene (40 mL) was added ethylene glycol (4 mL, 71.73 mmol, 8.9 eq) and PPTS (606 mg, 1.5 mmol, 0.3 eq). The flask was equipped with a Dean-Stark and the mixture was stirred for 4 h at 130 °C. The solution was then allowed to cool down to room temperature and sat. aq. $NaHCO_3$ (40 mL) was added. The different layers were separated and the aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude was purified with column chromatography (PE/EA (90:10)) to afford **35** (2.1 g, 94%) as a colorless oil. $[\alpha]^{20}_D$ = + 0.75 (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.19 (m, 5 H), 4.94 (t, *J* = 4.3 Hz, 1 H), 4.51 (s, 2 H), 3.97 – 3.88 (m, 2 H), 3.86 – 3.78 (m, 2 H), 3.70 (s, 3 H), 3.65 (dd, *J* = 9.2, 7.6 Hz, 1 H), 3.58 (dd, *J* = 9.2, 5.7 Hz, 1 H), 2.97 – 2.91 (m, 1 H), 2.11 (ddd, *J* = 13.7, 9.2, 4.5 Hz, 1 H), 1.85 (dt, *J* = 14.2, 4.5 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 138.1, 128.4, 127.7, 127.7, 102.7, 73.1, 71.0, 65.1, 51.9, 41.3, 32.9. IR ν□ (cm^{-1}): 1736, 1436, 1196, 1172, 993, 918, 737, 697. HRMS calculated for: $[C_{15}H_{20}O_5 + H]^+$ 281.1384, found: 281.1383 $[M + H]^+$.

(*R*)-2-((1,3-dioxolan-2-yl)methyl)-3-(benzyloxy)propan-1-ol 36

To a stirred solution of $LiAlH_4$ (380 mg, 10.0 mmol, 2.0 eq) in THF (50 mL) was added a solution of the ester **35** (1.40 g, 4.99 mmol) in THF (5 mL), at 0 °C under argon. The mixture was stirred at this temperature for 30 min. It was then allowed to warm up to room temperature and stirred overnight at this temperature. Water (2 mL), NaOH (15%) (2 mL) and water (6 mL) were then successively added to the mixture. It was then filtered through a pad of Celite®, washed with THF and the solvent was evaporated. The crude was then extracted with $CHCl_3$ (3 × 40 mL) and the combined organic layers were washed with water (60 mL), brine (60 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the alcohol **36** (1.1 g, 87%, colorless oil) was used without any further purification (^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.27 (m, 5 H), 4.94 (t, J = 4.7 Hz, 1 H), 4.51 (s, 2 H), 4.07 – 3.86 (m, 2 H), 3.94 – 3.78 (m, 2 H), 3.71 – 3.70 (m, 2 H), 3.58 (dd, J = 9.2, 5.0 Hz, 1 H), 3.50 (dd, J = 9.2, 7.0 Hz, 1 H), 2.79 (bs, 1 H), 2.15 – 2.09 (m, 1 H), 1.91 – 1.66 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 128.6, 127.7, 103.6, 73.4, 73.2, 65.5, 65.0, 64.9, 37.2, 32.9. IR ν □(cm^{-1}) 3435, 2883, 1454, 1410, 1364, 1092, 1028, 945, 737, 699, 532.

(*S*)-2-((1,3-dioxolan-2-yl)methyl)-3-(benzyloxy)propanal 37

DMP (2.77 g, 6.53 mmol, 1.5 eq) was added to a stirred solution of the alcohol **36** (1.1 g, 4.36 mmol) in DCM (15 mL). The mixture was stirred for 3 h at room temperature. A mixture of sat. aq. $NaHCO_3$:$Na_2S_2O_3$ (1N) (1:1) (40 mL) was then added and the different layers were separated. The aqueous layer was extracted with Et_2O (2 × 30 mL). The combined organic layers were washed with water (30 mL), brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to get the crude aldehyde **37** (960 mg, 88%, colorless oil) without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (d, J = 2.2 Hz, 1 H), 7.67 – 6.74 (m, 5 H), 4.99 (t, J = 4.1 Hz, 1 H), 4.51 (s, 2 H), 4.01 – 3.87 (m, 2 H), 3.87 – 3.78 (m, 2 H), 3.77 – 3.62 (m, 2 H), 2.81 (ttd, J = 7.4,

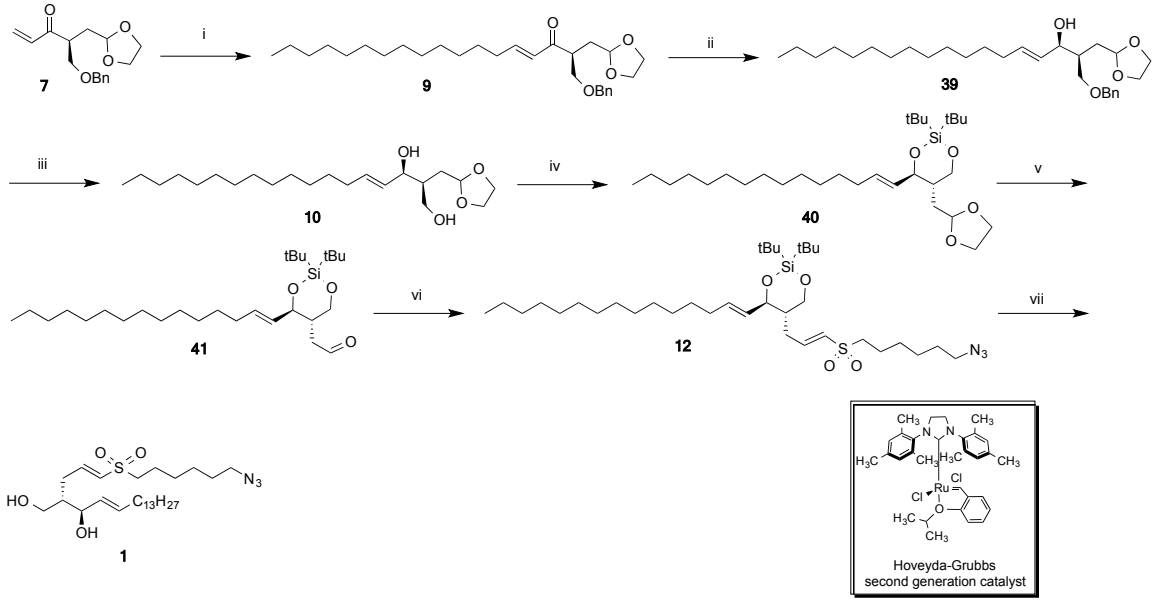
5.2, 2.2 Hz, 1 H), 2.23 (ddd, *J* = 14.6, 7.4, 4.1 Hz, 1 H), 1.89 (ddd, *J* = 14.6, 5.2, 4.0 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.2, 138.0, 128.6, 127.8, 102.7, 73.4, 69.1, 65.2, 65.1, 47.6, 30.4. IR ν □(cm^{-1}) 1722, 1454, 1436, 1364, 1208, 1174, 1098, 739, 699, 531. HRMS calculated for: $[C_{14}H_{18}O_4 + H]^+$ 251.1278, found: 251.1279 $[M + H]^+$.

(4*S*)-4-((1,3-dioxolan-2-yl)methyl)-5-(benzyloxy)pent-1-en-3-ol 38

To a stirred solution of the aldehyde **37** (1.29 g, 5.15 mmol) in THF (100 mL) was added vinylmagnesium bromide (10.3 mL, 10.3 mmol, 2.0 eq). The reaction mixture was stirred 30 min, at room temperature, under argon, after which a TLC plate indicated completion of the reaction. Sat. NH_4Cl (60 mL) was added and the two layers were separated. The aqueous layer was then extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with water (100 mL), brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to get the crude, which was purified by column chromatography (PE/EA (60:40)). The vinyl alcohol **38** (904 mg, 63%, colorless oil) was obtained as a mixture of diastereoisomers in a 1:1 ratio. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.23 (m, 5 H), 5.86 (dddd, *J* = 17.2, 10.5, 5.4, 3.3 Hz, 1 H), 5.30 (dtd, *J* = 17.2, 1.7, 0.8 Hz, 1 H), 5.18 (ddt, *J* = 10.5, 3.2, 1.6 Hz, 1 H), 4.94 (dt, *J* = 10.9, 4.8 Hz, 1 H), 4.52 – 4.48 (m, 2 H), 4.35 – 4.32 (m, 0.5 H), 4.31 – 4.22 (m, 0.5 H), 3.98 – 3.95 (m, 2 H), 3.90 – 3.80 (m, 2 H), 3.70 (dd, *J* = 9.3, 4.3 Hz, 0.5 H), 3.68 – 3.59 (m, 1 H), 3.51 (dd, *J* = 9.3, 5.5 Hz, 0.5 H), 3.27 (dd, *J* = 15.4, 6.0 Hz, 1 H), 2.17 – 2.06 (m, 0.5 H), 2.05 – 2.03 (m, 0.5 H), 1.95 – 1.90 (m, 0.5 H), 1.87 – 1.71 (m, 1.5 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.3, 138.5, 137.9, 128.44, 128.42, 127.8, 127.7, 127.7, 127.7, 115.5, 103.5, 74.8, 73.5, 73.4, 72.2, 71.3, 64.9, 64.8, 64.8, 39.5, 32.1, 30.6. IR ν□ (cm^{-1}): 3670 – 3132, 1411, 1365, 1097, 1027, 989, 738. HRMS calculated for: $[C_{16}H_{20}O_4 + H]^+$ 277.1434, found: 277.1435 $[M + H]^+$.

(*S*)-4-((1,3-dioxolan-2-yl)methyl)-5-(benzyloxy)pent-1-en-3-one 7

DMP (1.57 g, 3.70 mmol, 1.5 eq) was added to a stirred solution of the mixture of vinyl alcohol diastereoisomers **38** (687 mg, 2.47 mmol) in DCM (10 mL). The mixture was stirred for 3 h at room temperature. A mixture of sat. aq. $NaHCO_3$:$Na_2S_2O_3$ (1N) (1:1) (40 mL) was then added and the different layers were separated. The aqueous layer was extracted with diethyl ether (2 × 30 mL). The combined organic layers were washed with water (30 mL), brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to get the crude was purified by column chromatography (PE/EA (80:20)) to yield ketone **7** as a colorless oil (682 mg, quantitative). $[\alpha]^{20}_D$ = + 2.0 (c = 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.09 (m, 5 H), 6.47 (dd, *J* = 17.5, 10.5 Hz, 1 H), 6.30 (dd, *J* = 17.5, 1.3 Hz, 1 H), 5.81 (dd, *J* = 10.5, 1.3 Hz, 1 H), 4.88 (t, *J* = 4.2 Hz, 1 H), 4.55 – 4.35 (2xd, *J* = 12.1 Hz, 2 H), 3.99 – 3.73 (m, 4 H), 3.65 (dd, *J* = 9.0, 7.8 Hz, 1 H), 3.56 (dd, *J* = 9.0, 5.6 Hz, 1 H), 3.41 (tdd, *J* = 8.1, 5.6, 4.6 Hz, 1 H), 2.16 (ddd, *J* = 14.2, 8.7, 4.2 Hz, 1 H), 1.82 (dt, *J* = 14.2, 4.5 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.9, 172.4, 138.2, 136.6, 128.5, 128.5, 128.3, 127.7, 127.6, 102.7, 73.3, 71.6, 65.0, 44.1, 32.8. IR ν □(cm^{-1}) 1708, 1097, 1024, 738, 698. HRMS calculated for: $[C_{16}H_{20}O_4 + H]^+$ 277.1434, found: 277.1435 $[M + H]^+$.



Reagents and conditions: i) **8** (2 eq), Hoveyda-Grubbs second generation catalyst (5 mol %), DCM, AcOH (20 mol %), 40 °C, overnight, 78%. ii) $BH_3.Me_2S$ (2.5 eq), (*R*)-MeCBS (1 eq), THF, 0 °C, 1 h 30 min, 78%. iii) NH_3 (l), Li, THF, 0 °C, 82%. iv) *t*-$Bu_2Si(OTf)_2$ (1.8 eq), 2,6-lutidine (3 eq), DMF, 0 °C, 2 h, 68%. v) HCl (37%)/THF (1:3), rt, 3 h. vi) a/ **11** (1.5 eq), NaH (1.5 eq), 0 °C then rt, 1 h ; b/ THF, 0 °C then rt, overnight, 34% (2 steps). vii) 3HF-NEt_3 (1.9 eq), pyridine, rt, 2 h 30 min, 69%.

Supporting Scheme 4. Synthesis of final compound **1.**

(*S*,*E*)-2-((1,3-dioxolan-2-yl)methyl)-1-(benzyloxy)octadec-4-en-3-one 9

To a stirred solution of the α,β-unsaturated ketone **7** (310 mg, 1.12 mmol) in DCM (3 mL) was added the alkene **8** (472 mg, 2.24 mmol, 2 eq), Hoveyda-Grubbs second-generation catalyst (1,3-Bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene)ruthenium) (35 mg, 0.06 mmol, 0.05 eq) and acetic acid (13 µL, 0.22 mmol, 0.2 eq). The reaction mixture was stirred overnight, at 40 °C under argon. After evaporation of the solvent, the crude was purified by column chromatography (PE/EA (95:5)) to afford the long hydrophobic α,β-unsaturated ketone **9** (401 mg, 78%) as a colorless oil. $[\alpha]^{20}_D$ = + 7.5 (c = 0.08, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.26 (m, 5 H), 6.90 (dt, *J* = 15.7, 7.0 Hz, 1 H), 6.19 (d, *J* = 15.7 Hz, 1 H), 4.87 (t, *J* = 4.5 Hz, 1 H), 4.48 (2×d, *J* = 12.1 Hz, 2 H), 4.47 (d, *J* = 15 Hz, 1 H), 3.95 – 3.88 (m, 2 H), 3.83 – 3.76 (m, 2 H), 3.65 (dd, *J* = 9.2, 7.6 Hz, 1 H), 3.54 (dd, *J* = 9.2, 5.7 Hz, 1 H), 3.41 – 3.25 (m, 1 H), 2.21 (q, *J* = 6.6 Hz, 2 H), 2.18 – 2.09 (m, 1 H), 1.80 (dt, *J* = 14.1, 4.5 Hz, 1 H), 1.46 – 1.41 (m, 2 H), 1.34 – 1.20 (m, 20 H), 0.88 (t, *J* = 6.6 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ

201.5, 148.3, 138.3, 130.2, 128.4, 127.6, 102.9, 73.3, 71.7, 65.1, 44.6, 33.0, 32.7, 32.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 28.3, 22.8, 14.3. IR ν □(cm^{-1}) 1695, 1624, 1104, 1023, 1735, 697. HRMS calculated for: [$C_{29}H_{46}O_4$+ H]$^+$ 459.3469, found: 459.3469 [M + H]$^+$.

(2*S*,3*R*,*E*)-2-((1,3-dioxolan-2-yl)methyl)-1-(benzyloxy)octadec-4-en-3-ol 39

BH_3.DMS (200 µL, 2.11 mmol, 2.5 eq) was added to a stirred solution (*R*)-MeCBS (236 mg, 0.85 mmol, 1 eq) in THF (8.5 mL) at 0 °C, under argon. The reaction mixture was stirred 15 min at this temperature, after which the long hydrophobic *α,β*-unsaturated ketone **9** (390 mg, 0.85 mmol) in THF (8.5 mL) was slowly added. The mixture was then stirred for 1 h 30 min at 0 °C. Sat. aq. NH_4Cl (80 mL) was added and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude, was purified by column chromatography (PE/EA (90:10) to 100 % EA) to give the corresponding alcohol **39** (302 mg, 78%) as a colorless oil. $[\alpha]^{20}_D$ = + 7.4 (c = 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.39 – 7.26 (m, 5 H), 5.75 – 5.60 (m, 1 H), 5.44 (dd, *J* = 15.4, 6.6 Hz, 1 H), 4.95 (t, *J* = 4.9 Hz, 1 H), 4.49 (d, *J* = 15 Hz, 1 H), 4.46 (d, *J* = 15 Hz, 1 H), 4.18 (d, *J* = 5.9 Hz, 1 H), 3.98 – 3.88 (m, 2 H), 3.86 – 3.84 (m, 2 H), 3.70 (dd, *J* = 9.3, 4.3 Hz, 1 H), 3.49 (dd, *J* = 9.3, 5.8 Hz, 1 H), 3.18 (d, *J* = 5.8 Hz, 1 H), 2.04 – 1.99 (m, 3 H), 1.94 – 1.82 (m, 1 H), 1.77 – 1.71 (m, 1 H), 1.58 (s, 1 H), 1.38 – 1.26 (m, 22 H), 0.88 (t, *J* = 6.7 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 138.1, 133.0, 130.8, 128.5, 127.8, 103.8, 100.1, 74.9, 73.5, 71.7, 65.0, 64.9, 40.1, 32.5, 32.3, 32.1, 29.9, 29.8, 29.7, 29.5, 29.4, 22.9, 14.3. IR ν □(cm^{-1}) 3674 – 3105, 1454, 1116, 1095, 970, 733, 697. HRMS calculated for: [$C_{29}H_{48}O_4$ + Na]$^+$ 483.3445, found: 483.3443 [M + Na]$^+$.

(2*S*,3*R*,*E*)-2-((1,3-dioxolan-2-yl)methyl)octadec-4-ene-1,3-diol 10

In a three-neck round bottomed flask at – 70 °C, under a flow of argon, was distilled NH_3 (25 mL) and lithium wire (50 mg) cut up into small pieces was added in one portion. After the entire solution had been deep blue, compound **39** (221 mg, 0.48 mmol) in THF (2.5 mL) was added dropwise and the mixture was allowed to warm up to – 50 °C and stirred for an extra hour at – 50 °C. It was then cooled down to – 78 °C and the reaction was quenched by solid NH_4Cl. After evaporation of the ammonia, water (20 mL) was added and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude diol **10** (146 mg, 82%) was directly used in the next step (decomposition was observed after one night). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.77 – 5.63 (m, 1 H), 5.50 (dd, J = 15.4, 7.0 Hz, 1 H), 4.98 (t, J = 4.4 Hz, 1 H), 4.17 (t, J = 6.1 Hz, 1 H), 4.05 – 3.95 (m, 2 H), 3.91 – 3.80 (m, 3 H), 3.69 (dd, J = 11.3, 4.9 Hz, 1 H), 2.04 (q, J = 6.9 Hz, 2 H), 1.95 – 1.71 (m, 3 H), 1.39 – 1.23 (m, 22 H), 0.88 (t, J = 6.8 Hz, 3 H). HRMS calculated for: $[C_{22}H_{42}O_4 + Na]^+$ 393.2975, found: 393.2972 $[M + Na]^+$.

(4*R*,5*S*)-5-((1,3-dioxolan-2-yl)methyl)-2,2-di-*tert*-butyl-4-((*E*)-pentadec-1-en-1-yl)-1,3,2-dioxasilinane 40

To a stirred solution of diol **10** (42 mg, 0.11 mmol) and 2,6-lutidine (40 μL, 0.34 mmol, 3 eq) in DMF (1 mL) at 0 °C under argon was added di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (65 μL, 0.20 mmol, 1.8 eq). The resulting mixture was stirred at 0 °C under argon for 2 h. Sat. aq. $NaHCO_3$ (15 mL) was then added and the mixture was extracted with Et_2O (3 × 15 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude, was purified by column chromatography (PE/EA (95:5) to afford the silylidene **40** (38 mg, 68%) as a colorless oil. $[\alpha]^{20}_D$ = + 14.0 (c = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (dt, J = 15.3, 6.7 Hz, 1 H), 5.40 (dd, J = 15.3, 7.7 Hz, 1 H), 4.84 (t, J =

4.9 Hz, 1 H), 4.19 (dd, *J* = 11.1, 4.0 Hz, 1 H), 4.10 (dd, *J* = 10.2, 7.6 Hz, 1 H), 3.99 – 3.89 (m, 2 H), 3.87 – 3.72 (m, 3 H), 2.11 – 1.87 (m, 3 H), 1.66 (ddd, *J* = 14.4, 4.8, 3.5 Hz, 1 H), 1.43 – 1.12 (m, 23 H), 1.02 (2xs, *J* = 9.5 Hz, 18 H), 0.88 (t, *J* = 6.8 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.8, 103.3, 80.6, 68.7, 65.1, 64.8, 40.6, 32.9, 32.4, 32.1, 29.9, 29.84, 29.82, 29.8, 29.7, 29.5, 29.4, 29.3, 27.7, 27.4, 22.9, 22.9, 20.0, 14.3. IR ν□ (cm^{-1}) 1470, 1143, 1113, 1025, 966, 835, 778, 650. HRMS calculated for: $[C_{30}H_{58}O_4Si+ H]^+$ 511.4177, found: 511.4172 $[M + H]^+$.

(4*R*,5*S*)-5-((*E*)-3-((6-azidohexyl)sulfonyl)allyl)-2,2-di-*tert*-butyl-4-((*E*)-pentadec-1-en-1-yl)-1,3,2-dioxasilinane 12

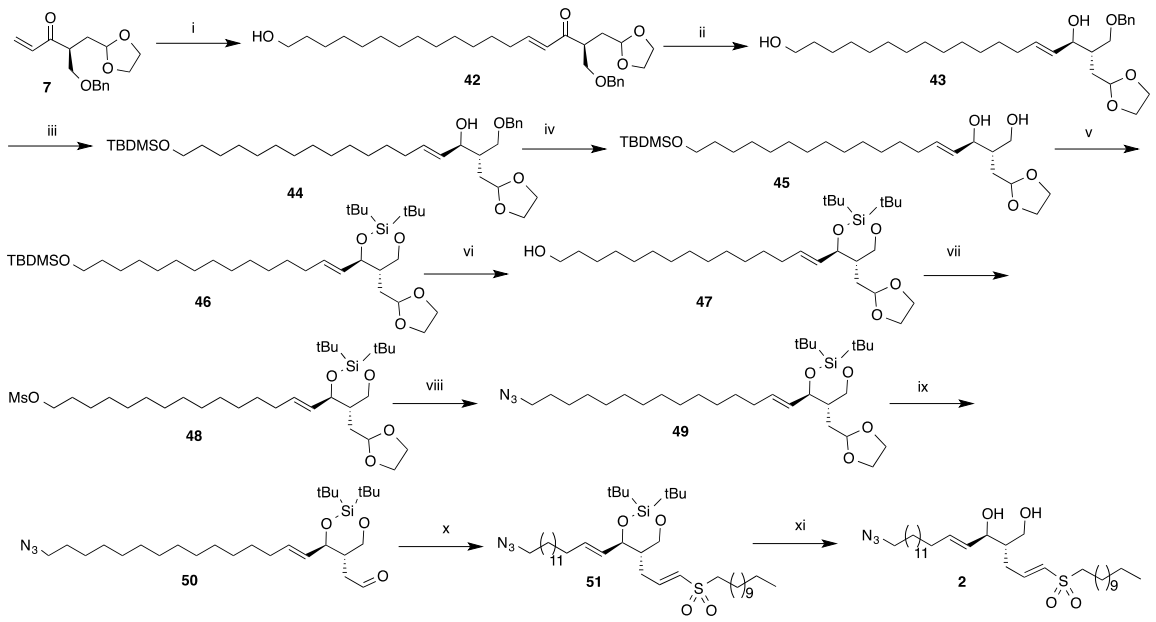
Concentrated aq. HCl (100 μL) was added to a solution of compound **40** (18 mg, 0.035 mmol) in THF (300 μL) and the mixture was stirred for 6 h, after which TLC analysis showed complete consumption of starting material. Water (10 mL) was added carefully and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were extracted with brine (15 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Aldehyde (13 mg, 79%) **41** was obtained as a colorless oil and directly used in the next step.

To a stirred solution of sodium hydride (60%) (1.7 mg, 0.043 mmol, 1.5 eq) in THF at 0 °C under argon, was carefully added azide **11** (15 mg, 0.043 mmol, 1.5 eq). The resulting mixture was sonicated for 1 h and cooled down again to 0 °C. Aldehyde **41** (13 mg, 0.028 mmol) was then added and the resulting mixture was stirred at room temperature, under argon, overnight. Sat. aq. NH_4Cl (10 mL) was added and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude, was purified by column chromatography (PE/EA (95:5) to (90:10)) to give vinyl sulfone **12** (7.5 mg, 34%) as a colorless oil. $[\alpha]^{20}_D$ = + 0.6 (c = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (ddd, *J* = 14.4, 8.2, 6.0 Hz, 1 H), 6.28 – 6.19 (d, *J* = 14.4 Hz, 1 H), 5.68 (dt, *J* = 15.4, 6.7 Hz, 1 H), 5.42 – 5.31 (dd, *J* = 15.4, 7.7, 1 H), 4.18 – 4.07 (dd, *J* = 7.7,

8.0 Hz, 1 H), 3.97 (dd, *J* = 10.9, 3.6 Hz, 1 H), 3.78 (t, *J* = 10.9 Hz, 1 H), 3.28 (t, *J* = 6.8 Hz, 2 H), 3.03 – 2.85 (m, 2 H), 2.35 – 2.30 (m, 1 H), 2.08 – 2.05 (m, 2 H), 1.96 – 1.72 (m, 3 H), 1.45 – 1.39 (m, 4 H), 1.38 – 1.17 (m, 26 H), 1.02 (2 x s, 18 H), 0.92 – 0.80 (t, *J* = 6.8 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.7, 134.6, 130.6, 129.7, 80.0, 68.1, 54.6, 51.4, 43.3, 32.4, 32.1, 31.0, 29.7, 29.83, 29.81, 29.80, 29.6, 29.5, 29.5, 29.3, 28.7, 28.1, 27.6, 27.3, 26.4, 22.9, 22.8, 22.4, 20.1, 14.3. IR ν □(cm^{-1}) 1400, 1124, 861, 829. HRMS calculated for: $[C_{35}H_{67}N_3O_4SSi + H]^+$ 654.4700, found: 654.4690 $[M + H]^+$.

(2*S*,3*R*,*E*)-2-((*E*)-3-((6-azidohexyl)sulfonyl)allyl)octadec-4-ene-1,3-diol 1

To a stirred solution of vinyl sulfone **12** (8.5 mg, 0.013 mmol) in pyridine (1 mL) at room temperature, under argon, was added $3HF.NEt_3$ (4 µL, 0.025 mmol, 1.9 eq). The resulting mixture was stirred for 2 h 30 min before being quenched with 1 mL H_2O and 1 mL sat. aq. $NaHCO_3$. Pyridine was evaporated and 10 mL H_2O was added. The mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography initially neutralized with 1% NEt_3 (PE/EA (60:40) to (40:60)) afforded compound **1** (4.6 mg, 69%) as a colorless oil. $[\alpha]^{20}_D$ = + 0.6 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.92 (ddd, *J* = 15.0, 8.0, 6.9 Hz, 1 H), 6.34 (d, *J* = 15.0, 1 H), 5.77 – 5.65 (dt, *J* = 15.4, 6.7 Hz, 1 H), 5.57 – 5.41 (dd, *J* = 15.4, 7.7 Hz, 1 H), 4.14 (t, *J* = 6.7 Hz, 1 H), 3.90 (dd, *J* = 11.2, 3.2 Hz, 1 H), 3.62 (dd, *J* = 11.2, 5.3 Hz, 1 H), 3.35 (sl, OH), 3.28 (t, *J* = 6.8 Hz, 2 H), 3.00 – 2.90 (m, 2 H), 2.51 – 2.38 (m, 2 H), 2.12 – 1.99 (m, 2 H), 1.85 – 1.73 (m, 3 H), 1.47 – 1.42 (m, 4 H), 1.52 – 1.19 (m, 26 H), 0.88 (t, *J* = 6.8 Hz, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 147.9, 134.7, 130.8, 129.5, 75.8, 63.0, 58.9, 54.7, 51.4, 44.4, 32.4, 32.1, 30.8, 29.9, 29.84, 29.82, 29.78, 29.6, 29.5, 29.4, 29.3, 28.7, 28.1, 26.4, 22.9, 22.5, 14.3, 8.5. IR ν □(cm^{-1}) 2095, 1729, 1669, 1463, 1283, 1125. HRMS calculated for: $[C_{27}H_{51}N_3O_4S + Na]^+$ 536.3498, found: 536.3487 $[M + Na]^+$.



Reagents and conditions: i) **21** (2 eq), Hoveyda-Grubbs second generation catalyst (5 mol %), DCM, AcOH (20 mol %), 40 °C, overnight 66%. ii) $BH_3.Me_2S$ (2.5 eq), (*R*)-MeCBS (1 eq), THF, 0 °C, 1 h 30 min, 74%. iii) TBDMSCl (1.1 eq), NEt_3 (1.3 eq), DMAP (1.1 eq), CH_2Cl_2, rt, overnight, 76%. iv) NH_3 (l), Li, THF, 0 °C, 94%. v) *t*-$Bu_2Si(OTf)_2$ (1.2 eq), 2,6-lutidine (2.5 eq), DMF, 0 °C, 2 h, 80%. vi) CSA (0.5 eq), MeOH, 0 °C, 2.5 h, 79% (crude). vii) MsCl (1.3 eq), NEt_3 (1.8 eq), DCM, 0 °C then rt, 2 h, quantitative (crude). viii) NaN_3 (1.5 eq), DMF, 60 °C, overnight, 72%. ix) HCl (37%)/THF (1:3), rt, 3 h. x) a/ **28** (1.6 eq), NaH (1.6 eq), 0 °C then rt, 1 h ; b/ THF, 0 °C then rt, overnight, 38% (2 steps). xi) 3HF-NEt_3 (3 eq), pyridine, rt, 2 h 30 min, 42%.

Supporting Scheme 5. Synthesis of final compound **2.**

(*S*,*E*)-2-((1,3-dioxolan-2-yl)methyl)-1-(benzyloxy)-18-hydroxyoctadec-4-en-3-one 42

To a stirred solution of the *α,β*-unsaturated ketone **7** (210 mg, 0.76 mmol) in DCM (2 mL) was added the alkene **21** (344 mg, 1.52 mmol), Hoveyda-Grubbs second-generation catalyst (1,3-Bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxyphenylmethylene)-ruthenium) (24 mg, 0.04 mmol, 0.05 eq) and AcOH (10 µL, 0.17 mmol, 0.2 eq). The reaction mixture was stirred overnight, at 40 °C under argon. After evaporation of the solvent, the crude was purified by column chromatography (PE/EA (95:5)) to afford the long hydrophobic *α,β*-unsaturated ketone **42** (238 mg, 66%) as a colorless oil. $[\alpha]^{20}_D$ = + 6.7 (c = 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.19 (m, 5 H), 6.92 (dt, *J* = 15.7, 6.9 Hz, 1 H), 6.19 (d, *J* = 15.7 Hz, 1 H), 5.43 – 5.40 (m, OH), 4.87 (t, *J* = 4.4 Hz, 1 H), 4.55 – 4.36 (2 x d, *J* = 12.1 Hz, 2 H), 3.97 – 3.76 (m, 4 H), 3.62 - 3.64 (m, 4 H), 3.54 (dd, *J* = 9.1, 5.7 Hz, 1 H), 3.34 (tt, *J* = 7.9, 5.3 Hz, 1 H), 2.26 – 2.07 (m, 3 H), 1.99-1.94 (m, 1 H), 1.80 (dt, *J* = 14.2, 4.8 Hz, 1 H), 1.58 – 1.55 (m, 2 H),

1.50 – 1.09 (m, 20 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.6, 148.3, 138.3, 130.5, 130.2, 128.4, 127.7, 102.8, 73.3, 71.6, 65.1, 65.0, 63.2, 44.6, 32.9, 32.7, 29.8, 29.8 29.70, 29.66, 29.58, 29.57, 29.5, 29.4, 28.3, 25.9. IR ν□ (cm^{-1}) 1454, 1363, 1028, 970, 735, 698. HRMS calculated for: $[C_{29}H_{46}O_5 + Na]^+$ 497.3234, found: 497.3234 $[M + Na]^+$.

(16*R*,17*S*,*E*)-17-((1,3-dioxolan-2-yl)methyl)-18-(benzyloxy)octadec-14-ene-1,16-diol 43

BH_3.DMS (360 µL, 3.80 mmol, 2.5 eq) was added to a stirred solution (*R*)-MeCBS (420 mg, 1.52 mmol, 1 eq) in THF (15 mL) at 0 °C, under argon. The reaction mixture was stirred 15 min at this temperature, after which the long hydrophobic α,β-unsaturated ketone **42** (720 mg, 1.52 mmol) in THF (15 mL) was slowly added. The mixture was then stirred for 1 h 30 min at 0 °C. Sat. aq. NH_4Cl (100 mL) was added and the mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude, was purified by column chromatography (PE/EA (90:10) to 100 % EtOAc) to give the corresponding alcohol **43** (541 mg, 74%) as a colorless oil. $[\alpha]^{20}_D$ = + 7.5 (c = 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 7.14 (m, 5 H), 5.66 (dt, *J* = 14.7, 6.7 Hz, 1 H), 5.49 – 5.36 (dd, *J* = 14.7, 5.9 Hz, 1 H), 4.95 (t, *J* = 4.9 Hz, 1 H), 4.57 – 4.42 (2 x d, *J* = 12.1 Hz, 2 H), 4.18 (t, *J* = 5.9 Hz, 1 H), 3.88 – 3.80 (m, 4 H), 3.70 (dd, *J* = 9.3, 4.4 Hz, 1 H), 3.64 (t, *J* = 6.6 Hz, 2 H), 3.49 (dd, *J* = 9.3, 5.9 Hz, 1 H), 2.06 – 1.98 (m, 3 H), 1.89 (dt, *J* = 14.4, 5.5 Hz, 1 H), 1.74 (ddd, *J* = 14.4, 7.4, 4.5 Hz, 1 H), 1.61 – 1.52 (m, 2 H), 1.41 – 1.21 (m, 20 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 133.0, 130.8, 128.5, 127.8, 103.8, 74.9, 73.5, 71.7, 65.0, 64.9, 63.2, 40.1, 33.0, 32.4, 32.3, 29.78, 29.75, 29.71, 29.6, 29.6, 29.4, 25.9. IR ν□ (cm^{-1}) 3671 – 3114, 1454, 1362, 1027, 970, 732, 698. HRMS calculated for: $[C_{29}H_{48}O_5 + Na]^+$ 439.3394, found: 439.3338 $[M + H]^+$.

(2*S*,3*R*,*E*)-2-((1,3-dioxolan-2-yl)methyl)-1-(benzyloxy)-18-((*tert*-butyldimethylsilyl)oxy)-octadec-4-en-3-ol 44

To a stirred solution of compound **43** (398 mg, 0.83 mmol) at room temperature, under argon, in DCM (4.2 mL) was added DMAP (4 mg, 0.033 mmol, 0.04 eq), NEt_3 (150 µL, 1.08 mmol, 1.3 eq) and TBDMSCl (160 µL, 0.92 mmol, 1.1 eq). The resulting mixture was stirred overnight at room temperature. Water (20 mL) was then added and the resulting mixture was extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the crude mixture by column chromatography (PE/EA (90:10) to (80:20)) afforded the title compound **44** (374 mg, 76%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.16 (m, 5 H), 5.61 (dt, *J* = 14.7, 6.7 Hz, 1 H), 5.39 (dd, *J* = 15.3, 6.5 Hz, 1 H), 4.90 (t, *J* = 4.9 Hz, 1 H), 4.44 (2 x d, *J* = 12.1 Hz, 2 H), 4.13 (d, *J* = 5.5 Hz, 1 H), 4.00 – 3.84 (m, 2 H), 3.85 – 3.75 (m, 2 H), 3.65 (dd, *J* = 9.3, 4.4 Hz, 1 H), 3.55 (t, *J* = 6.6 Hz, 2 H), 3.44 (dd, *J* = 9.3, 5.8 Hz, 1 H), 3.16 (d, *J* = 5.5 Hz, OH), 1.97 (m, 3 H), 1.84 (dt, *J* = 14.4, 5.5 Hz, 1 H), 1.69 (ddd, *J* = 14.4, 7.4, 4.5 Hz, 1 H), 1.46 (q, *J* = 6.9 Hz, 2 H), 1.21 (d, *J* = 7.4 Hz, 20 H), 0.85 (s, 9 H), -0.002 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 133.0, 131.0, 128.5, 127.8, 103.8, 74.8, 73.5, 73.5, 71.6, 65.0, 64.9, 64.8, 63.5, 40.1, 33.0, 32.4, 32.3, 29.82, 29.81, 29.79, 29.76, 29.7, 29.7, 29.6, 29.4, 26.1, 25.9, 18.5, -5.1. IR ν□ (cm^{-1}) 1465, 1253, 1097, 834, 774, 734, 698. HRMS calculated for: $[C_{35}H_{62}O_5Si + Na]^+$ 613.4259, found: 613.4252 $[M + Na]^+$.

(2*S*,3*R*,*E*)-2-((1,3-dioxolan-2-yl)methyl)-18-((*tert*-butyldimethylsilyl)oxy)octadec-4-ene-1,3-diol 45

In a three-neck round bottomed flask at – 70 °C, under a flow of argon, was distilled NH_3 (25 mL) and lithium wire (50 mg) cut up into small pieces was added in one portion. After the entire solution had been deep blue, compound **44** (374 mg, 0.63 mmol) in THF (5 mL) was added

dropwise and the mixture was allowed to warm up to – 50 °C and stirred for an extra hour at – 50 °C. It was then cooled down to – 78 °C and the reaction was quenched by solid NH_4Cl. After evaporation of the ammonia, water (30 mL) was added and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude **45** (298 mg, 94%) was directly used in the next step. (decomposition was observed after one night). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.65 (dt, J = 14.7, 6.7 Hz, 1 H), 5.44 (dd, J = 15.4, 7.0 Hz, 1 H), 4.92 (t, J = 4.4 Hz, 1 H), 4.11 (t, J = 6.1 Hz, 1 H), 4.04 – 3.89 (m, 2 H), 3.87 – 3.75 (m, 3 H), 3.63 (dd, J = 11.2, 4.8 Hz, 1 H), 3.55 (t, J = 6.7 Hz, 2 H), 1.99 (q, J = 7.4 Hz, 2 H), 1.85 – 1.78 (m, 2 H), 1.73 – 1.68 (m, 1 H), 1.48 – 1.44 (m, 2 H), 1.34 - 1.21 (m, 20 H), 0.85 (s, 9 H), 0.047 (s, 6 H). IR ν□ (cm^{-1}) 1741, 1463, 1252, 1098, 970, 834, 774, 734. HRMS calculated for: $[C_{28}H_{56}O_5Si + Na]^+$ 523.3789, found: 523.3786 [M + Na]$^+$.

(4*R*,5*S*)-5-((1,3-dioxolan-2-yl)methyl)-2,2-di-*tert*-butyl-4-((*E*)-15-((*tert*-butyldimethylsilyl)-oxy) pentadec-1-en-1-yl)-1,3,2-dioxasilinane 46

To a stirred solution of diol **45** (298 mg, 0.60 mmol) and 2,6-lutidine (170 µL, 1.47 mmol, 2.5 eq) in DMF (6 mL) at 0 °C under argon was added di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (230 µL, 0.71 mmol, 1.2 eq). The resulting mixture was stirred at 0 °C under argon for 2 h. Sat. aq. $NaHCO_3$ (30 mL) was then added and the mixture was extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude, was purified by column chromatography (PE/EA (95:5)) to afford the silylidene **46** (306 mg, 80%) as a colorless oil. $[\alpha]^{20}_D$ = + 12.2 (c =1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.59 (dt, J = 15.2, 6.7 Hz, 1 H), 5.35 (dd, J = 15.3, 7.8 Hz, 1 H), 4.79 (t, J = 4.9 Hz, 1 H), 4.14 (dd, J = 11.1, 4.0 Hz, 1 H), 4.05 (dd, J = 10.2, 7.7 Hz, 1 H), 3.95 – 3.82 (m, 2 H), 3.83 – 3.70 (m, 3 H), 3.55 (t, J = 6.6 Hz, 2 H), 2.02 – 1.99 (m, 3 H), 1.61 (ddd, J = 14.4,

4.8, 3.3 Hz, 1 H), 1.45 (q, *J* = 6.8 Hz, 2 H), 1.30 - 1.21 (m, 21 H), 0.98 (s, 9 H), 0.96 (s, 9 H), 0.85 (s, 9 H), 0.047 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.8, 131.2, 103.3, 80.6, 68.7, 65.1, 64.8, 63.5, 40.7, 33.1, 32.9, 32.4, 29.83, 29.79, 29.72, 29.67, 29.6, 29.4, 29.3, 27.7, 27.4, 26.2, 26.0, 22.7, 20.0, 18.5, -5.1. IR ν□ (cm^{-1}) 1472, 1099, 826, 774, 650. HRMS calculated for: $[C_{36}H_{72}O_5Si_2 + H]^+$ 641.4991, found: 641.4987 $[M + H]^+$.

(*E*)-15-((4*R*,5*S*)-5-((1,3-dioxolan-2-yl)methyl)-2,2-di-*tert*-butyl-1,3,2-dioxasilinan-4-yl)pentadec-14-en-1-ol 47

To a stirred solution of silylidene **46** (306 mg, 0.48 mmol) in MeOH (24 mL) at 0 °C under argon was added CSA (12 mg, 0.05 mmol, 0.1 eq). The resulting mixture was stirred at 0 °C under argon for 2 h 30 min. After evaporation MeOH, sat. aq. $NaHCO_3$ (50 mL) was then added and the mixture was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude deprotected primary alcohol **47** (199 mg, 79%) was directly used in the next step without further purification. $[\alpha]^{20}_D$ = + 11.6 (c = 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (dt, *J* = 15.3, 6.6 Hz, 1 H), 5.41 (dd, *J* = 15.3, 7.8 Hz, 1 H), 4.84 (t, *J* = 4.9 Hz, 1 H), 4.19 (dd, *J* = 11.1, 4.0 Hz, 1 H), 4.10 (dd, *J* = 10.2, 7.6 Hz, 1 H), 4.00 – 3.88 (m, 2 H), 3.86 – 3.77 (m, 3 H), 3.64 (t, *J* = 6.6 Hz, 2 H), 2.11 – 1.81 (m, 3 H), 1.66 (ddd, *J* = 14.5, 4.8, 3.3 Hz, 1 H), 1.61 – 1.52 (m, 2 H), 1.40 – 1.23 (m, 20 H), 1.19 (td, *J* = 9.0, 4.4 Hz, 1 H), 1.03 (s, 9 H), 1.01 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.8, 131.2, 103.3, 80.6, 68.7, 65.1, 64.8, 63.2, 40.7, 33.0, 32.9, 32.4, 29.79, 29.76, 29.7, 29.64, 29.58, 29.4, 29.3, 27.6, 27.4, 25.9, 22.9, 20.0. IR ν□ (cm^{-1}) 1471, 1112, 1025, 825, 650. HRMS calculated for: $[C_{30}H_{58}O_5Si_2 + H]^+$ 527.4126, found: 527.4125 $[M + H]^+$.

(4*R*,5*S*)-5-((1,3-dioxolan-2-yl)methyl)-4-((*E*)-15-azidopentadec-1-en-1-yl)-2,2-di-*tert*-butyl-1,3,2-dioxasilinane 49

To a stirred solution of compound **47** (199 mg, 0.38 mmol) and NEt_3 (95 μL, 0.68 mmol, 1.8 eq) in DCM (2 mL) at 0 °C under argon was added MsCl (40 μL, 0.52 mmol, 1.4 eq). The resulting mixture was stirred at room temperature for 2 h. Water (10 mL) was then added and the mixture was extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude mesylated alcohol **48** (230 mg, quantitative) was directly used in the next step without further purification.

To a stirred solution of mesylate **48** (230 mg, 0.38 mmol) in DMF (2 mL) under argon was added NaN_3 (38 mg, 0.58 mmol, 1.5 eq). The resulting mixture was stirred overnight at 60 °C. Water (30 mL) was then added and the mixture was extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude mixture was purified by column chromatography (PE/EA (95:5)) to afford the azide **49** (152 mg, 72%) as a colorless oil. $[\alpha]^{20}_D$ = + 11.4 (c = 1.1, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (dt, *J* = 15.2, 6.7 Hz, 1 H), 5.40 (dd, *J* = 15.3, 7.8 Hz, 1 H), 4.84 (t, *J* = 4.9 Hz, 1 H), 4.19 (dd, *J* = 11.1, 4.0 Hz, 1 H), 4.10 (dd, *J* = 10.2, 7.6 Hz, 1 H), 3.98 – 3.88 (m, 2 H), 3.87 – 3.73 (m, 3 H), 3.26 (t, *J* = 7.0 Hz, 2 H), 2.05 (q, *J* = 7.1, Hz, 2 H), 1.93 – 1.90 (m, 1 H), 1.72 – 1.63 (m, 3 H), 1.44 – 1.14 (m, 21 H), 1.03 (s, 9 H), 1.01 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.7, 131.0, 103.1, 80.5, 68.5, 65.0, 64.7, 51.5, 40.5, 32.7, 32.2, 29.67, 29.65, 29.6, 29.5, 29.3, 29.2, 29.1, 28.9, 27.5, 27.3, 26.7, 22.7, 19.9. IR ν □(cm^{-1}) 2095, 1469, 1143, 1112, 1024, 967, 825, 778, 650. HRMS calculated for: $[C_{30}H_{57}N_3O_4Si + H]^+$ 552.4191, found: 552.4190 $[M + H]^+$.

(4*R*,5*S*)-4-((*E*)-15-azidopentadec-1-en-1-yl)-2,2-di-*tert*-butyl-5-((*E*)-3-(nonylsulfonyl)allyl)-1,3,2-dioxasilinane 51

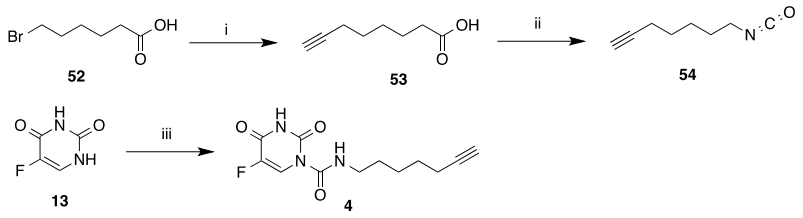
Concentrated aq. HCl (270 μL) was added to a solution of compound **49** (90 mg, 0.16 mmol) in THF (815 μL) and the mixture was stirred for 6 h, after which TLC analysis showed complete consumption of starting material. Water (10 mL) was added carefully and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were extracted with brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Aldehyde **50** (83 mg, quantitative) was obtained as a colorless oil and directly use in the next step.

To a stirred solution of sodium hydride (60%) (10 mg, 0.25 mmol, 1.6 eq) in THF at 0 °C under argon, was carefully added sulfone **28** (102 mg, 0.26 mmol, 1.6 eq). The resulting mixture was sonicated for 1 h and cooled down again to 0 °C. Aldehyde **50** (83 mg, 0.16 mmol) was then added and the resulting mixture was stirred at room temperature, under argon, overnight. Sat. aq. NH_4Cl (10 mL) was added and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude, was purified by column chromatography (PE/EA (95:5)) to (90:10)) to give vinyl sulfone **51** (45 mg, 38%, colorless oil) as a non-separable diastereoisomeric mixture (7:3 according to ^{1}H NMR). ^{1}H NMR (400 MHz, $CDCl_3$) (mixture of diastereoisomers) δ 6.80 (ddd, J = 14.5, 8.1, 6.0 Hz, 1 H), 6.24 (d, J = 15.4 Hz, 1 H), 5.76 – 5.60 (m, 1.8 H), 5.44 – 5.32 (m, 1.8 H), 4.39 – 4.24 (m, 0.4 H), 4.13 (dd, J = 9.4, 7.8 Hz, 1 H), 4.01 – 3.94 (m, 1.8 H), 3.78 (t, J = 10.8 Hz, 1 H), 3.64 – 3.50 (m, 1 H), 3.26 (t, J = 7.0 Hz, 2.8 H), 2.99 – 2.85 (m, 2.8 H), 2.91 – 2.83 (m, 1 H), 2.09 – 1.79 (m, 8.4 H), 1.45 – 1.18 (m, 56 H), 1.05 – 0.98 (m, 25.2 H), 0.88 (t, J = 6.8 Hz, 4.2 H). ^{13}C NMR (100 MHz, $CDCl_3$) (mixture of diastereoisomers) δ 146.5, 136.9, 132.8, 130.8, 130.6, 129.6, 120.5, 80.0, 78.2, 68.1, 56.8, 54.9, 51.6, 51.3, 49.4, 43.2, 32.4, 32.1, 31.0, 29.79, 29.76, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.3, 29.2, 29.0, 28.7, 28.5, 27.6, 27.3, 26.9, 22.9, 22.8, 22.6, 21.9, 20.0, 14.3.

(2*S*,3*R*,*E*)-18-azido-2-((*E*)-3-(nonylsulfonyl)allyl)octadec-4-ene-1,3-diol 2

To a stirred solution of the diastereoisomeric mixture of vinyl sulfone **51** (15 mg, 0.02 mmol) in pyridine (2 mL) at room temperature, under argon, was added 3HF.NEt_3 (10 μL, 0.06 mmol, 3.0 eq). The resulting mixture was stirred for 2 h 30 min before being quenched with 1 mL H_2O and 1 mL sat. aq. $NaHCO_3$. Pyridine was evaporated and H_2O (10 mL) was added. The mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography initially neutralized with 1% NEt_3 (PE/EA (60:40) to (40:60)) could provide the desired compound **2** (5 mg, 42%) as a colorless oil. $[\alpha]^{20}_D$ = + 4.0 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.90 (dt, *J* = 15.0, 7.5 Hz, 1 H), 6.33 (d, *J* = 15.1 Hz, 1 H), 5.73 (dt, *J* = 15.7, 6.8 Hz, 1 H), 5.55 – 5.44 (dd, *J* = 15.7, 6.7 Hz, 1 H), 4.15 (t, *J* = 6.7 Hz, 1 H), 3.89 (dd, *J* = 11.1, 3.2 Hz, 1 H), 3.64 (dd, *J* = 11.1, 5.5 Hz, 1 H), 3.26 (t, *J* = 7.0 Hz, 2 H), 2.97 – 2.82 (m, 2 H), 2.47 (dddd, *J* = 12.6, 7.1, 5.5, 2.7 Hz, 1 H), 2.42 – 2.32 (m, 1 H), 2.08 – 2.04 (m, 3 H), 1.79 – 1.75 (m, 2 H), 1.62 – 1.58 (m, 2 H), 1.45 – 1.26 (m, 38 H), 0.88 (t, *J* = 7.0 Hz, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 147.5, 134.7, 130.8, 129.7, 75.9, 63.2, 54.9, 51.7, 44.4, 32.4, 32.1, 30.8, 29.79, 29.76, 29.75, 29.69, 29.67, 29.64, 29.62, 29.49, 29.45, 29.4, 29.3, 29.28, 29.25, 29.0, 28.6, 26.9, 22.8, 22.6, 14.3. IR ν □(cm^{-1}) 3650 – 3129, 2094, 1466, 1282, 1127, 971. HRMS calculated for: $[C_{33}H_{63}N_3O_4S + Na]^+$ 620.4430, found: 620.4431$[M + H]^+$.

Synthesis of probes 3, 4, 5 and 6



Reagents and conditions: i) Lithium acetylide, ethylenediamine complex (3 eq), DMSO, 0 °C then rt, 1 h 30 min, 77% ii) DPPA (1eq), NEt_3 (1 eq), ACN, 50 °C, 2 h, quantitative. iii) a/ DMAP (1.1 eq), 30 min, rt ; b/ **54** (1.7 eq), pyridine,rt, overnight, 58%.

Supporting Scheme 6. Synthesis of final compound **4.**

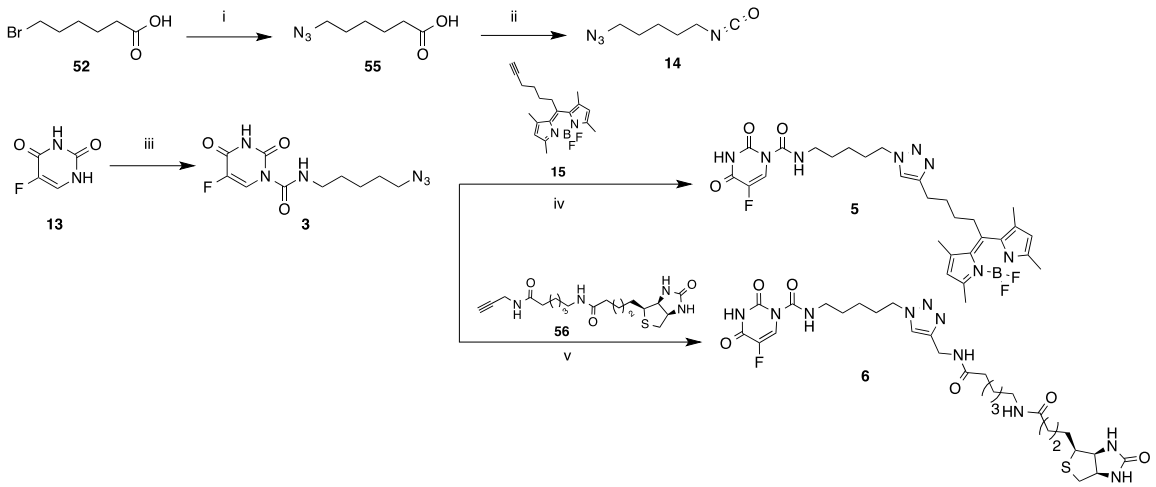
Oct-7-ynoic acid 53

To a solution of the lithium acetylide ethelenediamine complex (3.9 g, 38.1 mmol, 3 eq) in DMSO (8 mL) was added 6-bromohexanoic acid **52** (2.5 g, 12.8 mmol, 1 eq) in DMSO (2 mL) at 0 °C. After stirring at room temperature for 90 min, the mixture was poured into brine with ice and acidified with aqueous HCl (2N). The aqueous layer was extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture was purified with column chromatography (100% EA) to give oct-7-ynoic acid **53** (1.39 g, 77%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (t, J = 7.5 Hz, 2 H), 2.20 (td, J = 6.9, 2.6 Hz, 2 H), 1.94 (t, J = 2.7 Hz, 1 H), 1.66 (p, J = 7.5 Hz, 2 H), 1.60 – 1.37 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.1, 84.4, 68.5, 34.0, 28.23, 28.18, 24.3, 18.4. The spectral data are comparable to the literature.[7]

5-fluoro-*N*-(hept-6-yn-1-yl)-2,4-dioxo-3,4-dihydropyrimidine-1(2*H*)-carboxamide 4

To oct-7-ynoic acid **53** (360 mg, 2.57 mmol) and NEt_3 (360 μL, 2.59 mmol, 1 eq) in ACN (3 mL), DPPA (560 μL, 2.60 mmol, 1 eq) was added. The resulting mixture was stirred for 2 h at 50

°C after what TLC-MS showed consumption of the starting material. The solvent was evaporated and the crude 7-isocyanatohept-1-yne **54** (350 mg, quantitative) was directly used in the next step. Fluorouracil **13** (200 mg, 1.54 mmol) was dissolved in pyridine (4 mL). DMAP (207 mg, 1.69 mmol, 1.1 eq) was added and the resulting mixture was stirred at room temperature under argon for 30 min. Then the isocyanate **54** (350 mg, 2.55 mmol, 1.7 eq) in pyridine (1 mL) was added and the mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the compound was purified with column chromatography (80:20 then 70:30 pentane/EA) to afford the fluorouracil derivative equipped with an alkyne tag **4** as a beige solid (239 mg, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1 H), 8.81 (s, 1 H), 8.48 (d, J = 6.7 Hz, 1 H), 3.41 (q, J = 6.7 Hz, 2 H), 2.21 (td, J = 6.7, 2.6 Hz, 2 H), 2.02 – 1.88 (m, 1 H), 1.80 – 1.34 (m, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.9, 149.1, 140.9 (d, J = 240 Hz), 123.4 (d, J = 37 Hz), 84.2, 68.7, 41.4, 28.8, 28.1, 26.0, 18.4. IR ν□ (cm^{-1}): 3269, 1721, 1686, 1533, 1335, 1200, 1092, 904, 740, 665, 607, 512. HRMS calculated for: $[C_{12}H_{14}FN_3O_3 + H]^+$ 268.1097, found: 268.1095.



Reagents and conditions: i) NaN_3 (3 eq), DMF, 50 °C, 3 h, 66%. ii) DPPA (1eq), NEt_3 (1 eq), ACN, 50 °C, 2 h, quantitative. iii) a/ DMAP (1.1 eq), 30 min, rt ; b/ **14** (1.6 eq), pyridine,rt, overnight, 84%. iv) **15** (1 eq), $CuSO_4$ (0.2 eq), sodium ascorbate (1.6 eq), sonication, 6 h, 25%. v) **56** (1 eq), $CuSO_4$ (0.2 eq), sodium ascorbate (1.5 eq), sonication, 6 h, 5%.

Supporting Scheme 7: Synthesis of final compounds **3**, **5** and **6**

6-Azidohexanoic acid 55

To a solution of 6-bromohexanoic acid **52** (2.5 g, 12.8 mmol) in DMF (15 mL) was added sodium azide (2.5 g, 38.5 mmol, 3 eq) and the resulting mixture was heated for 3 h at 50 °C. H_2O (45 mL) was then added and the mixture was extracted with Et_2O (3 × 45 mL). The combined organic layers were washed with brine (45 mL) and dried over $MgSO_4$ before being evaporated. The residue was purified with column chromatography (100% EA) to afford 6-azidohexanoic acid **55** (1.33 g, 66%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.27 (t, J = 6.9 Hz, 2 H), 2.37 t, J = 6.9 Hz, 2 H), 1.63 (m, 4 H), 1.51 – 1.34 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.2, 51.3, 34.0, 28.6, 26.2, 24.2. The spectral data are comparable to the literature.[7]

***N*-(5-azidopentyl)-5-fluoro-2,4-dioxo-3,4-dihydropyrimidine-1(2*H*)-carboxamide 3**

To 6-azidohexanoic acid **52** (395 mg, 2.51 mmol) and NEt_3 (350 µL, 2.52 mmol, 1 eq) in ACN (3 mL), DPPA (540 µL, 2.51 mmol, 1 eq) was added. The resulting mixture was stirred for 2 h at 50 °C after what TLC-MS showed consumption of the starting material. The solvent was evaporated and the crude 1-Azido-5-isocyanatopentane **14** (388 mg, quantitative) was directly used in the next step.

Fluorouracil **13** (200 mg, 1.54 mmol) was dissolved in pyridine (4 mL). DMAP (207 mg, 1.69 mmol, 1.1 eq) was added and the resulting mixture was stirred at room temperature under argon for 30 min. Then the isocyanate **14** (388 mg, 2.52 mmol, 1.6 eq) in pyridine (1 mL) was added and the mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the compound was purified with column chromatography (80:20 then 70:30 pentane/EA) to afford the fluorouracil derivative equipped with an azido tag **3** as a white solid (369 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1 H), 8.86 (s, 1H), 8.47 (d, J = 6.7 Hz, 1 H), 3.41 (q, J = 6.7 Hz, 2 H), 3.29 (t, J = 6.7 Hz, 2 H), 1.70 – 1.60 (m, 4 H), 1.49 – 1.41 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 150.0, 149.2, 140.9 (d, J = 240 Hz), 123.3 (d, J = 37 Hz),

51.3, 41.3, 28.9, 28.6, 24.1. IR ν □(cm^{-1}): 3303, 2087, 1739, 1666, 1518, 1463, 1331, 1261, 849, 759, 677, 613, 537. HRMS calculated for: $[C_{10}H_{13}FN_6O_3 + H]^+$ 285.1111, found: 285.1110.

5,5-difluoro-10-(4-(1-(5-(5-fluoro-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-1-carboxamido)pentyl)-1*H* 1,2,3-triazol-4-yl)butyl)-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide 5

To a solution of fluorouracil derivative **3** (20 mg, 0.07 mmol, 1 eq) and Bodipy[8] **15** (23 mg, 0.07 mmol, 1 eq) in DMF (1 mL) was added sodium ascorbate (105 μL (1M stock solution), 0.11 mmol, 1.6 eq) followed by copper sulfate (70 μL (200mM stock solution), 0.014 mmol, 0.2 eq). The resulting mixture was sonicated for 6 h, after what TLC showed completion of the reaction. The solvent was evaporated under reduced pressure and the compound **5** was purified by HPLC (52% to 58% ACN) and obtained as a red powder (10.7 mg, 25%). ^{1}H NMR (600 MHz, MeOD) δ 8.39 (d, J = 7.3 Hz, 1 H), 7.78 (s, 1 H), 6.11 (s, 2 H), 4.39 (t, J = 6.8 Hz, 2 H), 3.28 (t, J = 6.8 Hz, 2 H), 3.05 – 2.95 (m, 2 H), 2.80 (t, J = 7.1 Hz, 3 H), 2.43 (s, 6 H), 2.38 (s, 6 H), 1.99 – 1.86 (m, 4 H), 1.72 – 1.53 (m, 4 H), 1.36 – 1.23 (m, 2 H). ^{13}C NMR (150 MHz, MeOD) δ 159.0, 158.8, 155.0, 154.9, 151.81, 151.76, 151.4, 151.3, 148.5, 147.9, 142.3 (d, J = 234 Hz), 142.2, 132.6, 123.9 (d, J = 39 Hz), 123.5, 122.6, 51.0, 41.7, 41.6, 32.1, 31.1, 30.74, 30.71, 29.5, 29.4, 29.1, 25.9, 24.6, 16.5, 14.4. IR ν□ (cm^{-1}): 3269, 1721, 1686, 1533, 1335, 1200, 1092, 904, 740, 665, 607, 512. HRMS calculated for: $[C_{29}H_{36}BF_3N_8O_3 + H]^+$ 613.3033, found: 613.3026 $[M + H]^+$.

5-fluoro-2,4-dioxo-*N*-(5-(4-((6-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexanamido)methyl)-1*H*-1,2,3-triazol-1-yl)pentyl)-3,4-dihydropyrimidine-1(2*H*)-carboxamid 6

To a solution of fluorouracil derivative **3** (40 mg, 0.14 mmol, 1 eq) and biotin **56**[7] (56 mg, 0.14 mmol, 1 eq) in DMF (1 mL) was added sodium ascorbate (210 μL (1M stock solution), 0.21

mmol, 1.5 eq) followed by copper sulfate (140 μL (200 mM stock solution), 0.028 mmol, 0.2 eq). The resulting mixture was sonicated for 6 h, after what TLC showed completion of the reaction. The solvent was evaporated under reduced pressure and the compound was purified by HPLC (20% to 26% ACN) to afford the desired compound **6** as a beige powder (5 mg, 5%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.11 (t, J = 5.7 Hz, 1 H), 8.38 (d, J = 7.4 Hz, 1 H), 8.24 (t, J = 5.6 Hz, 1 H), 7.88 (s, 1 H), 7.72 (t, J = 5.6 Hz, 1 H), 7.19 – 7.02 (bs, 4 H), 6.53 (s, 1 H), 6.40 (d, J = 24 Hz, 1 H), 4.32 - 4.26 (m, 5 H), 4.13 – 4.10 (m, 1 H), 3.27 – 3.25 (m, 1 H), 3.17 (d, J = 5.0 Hz, 2 H), 3.12 – 3.03 (m, 1 H), 2.99 (q, J = 6.6 Hz, 2 H), 2.81 (dd, J = 12.5, 5.1 Hz, 1 H), 2.07 (t, J = 7.5 Hz, 2 H), 2.03 (t, J = 7.5 Hz, 2 H), 1.85 – 1.75 (m, 2 H), 1.60 – 1.42 (m, 8 H), 1.42 – 1.19 (m, 8 H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.0, 171.8, 162.7, 157.6, 150.1, 149.5, 144.9, 140.6 (d, J = 233 Hz), 128.8, 122 .8 (d, J = 37.5 Hz), 122.6, 63.1, 61.0, 59.2, 55.4, 49.1, 39.9, 35.2, 35.1, 34.1, 29.3, 29.0, 28.2, 28.0, 26.1, 25.3, 24.9, 23.1. HRMS calculated for: $[C_{29}H_{43}FN_{10}O_6S + H]^+$ 679.3142, found: 679.3145 $[M + H]^+$.

Biochemistry

Cloning and generation of stable ASAH1 expressing Farber fibroblasts

The PCR-amplified acid ceramidase (ASAH1, acc. nr: NM_177924.3) coding sequence (using the following oligonucleotides: sense. 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGCCACCATGCCGGGCCGGAGTTG-3' and antisense 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCTCACCAACCTATACAAGGGT CAGGGC-3') was cloned into pDNOR-221 and sub-cloned in pLenti6.3/TO/V5-DEST using the Gateway system (Invitrogen). Mutagenesis was performed using the QuikChange Lightning Site-Directed Mutagenesis Kit in order to introduce the C122S mutation in the ASAH1 coding sequence using the following oligonucleotides: 5'-GAATTATTTACCATTaGTACTTCAATAGTAGCAGAAGAC-3' and 5'-GTCTTCTGCTACTATTGAAGTACtAATGGTAAATAATTC-3'. Correctness of all constructs was verified by sequencing. To produce lentiviral particles HEK293T cells were transfected with pLenti6.3-ASAH1 or pLenti6.3-ASAH1-C122S in combination with the envelope and packaging plasmids pMD2G, pRRE and pRSV. Subsequently, culture supernatant containing viral particles was collected and used for infection of Farber fibroblasts. Selection using blasticidin for several weeks rendered cells stably expressing either the wild type or mutant acid ceramidase as determined by activity assays and Western blot using monoclonal anti Acid Ceramidase (BD Biosciences (figure supporting 1).

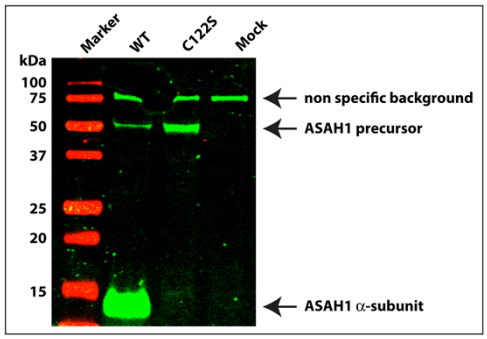


Figure Supporting 1. Western blot of Farber fibroblast stably expressing ASAH1 WT or the C122S mutant**.**

The monoclonal antibody recognizes the 13 kDa ASAH1 alpha-subunit and the 55 kDa ASAH1 precursor. In the case of WT ASAH1 expressing cells both precursor and mature alpha-subunit could be detected, while in the case of the C122S ASAH1 mutant only the ASAH1 precursor is visible due to the fact that this mutant is not able to auto-activate itself into mature alpha- and beta-subunits.

Cell Culture and lysates

Fibroblasts were established from normal individuals and Farber disease patients. Fibroblasts were grown on Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Calf Serum, 10 units/mL penicillin and 10 units/mL streptomycin in a 5% CO_2 humidified incubator at 37 °C. Cells lysates were prepared from cell pellets by resuspension in sucrose 25%. The protein concentration was determined using the Qubit® Protein Assay Kit.

Preparation of human (healthy and Gaucher) spleen homogentes/lysates.

The organs were thawed on ice and Dounce-homogenized in 0.3 M sucrose. The supernatant was subjected to ultracentrifugation (100.000 g, 45 min. at 4 °C, Beckman Coulter, Type Ti70 rotor) to yield the cytosolic fraction in the supernatant and the membrane fraction as a pellet. The pellet was discarded and the supernatant was stored resuspended in small aliquots at - 80 °C until use. The total protein concentration was determined with Qubit® Protein Assay Kit.

SDS-Page analysis: The gels were scanned with The ChemiDoc™ MP system (BioRad) using Cy2/TAMRA settings (excitation wavelength 532 nm, emission wavelength 580 nm) and analyzed using (ImageJ). Protein standard is Dual Color protein standard marker.

***In vitro* labeling of acid ceramidase using cell lysates**

Fibroblasts lysates (12.3 μg protein per experiment) from cells transfected with WT acid ceramidase were diluted in assay buffer (100 mM sodium citrate, 150 mM NaCl, 0.1% Igepal, 3 mM DTT, pH = 4.5, 10 μL) and exposed to the indicated concentration of ABP **5** for 30 min at 37 °C while shaking. The reaction mixtures were then quenched with 10 μL 3 × Laemmli's buffer and resolved on 12.5% SDS-PAGE. In-gel visualization of the fluorescent labeling was performed in the wet gel slabs using Cy2/TAMRA settings.

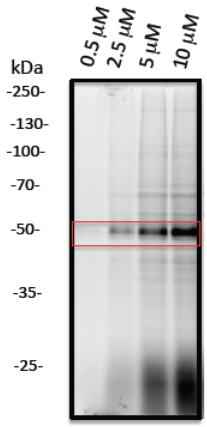


Figure Supporting 2. Labeling in fibroblasts transfected with WT acid ceramidase using ABP **5.**

***In vitro* competition assay versus ABP 5**

Fibroblasts lysates (9.4 μg protein per experiment) from cells transfected with WT acid ceramidase were diluted in assay buffer (100 mM sodium citrate, 150 mM NaCl, 0.1% Igepal, 3 mM DTT, pH = 4.5, 10 μL) and exposed to 10 μM of Carmofur, **1**, **2, 3** or **4** for 30 min at 37 °C while shaking. Afterwards the lysates were incubated with 2.5 μM ABP **5** for 30 min at 37 °C while shaking. The reaction mixtures were then quenched with 10 μL 3 × Laemmli's buffer and resolved on 12.5% SDS-PAGE. In gel visualization was measured using Cy2 settings.

***In vitro* assay using mutant C122S construct**

Fibroblasts lysates (9.4 μg protein per experiment) from cells transfected with C122S mutant were diluted in assay buffer (100 mM sodium citrate, 150 mM NaCl, 0.1% Igepal, 3 mM DTT, pH = 4.5, 10 μL) and exposed to 2.5 μM and 1.0 μM ABP **5** for 30 min at 37 °C while shaking. Cells transfected with WT acid ceramidase and incubated with 2.5 μM ABP **5** for 30 min at 37 °C with shaking were used as controls. The reaction mixtures were then quenched with 10 μL 3 × Laemmli's buffer and resolved on 12.5% SDS-PAGE. In gel-visualization were performed using Cy2 settings.

***In vitro* labeling and competition assay versus ABP 5 using tissues**

Tissue lysates (1.6 mg/mL final protein concentration) from healthy and Gaucher spleen were diluted in assay buffer (100 mM sodium citrate, 150 mM NaCl, 0.1% Igepal, 3 mM DTT, pH = 4.5, V_{final} = 20 μL) and exposed to DMSO (0.5 μL) or 10 μM of Carmofur for 30 min at 37 °C with shaking. Afterwards the lysates were incubated with 2.5 μM ABP **5** for 30 min at 37 °C while shaking. The reaction mixtures were then quenched with 10 μL 3 × Laemmli's buffer and resolved on 12.5% SDS-PAGE. As a control, the assay was reiterated on healthy and Gaucher tissues coming from two others individuals. In gel visualization was measured using Cy2 settings.

***In vitro* competition assay versus ABP 5 using tissues**

Tissue lysates (1.6 mg/mL final protein concentration) from Gaucher spleen were diluted in assay buffer (100 mM sodium citrate, 150 mM NaCl, 0.1% Igepal, 3 mM DTT, pH = 4.5, V_{final} = 20 μL) and exposed to DMSO (0.5 μL) or the indicated concentration of ABP **6** for 30 min at 37 °C after which, the lysates were incubated with 2.5 μM ABP **5** for 30 min at 37 °C while shaking. The reaction mixtures were then quenched with 10 μL 3 × Laemmli's buffer and resolved on 12.5% SDS-PAGE. In gel visualization was measured using Cy2 settings.

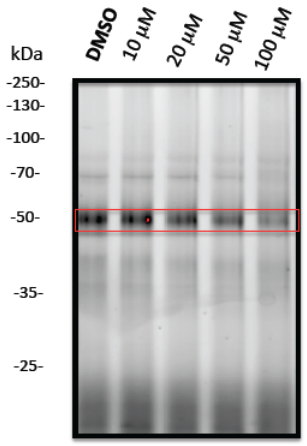


Figure Supporting 3: Competition assay versus **5** in Gaucher tissues (spleen).

Pulldown with 6

Tissue lysates (1440 µg protein) from Gaucher spleen in assay buffer (100 mM sodium citrate, 150 mM NaCl, 0.1% Igepal, 3 mM DTT, pH = 4.5, V_{final} = 200 µL) was incubated with biotin probe **6** (200 µM) for 30 minutes at 37 °C with shaking. The sample was denaturated with a 10% (wt/vol) SDS solution to a final SDS concentration of 1%, vortexed and heated at 100 ° C for 5 min. Chloroform/methanol precipitation was then carried out as described previously.[9] The cell pellet was dried for 5 minutes and rehydrated in 180 µL urea buffer (8 M urea/100 mM NH_4HCO_3). After addition of 10 µL DTT (90 mM stock solution in 8 M urea/100 mM NH_4HCO_3) and 15 µL iodoacetamide (200 mM stock solution in 8 M urea/100 mM NH_4HCO_3) for reduction and alkylation, the chloroform/methanol precipitation was repeated.[8] The sample was then diluted stepwise with PD buffer (50 mM Tris-HCl, pH = 7.5, 150 mM NaCl) to a final SDS concentration of 0.05% (wt/vol). Streptavidin magnetic beads 50 µL (beforehand washed 2 times with 500 µL PD buffer and 2 times with 500 µL 0.05% SDS PD buffer) were added. After addition of protease inhibitor (30 µL), the proteins were incubated overnight at 4 °C with vigorous shaking. The beads were washed with different stringent buffers before 100 µL on-bead digestion buffer (100 mM Tris-HCl, pH = 7.5, 100 mM NaCl, 1 mM CaCl2, 2 % ACN) and 1 µL

trypsin (500 ng) was added for the overnight on bead digestion at 37 °C with vigorous shaking. Finally, the pH was adjusted with formic acid to pH 3 and desalting and sample preparation was performed as described previously.[10]

Tryptic peptides were analyzed on a Surveyor nanoLC system (Thermo) hyphenated to a LTQ-Orbitrap mass spectrometer (Thermo). Gold and carbon coated emitters (OD/ID = 360/25 mm tip ID = 5 μm), trap column (OD/ID = 360/ 100 μm packed with 25 mm robust Poros10R2/ 15 mm BioSphere C18 5 μm 120 Å) and analytical columns (OD/ID = 360/75 μm packed with 20 cm BioSphere C18 5 mm 120 Å) were from Nanoseparations (Nieuwkoop, The Netherlands). The mobile phases (A: 0.1% FA/H_2O, B: 0.1% FA/ACN) were made with ULC/MS grade solvents (Biosolve). The emitter tip was coupled end-to-end with the analytical column via a 15 mm long TFE Teflon tubing sleeve (OD/ID 0.3 3 1.58 mm, Supelco, USA) and installed in a stainless steel holder mounted in a nano-source base (Upchurch scientific, Idex, USA). General mass spectrometric conditions were as follows: an electrospray voltage of 1.8 kV was applied to the emitter, no sheath and auxiliary gas flow, ion transfer tube temperature 150 °C, capillary voltage 41 V, tube lens voltage 150 V. Internal mass calibration was performed with air-borne protonated polydimethylcyclosiloxane (m/z = 445.12002) and the plasticizer protonated dioctyl phthalate ions (m/z = 391.28429) as lock mass. For shotgun proteomics analysis, 10 μl of the samples was pressure loaded on the trap column with a 10 μl/min flow for 5 min followed by peptide separation with a gradient of 35 min 5%–30% B, 15 min 30%–60% B, 5 min A at a flow of 300 ml/min split to 250 nl/min by the LTQ divert valve. For each data-dependent cycle, one full MS scan (300–2000 m/z) acquired at high mass resolution (60,000 at 400 m/z, AGC target 1 x 106, maximum injection time 1000 ms) in the Orbitrap was followed by three MS/MS fragmentations in the LTQ linear ion trap (AGC target 5 x 103, maximum injection time 120 ms) from the three most abundant ions. MS2 settings were as follows: collision gas pressure 1.3 mT, normalized collision energy 35%, ion selection threshold of 500 counts, activation q = 0.25 and activation S6 time of 30 ms. Fragmented precursor ions that were measured twice within 10 s were dynamically

excluded for 60 s and ions with z < 2 or unassigned were not analyzed. After LC-MS analysis, peak lists were extracted from the .raw files using the DTA supercharge software. The peak lists were searched using the Mascot software (Matrix science).

File F10920
Prot accession nr: E7EMM4, Acid ceramidase, Homo sapiens
Mw=42169
emPAI=0.16
peptide FDR = 1.24%
position 85 out 271 proteins found
coverage of amino acids : 15%

Start-end	z	ppm	pept score	pept seq
275-285	2	2.03	15	ESLDVYELDAK
289-298	2	-1.09	17	WYVVQTNYDR
342-353	2	-0.04	65	LTVYTTLIDVTK
354-361	2	-0.06	17	GQFETYLR
362-370	2	-1.47	37	DCPDPCIGW*

* C-terminal peptide

Table Supporting 1. Identified peptides of acid ceramidase

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