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Design and synthesis of a macrosphelide A-biotin chimera†

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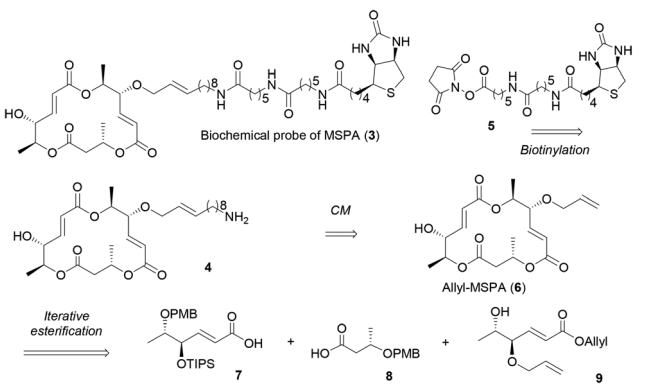
The rational design and synthesis of a biochemical probe of natural (+)-macrosphelide A, a potent cell–cell adhesion inhibitor, was completed to aid in the identification of its biological target. The key features of the synthesis include: (1) an efficient synthesis of the macrosphelide core structure using Yamaguchi–Hirao alkynylation, (2) a cross metathesis to connect a linker unit to the allyl-macrosphelide and (3) coupling of the linker-bound macrosphelide A with a chemical biotin tag.

Introduction

Macrosphelide A (MSPA), a natural polyketide with multiple biological characters, is affiliated with macrotriolide.[1] MSPA was first isolated from the fermentation broth of *Microsphaeropsis* sp. FO-5050 and was subsequently identified in the mycoparasite *Coniothyrium minitans*[2] and the fungus *Tritirachium* sp. HKI 0317.[3] This 16-membered macrolide dose-dependently inhibited the adhesion of human leukemia HL-60 cells to the monolayer of LPS-activated human-umbilical-vein endothelial cells (HUVECs) with an IC_{50} of 3.5 μM.[1a] MSPA also exhibited anticancer activity against lung metastasis of B16/BL6 melanoma in mice (50 mg kg^{-1}) while showing no growth inhibition of various mammalian cell lines (0.2 mg mL^{-1}). Notably, acute toxicity was not induced by the intraperitoneal injection of MSPA into BDF1 mice at 200 mg kg^{-1} for 5 days.[4] Thus, MSPA has attracted interest as a potential pharmaceutical lead for the treatment of tumor metastasis.[5–11] However, the molecular mode of action of MSPA remains unknown in spite of its promising biological activities and potential clinical use.

We have recently investigated the asymmetric synthesis of the macrosphelide series and their molecular mechanisms of action. We reported total syntheses of natural macrosphelides A, B, J and K, which were accomplished *via* optimized nitrile oxide-olefin cycloadditions.[12] We have also achieved a synthesis-based structure elucidation of macrosphelides J and K.[12c] Having established the synthetic procedures for macrosphelides, we recently focused on the preparation of biochemical probes of MSPA (Scheme 1, 3), a highly potent cell–cell adhesion inhibitor, for identification of its biological targets. Clearly, it is crucial not to compromise the active pharmacophores when designing such chemical probes. Macrosphelide B (MSPB) possesses a C14-carbonyl moiety rather than the C14-hydroxyl group of MSPA as shown in Fig. 1 and exhibits



Scheme 1 Retrosynthetic analysis.

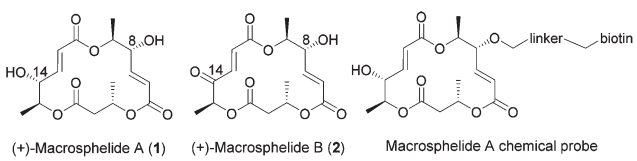


Fig. 1 Macrosphelides A, B and a chemical probe of MSPA.

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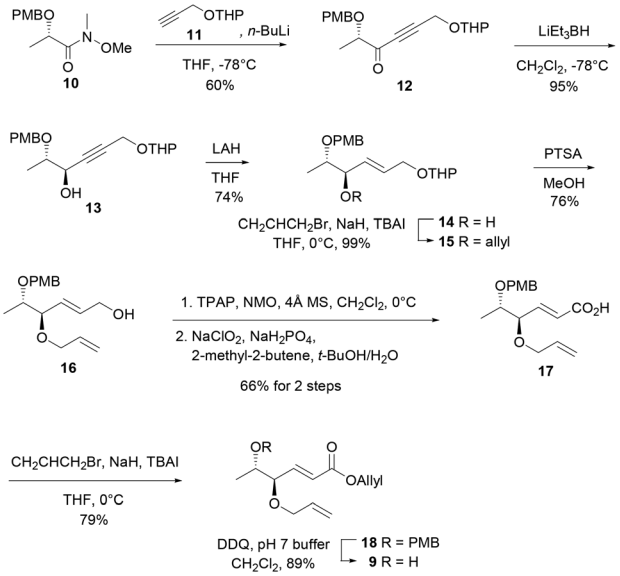
lower (IC_{50} 36 μM) inhibition of HL-60 cell adhesion compared to that of MSPA.[1a] Thus, we introduced an affinity tag to the C8-hydroxyl group rather than the C14-hydroxyl group of MSPA to maintain the inhibitory activity of HL-60 cell adhesion, although the role of the C8-hydroxyl group for the inhibition of HL-60 cell adhesion should be further examined. Biotin was selected as an affinity tag because biotin-bound proteins are easily isolated with streptavidin.[13] Herein we describe an efficient synthesis of biotin-tagged MSPA for elucidation of its mode of action.

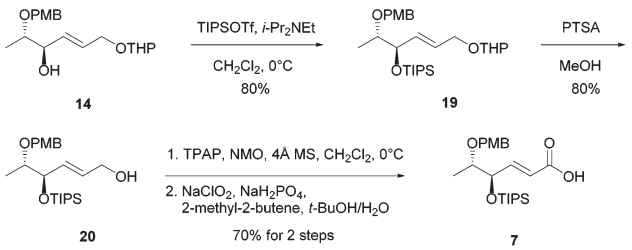
Results and discussion

Our synthetic strategy for the macrosphelide A-biotin chimera is outlined in Scheme 1. The final biochemical probe **3** is accessible *via* a Schotten–Baumann reaction of the linker-bound MSPA **4** and the *N*-hydroxysuccinimide (NHS)-biotin **5**. Prior to tagging MSPA with biotin, a long chain linker was connected to the allyl-MSPA **6** by employing cross metathesis. The allyl-MSPA **6** was strategically disassembled into three parts to maximize the synthetic convergence. The three fragments were efficiently coupled *via* iterative esterification.



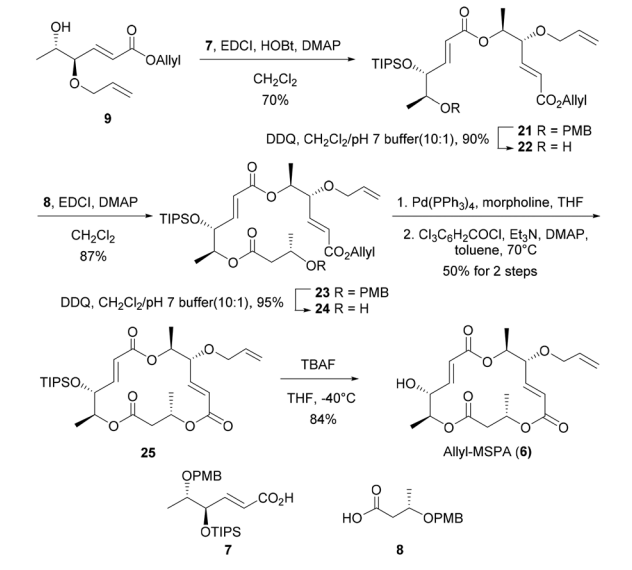
Scheme 2 Synthesis of building block **9**.

The synthesis commenced with Yamaguchi–Hirao alkynylation[14] of the optically active Weinreb amide **10**[12a] with propargylic ether **11** to furnish alkynone **12**, which was subjected to the chelation-controlled reduction using a super hydride at −78 °C in CH_2Cl_2.[15] A single detectable isomer was successfully isolated. The propargylic alcohol produced (**13**) was then reduced with LAH to afford allylic alcohol **14**.[16] Protection of allylic alcohol **14** with allyl bromide followed by acidic hydrolysis afforded the desired allylic alcohol **16**. The key building block **9** was successfully synthesized, as shown in Scheme 2, *via* consecutive oxidations, O-allylation of the resulting enoic acid **17**, and PMB deprotection under buffered conditions.



Scheme 3 Synthesis of building block **7**.

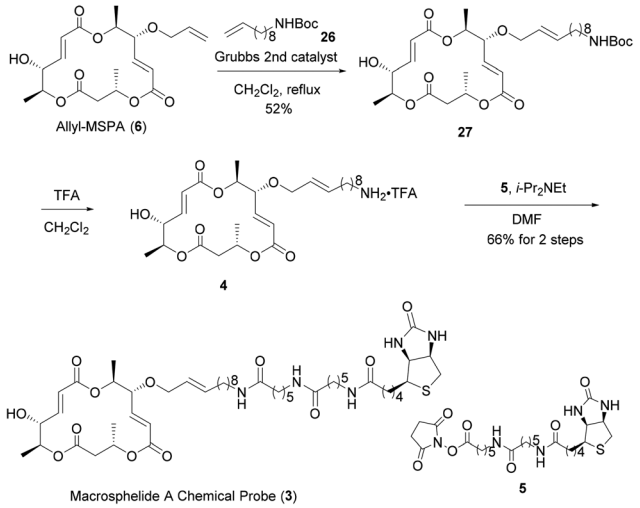
The second key building block **7** was prepared from allylic alcohol **14** (Scheme 3). Protection of **14** with TIPSOTf and THP deprotection of the resulting silyl ether **19** afforded the corresponding alcohol **20**, which was conveniently transformed into carboxylic acid **7** *via* sequential TPAP/NMO oxidation and Pinnick oxidation.[17]



Scheme 4 Synthesis of allyl-MSPA **6**.

Synthesis of allyl-MSPA **6** is outlined in Scheme 4. Alcohol **9** was coupled with acid **7** in the presence of EDCI to afford diester **21**. PMB ether was cleaved with DDQ to yield the free alcohol **22**. Alcohol **22** was coupled with the optically active butyric acid **8**[18] to provide triester **23**, which was treated with DDQ to furnish the Yamaguchi precursor **24**. The Pd-catalyzed deprotection of allyl ester **24** and subsequent macrolactonization of the resulting ω-hydroxy acid under the Yamaguchi con-

ditions[19] afforded the 16-membered macrolide **25**. Finally, removal of the silyl protective group with TBAF provided the key allyl-MSPA **6** in 84% yield.



Scheme 5 Synthesis of the MSPA-chemical probe **3**.

For completion of the synthesis, ruthenium-catalyzed cross metathesis of allyl-MSPA **6** and the Boc-protected amino alkene linker **26**[20] was carried out to provide exclusively the *trans*-isomer **27** (Scheme 5). Finally, Boc-deprotection of **27** in acidic medium and subsequent biotinylation with the commercially available **5** produced the MSPA-biotin chimera **3** in good yield.

Conclusions

In conclusion, rational design and synthesis of a biotin-affinity probe of MSPA was accomplished. Our strategy involved an initial preparation of the allyl-MSPA, linker connection using cross metathesis and biotinylation of the linker-bound MSPA **4** under Schotten–Baumann conditions. Considering that natural MSPA is a promising lead compound for the development of cell–cell adhesion inhibitors, our chemical probe could serve as an important tool for the elucidation of the molecular mode of action of MSPA. Further studies using **3** as a chemical probe for the identification of MSPA's target proteins are currently underway.

Experimental

Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran and Et_2O were distilled from sodium benzophenone ketyl. Dichloromethane, chloroform, triethylamine, acetonitrile and pyridine were freshly distilled from calcium hydride. All solvents used for routine isolation of products and chromatography were reagent grade and glass distilled. Reaction flasks were dried at 100 °C. Air and moisture sensitive reactions were performed under an argon atmosphere. Flash column chromatography was performed using silica gel 60 (230–400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Optical rotations were measured with a JASCO DIP-1000 digital polarimeter at ambient temperature using 100 mm cell of 2 mL capacity. Infrared spectra were recorded on a Perkin-Elmer 1710 FT-IR spectrometer. Mass spectra were obtained with a VG Trio-2 GC-MS instrument. High resolution mass spectra were obtained with a JEOL JMS-AX 505WA instrument. ^{1}H and ^{13}C NMR spectra were recorded on either a JEOL JNM-GCX 400 or a JEOL JNM-LA 300 spectrometer as solutions in deuteriochloroform ($CDCl_3$). Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane and are referenced to the deuterated solvent ($CHCl_3$). ^{1}H-NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and/or multiple resonances), number of protons, and coupling constant in hertz (Hz).

(2*S*)-2-(4-Methoxybenzyloxy)-6-(tetrahydro-2*H*-pyran-2-yloxy)-hex-4-yn-3-one (12)

To a solution of alkyne **11** (1.03 g, 7.4 mmol) in THF (10 mL) was added *n*-BuLi (3.98 mL, 6.4 mmol, 1.6 M in hexane) at −78 °C. The mixture was stirred for 1 h at the same temperature and a solution of amide **10** (1.24 g, 4.9 mmol) in THF (10 mL) was added at −78 °C. The reaction mixture was again stirred for 2 h at −40 °C, quenched with H_2O, and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 3) to afford 977 mg (60%) of ketone **12** as a pale yellow oil: $[\alpha]_D^{25}$ −23.5 (*c* 0.293, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 2943, 2212, 1681, 1613, 1514, 1442, 1303 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.27 (d, 2H, J = 8.6 Hz), 6.85 (d, 2H, J = 8.6 Hz), 4.80 (q, 1H, J = 3.3 Hz), 4.50 (m, 2H), 4.43 (s, 2H), 4.00 (q, 1H, J = 6.9 Hz), 3.78 (s, 3H), 3.82–3.50 (m, 2H), 1.82–1.67 (m, 2H), 1.60 (m, 2H), 1.53 (m, 2H), 1.38 (d, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.2, 159.4, 129.6, 129.4, 113.8, 97.1, 91.4, 83.1, 80.3, 71.8, 62.0, 55.2, 53.8, 30.0, 25.2, 18.8, 17.6; LR-MS (CI+) m/z 333 (M + H$^+$); HR-MS (CI+) calcd for $C_{19}H_{25}O_5$ (M + H$^+$) 333.1702; found 333.1701.

(2*S*,3*R*)-2-(4-Methoxybenzyloxy)-6-(tetrahydro-2*H*-pyran-2-yloxy)-hex-4-yn-3-ol (13)

To a solution of ketone **12** (5.67 g, 17.1 mmol) in CH_2Cl_2 (100 mL) was added $LiEt_3BH$ (25.7 mL, 25.7 mmol, 1.0 M in THF) at −78 °C. The reaction mixture was stirred for 0.5 h at the same temperature and quenched with H_2O. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 2) to afford 5.42 g (95%) of alcohol

13 as a pale yellow oil: $[\alpha]_D^{24}$ −11.2 (*c* 0.173, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3428, 2941, 1612, 1514, 1455, 1375, 1302 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.24 (d, 2H, *J* = 8.5 Hz), 6.85 (d, 2H, *J* = 8.6 Hz), 4.78 (t, 1H, *J* = 3.2 Hz), 4.50 (m, 2H), 4.43 (m, 1H), 4.27 (m, 2H), 3.77 (s, 3H), 3.82–3.61 (m, 2H), 3.49 (m, 1H), 2.52 (d, 1H, *J* = 6.1 Hz), 1.82–1.66 (m, 2H), 1.58 (m, 2H), 1.51 (m, 2H), 1.23 (d, 3H, *J* = 6.3 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.2, 130.1, 129.3, 113.8, 96.7, 83.8, 82.0, 76.4, 70.7, 65.1, 62.0, 55.2, 54.2, 30.2, 25.3, 19.0, 14.6; LR-MS (CI+) *m*/*z* 333 ($M - H^+$); HR-MS (CI+) calcd for $C_{19}H_{25}O_5$ ($M - H^+$) 333.1702; found 333.1703.

(2*S*,3*R*,*E*)-2-(4-Methoxybenzyloxy)-6-(tetrahydro-2*H*-pyran-2-yloxy)hex-4-en-3-ol (14)

To a solution of alcohol **13** (5.42 g, 16.2 mmol) in THF (100 mL) was added LAH (736 mg, 19.4 mmol) at 0 °C. The reaction mixture was stirred for 2 h at ambient temperature and H_2O (0.7 mL), 10% aqueous NaOH (1.4 mL), and then H_2O (2.1 mL) were sequentially added. Aluminium salt was filtered and the solution was carefully dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 2) to afford 4.04 g (74%) of alcohol **14** as a pale yellow oil: $[\alpha]_D^{24}$ 20.6 (*c* 0.107, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3444, 2940, 1612, 1514, 1455, 1374, 1302 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.24 (d, 2H, *J* = 8.8 Hz), 6.86 (d, 2H, *J* = 8.6 Hz), 5.84 (m, 1H), 5.71 (m, 1H), 4.62 (m, 1H), 4.49 (m, 2H), 4.22 (m, 2H), 3.98 (m, 1H), 3.79 (s, 3H), 3.87–3.55 (m, 2H), 3.49 (m, 1H), 2.19 (d, 1H, *J* = 4.4 Hz), 1.82–1.66 (m, 2H), 1.59 (m, 2H), 1.51 (m, 2H), 1.11 (d, 3H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.2, 130.9, 130.4, 129.2, 128.8, 113.8, 97.8, 77.1, 73.8, 70.4, 67.0, 62.1, 55.2, 30.5, 25.4, 19.4, 14.1; LR-MS (FAB+) *m*/*z* 337 ($M + H^+$); HR-MS (FAB+) calcd for $C_{19}H_{29}O_5$ ($M + H^+$) 337.2015; found 337.2029.

2-((4*R*,5*S*,*E*)-4-(Allyloxy)-5-(4-methoxybenzyloxy)hex-2-enyloxy)-tetrahydro-2*H*-pyran (15)

To a solution of alcohol **14** (774 mg, 2.3 mmol) in THF (10 mL) were added TBAI (170 mg, 0.5 mmol) and NaH (230 mg, 5.8 mmol, 60% dispersion in mineral oil) at 0 °C. The reaction mixture was stirred for 10 min at the same temperature and allyl bromide (0.60 mL, 6.9 mmol) was added. The reaction mixture was again stirred for 12 h at ambient temperature and quenched with H_2O. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 3) to afford 858 mg (99%) of allyl ether **15** as a pale yellow oil: $[\alpha]_D^{25}$ −22.5 (*c* 0.153, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 2940, 2868, 1613, 1514, 1465, 1368, 1302 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.18 (d, 2H, *J* = 8.2 Hz), 6.77 (d, 2H, *J* = 8.6 Hz), 5.82 (m, 1H), 5.72 (m, 1H), 5.61 (dd, 1H, *J* = 15.7, 7.5 Hz), 5.19 (dd, 1H, *J* = 17.3, 1.7 Hz), 5.07 (d, 1H, *J* = 10.4 Hz), 4.56 (t, 1H, *J* = 3.6 Hz), 4.45 (s, 2H), 4.21–3.94 (m, 2H), 4.01 (m, 1H), 3.81 (m, 2H), 3.71 (s, 3H), 3.69–3.46 (m, 2H), 3.42 (m, 1H), 1.79–1.60 (m, 2H), 1.51 (m, 2H), 1.44 (m, 2H), 1.10 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.0, 135.1, 130.9, 130.7, 130.1, 129.1, 116.3, 113.6, 97.8, 82.4, 76.7, 70.9, 69.6, 66.9, 62.1, 55.1, 30.6, 25.4, 19.4, 16.1; LR-MS (FAB+) *m*/*z* 377 ($M + H^+$); HR-MS (FAB+) calcd for $C_{22}H_{33}O_5$ ($M + H^+$) 377.2328; found 377.2314.

(4*R*,5*S*,*E*)-4-(Allyloxy)-5-(4-methoxybenzyloxy)hex-2-en-1-ol (16)

To a solution of allyl ether **15** (820 mg, 2.2 mmol) in MeOH (10 mL) was added *p*-TsOH·H_2O (42 mg, 0.2 mmol) at ambient temperature. The reaction mixture was stirred for 2 h at the same temperature and quenched with $NaHCO_3$. The mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 1) to afford 485 mg (76%) of allyl alcohol **16** as a pale yellow oil: $[\alpha]_D^{24}$ −24.8 (*c* 0.287, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3417, 2864, 1613, 1514, 1463, 1372, 1302 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.18 (d, 2H, *J* = 8.6 Hz), 6.78 (d, 2H, *J* = 8.7 Hz), 5.81 (m, 1H), 5.74 (t, 1H, *J* = 5.4 Hz), 5.58 (dd, 1H, *J* = 15.7, 7.5 Hz), 5.19 (dd, 1H, *J* = 17.3, 1.7 Hz), 5.07 (dd, 1H, *J* = 10.5, 1.5 Hz), 4.46 (s, 2H), 4.05 (d, 2H, *J* = 5.2 Hz), 4.01–3.78 (m, 2H), 3.71 (s, 3H), 3.67 (dd, 1H, *J* = 7.5, 4.3 Hz), 3.51 (m, 1H), 1.97 (s, 1H), 1.09 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.0, 135.0, 133.4, 130.8, 129.2, 128.6, 116.4, 113.6, 82.4, 76.7, 71.0, 69.5, 62.8, 55.2, 16.2; LR-MS (CI+) *m*/*z* 291 ($M - H^+$); HR-MS (CI+) calcd for $C_{17}H_{23}O_4$ ($M - H^+$) 291.1596; found 291.1603.

(4*R*,5*S*,*E*)-4-(Allyloxy)-5-(4-methoxybenzyloxy)hex-2-enoic acid (17)

To a solution of allyl alcohol **16** (141 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) were added 4 Å MS (145 mg, 300 mg $mmol^{-1}$), 4-methylmorpholine *N*-oxide (85 mg, 0.7 mmol), and tetrapropylammonium perruthenate (25 mg, 70 μmol) at 0 °C. The reaction mixture was stirred for 12 h at ambient temperature, filtered through silica gel, and concentrated *in vacuo*. The crude mixture was used for the next step without further purification. To a solution of the above aldehyde in *t*-BuOH (3 mL) and H_2O (2 mL) was added 2-methyl-2-butene (1.55 mL, 14.6 mmol) at ambient temperature. The reaction mixture was stirred for 5 min at the same temperature and a solution of $NaClO_2$ (165 mg, 1.5 mmol, 80%) and $NaH_2PO_4 \cdot 2H_2O$ (379 mg, 2.4 mmol) in H_2O (1.5 mL) was added. The reaction mixture was again stirred for 12 h at the same temperature and concentrated. The residue was diluted with EtOAc and carefully acidified with 2 N HCl. The mixture was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 2 : 1) to afford 97.4 mg (66%) of acid **17** as a pale yellow oil: $[\alpha]_D^{24}$ −12.3 (*c* 0.480, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 2978, 2934, 1699, 1656, 1613, 1586, 1514, 1458, 1420, 1376, 1302 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.23 (d, 2H, *J* = 8.4 Hz), 7.03 (dd, 1H, *J* = 15.8, 5.7 Hz), 6.85 (d, 2H, *J* = 8.5 Hz), 6.05 (d, 1H, *J* = 15.9 Hz), 5.87 (m, 1H), 5.26 (dd, 1H, *J* = 17.3, 1.3 Hz), 5.17 (d, 1H, *J* = 10.5 Hz), 4.49 (m, 2H), 4.07 (dd, 1H, *J* = 12.8, 5.1 Hz), 3.94 (m, 2H), 3.78 (s, 3H), 3.57 (m, 1H), 1.20 (d, 3H, *J* = 6.3 Hz);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.1, 159.2, 148.7, 134.4, 130.4, 129.3, 122.2, 117.2, 113.8, 81.0, 76.4, 71.0, 70.9, 55.2, 16.1.

(4*R*,5*S*,*E*)-Allyl 4-(allyloxy)-5-(4-methoxybenzyloxy)hex-2-enoate (18)

To a solution of acid **17** (350 mg, 1.1 mmol) in THF (10 mL) were added TBAI (42 mg, 0.1 mmol) and NaH (91.2 mg, 2.3 mmol, 60% dispersion in mineral oil) at 0 °C. The reaction mixture was stirred for 10 min at the same temperature and allyl bromide (0.3 mL, 3.4 mmol) was added. The reaction mixture was again stirred for 12 h at ambient temperature and quenched with H_2O. The mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 10) to afford 312 mg (79%) of ester **18** as a yellow oil: $[\alpha]_D^{23}$ −15.2 (*c* 0.467, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 2935, 2867, 1721, 1655, 1613, 1586, 1514, 1458, 1370 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.22 (d, 2H, *J* = 8.6 Hz), 6.93 (dd, 1H, *J* = 15.8, 5.9 Hz), 6.84 (d, 2H, *J* = 8.6 Hz), 6.05 (d, 1H, *J* = 15.8 Hz), 5.94 (m, 1H), 5.87 (m, 1H), 5.33 (d, 1H, *J* = 17.2 Hz), 5.26 (d, 1H, *J* = 17.5 Hz), 5.24 (d, 1H, *J* = 10.4 Hz), 5.16 (d, 1H, *J* = 10.4 Hz), 4.64 (d, 2H, *J* = 5.7 Hz), 4.49 (m, 2H), 4.06 (dd, 1H, *J* = 12.8, 3.8 Hz), 3.91 (m, 2H), 3.78 (s, 3H), 3.56 (m, 1H), 1.18 (d, 3H, *J* = 6.3 Hz); LR-MS (CI+) *m*/*z* 345 (M − H$^+$); HR-MS (CI+) calcd for $C_{20}H_{25}O_5$ (M − H$^+$) 345.1702; found 345.1703.

(4*R*,5*S*,*E*)-Allyl 4-(allyloxy)-5-hydroxyhex-2-enoate (9)

To a solution of allyl ester **18** (3.08 g, 8.9 mmol) in CH_2Cl_2 (50 mL) and phosphate buffer solution (5.0 mL, pH 7.0) was added DDQ (6.06 g, 26.7 mmol) at ambient temperature. The reaction mixture was stirred for 1 h, diluted with CH_2Cl_2, and filtered under reduced pressure. The organic layer was washed with H_2O and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 3) to afford 1.79 g (89%) of alcohol **9** as a yellow oil: $[\alpha]_D^{23}$ −35.5 (*c* 0.220, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3461, 3083, 2980, 2934, 2873, 1722, 1657, 1455, 1424, 1367 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.87 (dd, 1H, *J* = 15.8, 6.4 Hz), 6.04 (dd, 1H, *J* = 15.8, 1.1 Hz), 5.89 (m, 2H), 5.35–5.15 (m, 4H), 4.63 (dt, 2H, *J* = 5.7, 1.4 Hz), 4.08 (m, 1H), 3.89 (m, 3H), 2.23 (m, 1H), 1.13 (d, 3H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.4, 144.6, 134.1, 132.0, 123.9, 118.4, 117.4, 81.8, 70.4, 69.1, 65.2, 17.9; LR-MS (FAB+) *m*/*z* 227 (M + H$^+$); HR-MS (FAB+) calcd for $C_{12}H_{19}O_5$ (M + H$^+$) 227.1283; found 227.1277.

Triisopropyl((2*S*,3*R*,*E*)-2-(4-methoxybenzyloxy)-6-(tetrahydro-2*H*-pyran-2-yloxy)hex-4-en-3-yloxy)silane (19)

To a solution of alcohol **14** (336 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) were added i-Pr_2NEt (0.3 mL, 1.5 mmol) and TIPSOTf (0.4 mL, 1.3 mmol) at 0 °C. The mixture was stirred for 10 min at ambient temperature and quenched with H_2O. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 5) to afford 394 mg (80%) of silyl ether **19** as a yellow oil: $[\alpha]_D^{23}$ −16.5 (*c* 2.90, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3480, 2942, 2892, 2866, 1613, 1586, 1514, 1465, 1383, 1365, 1322, 1302 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.24 (d, 2H, *J* = 8.6 Hz), 6.83 (d, 2H, *J* = 8.6 Hz), 5.75 (m, 2H), 4.62 (m, 1H), 4.53 (m, 2H), 4.21 (m, 2H), 4.00 (m, 1H), 3.84 (m, 1H), 3.77 (s, 3H), 3.50 (m, 2H), 1.84–1.65 (m, 2H), 1.57 (m, 2H), 1.50 (m, 2H), 1.13 (dd, 3H, *J* = 6.4, 3.7 Hz), 1.03 (m, 21H); ^{13}C NMR ($CDCl_3$, 125 MHz, mixture of diastereomers) δ 158.9, 133.4, 133.2, 131.2, 129.1, 128.3, 128.2, 113.6, 97.6, 97.4, 78.7, 78.7, 77.1, 76.9, 71.3, 71.2, 66.9, 66.8, 62.0, 61.9, 55.2, 30.6, 30.5, 25.5, 19.4, 19.3, 18.1, 18.0, 17.7, 16.0, 12.4, 12.3; LR-MS (CI+) *m*/*z* 491 (M − H$^+$); HR-MS (CI+) calcd for $C_{28}H_{47}O_5Si$ (M − H$^+$) 491.3193; found 491.3194.

(4*R*,5*S*,*E*)-5-(4-Methoxybenzyloxy)-4-(triisopropylsilyloxy)hex-2-en-1-ol (20)

To a solution of the allyl ether **19** (11.8 g, 23.9 mmol) in MeOH (60 mL) was added *p*-TsOH·H_2O (455 mg, 2.4 mmol) at ambient temperature. The reaction mixture was stirred for 2 h at the same temperature and quenched with saturated aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 3) to afford 7.82 g (80%) of allyl alcohol **20** as a pale yellow oil: $[\alpha]_D^{23}$ −20.3 (*c* 1.13, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3408, 2943, 2866, 1613, 1587, 1514, 1464, 1381, 1302 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.24 (d, 2H, *J* = 8.5 Hz), 6.84 (d, 2H, *J* = 8.6 Hz), 5.76 (m, 2H), 4.55 (m, 2H), 4.21 (dd, 1H, *J* = 6.4, 3.4 Hz), 4.12 (m, 2H), 3.78 (s, 3H), 3.54 (m, 1H), 1.41 (s, 1H), 1.12 (d, 3H, *J* = 6.5 Hz), 1.04 (m, 21H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.0, 132.0, 131.2, 130.8, 129.2, 113.6, 78.8, 71.5, 63.2, 55.3, 18.1, 18.1, 18.0, 16.2, 12.4; LR-MS (FAB+) *m*/*z* 431 (M + Na$^+$); HR-MS (FAB+) calcd for $C_{23}H_{40}O_4SiNa$ (M + Na$^+$) 431.2594; found 431.2594.

(4*R*,5*S*,*E*)-5-(4-Methoxybenzyloxy)-4-(triisopropylsilyloxy)hex-2-enoic acid (7)

To a solution of the allyl alcohol **20** (433 mg, 1.1 mmol) in CH_2Cl_2 (10 mL) were added 4 Å MS (318 mg, 300 mg mmol^{-1}), 4-methylmorpholine *N*-oxide (186 mg, 1.6 mmol), and tetrapropylammonium perruthenate (56 mg, 0.2 mmol) at 0 °C. The reaction mixture was stirred for 12 h at ambient temperature, filtered through silica gel, and concentrated *in vacuo*. The crude mixture was used for the next step without further purification. To a solution of the above aldehyde in *t*-BuOH (6 mL) and H_2O (3 mL) was added 2-methyl-2-butene (3.4 mL, 31.8 mmol) at ambient temperature. The reaction mixture was stirred for 5 min at the same temperature and a solution of $NaClO_2$ (359 mg, 3.2 mmol, 80%) and NaH_2PO_4·$2H_2O$ (636 mg, 5.3 mmol) in H_2O (3 mL) was added. The reaction mixture was again stirred for 8 h at the same temperature and concentrated. The residue was diluted with EtOAc and carefully acidified with 1 N HCl. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and

concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 5) to afford 314 mg (70%) of acid **7** as a pale yellow oil: $[\alpha]_D^{25}$ −25.9 (*c* 0.393, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 2944, 2867, 1699, 1656, 1613, 1514, 1464, 1420, 1382, 1302 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.22 (d, 2H, *J* = 8.4 Hz), 7.09 (dd, 1H, *J* = 15.7, 5.6 Hz), 6.85 (d, 2H, *J* = 8.6 Hz), 6.05 (d, 1H, *J* = 15.6 Hz), 4.51 (m, 2H), 4.44 (m, 1H), 3.78 (s, 3H), 3.57 (m, 1H), 1.16 (d, 3H, *J* = 6.4 Hz), 1.05 (m, 21H); LR-MS (CI+) *m*/*z* 421 (M − H^+); HR-MS (CI+) calcd for $C_{23}H_{37}O_5Si$ (M − H^+) 421.2410; found 421.2407.

(4*R*,5*S*,*E*)-Allyl 4-(allyloxy)-5-((4*R*,5*S*,*E*)-5-(4-methoxybenzyloxy)-4-(triisopropylsilyloxy)hex-2-enoyloxy)hex-2-enoate (21)

To a solution of alcohol **9** (50 mg, 0.2 mmol) and acid **7** (112 mg, 0.3 mmol) in CH_2Cl_2 (5 mL) were added HOBt (101 mg, 0.7 mmol), DMAP (81.0 mg, 0.663 mmol), and EDCI (127 mg, 0.663 mmol) at ambient temperature. The reaction mixture was stirred for 2 h at the same temperature and quenched with H_2O. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 10) to afford 97.8 mg (70%) of PMB ether **21** as a yellow oil: $[\alpha]_D^{25}$ −15.9 (*c* 0.227, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3438, 3080, 2943, 2892, 2867, 2725, 1724, 1659, 1612, 1586, 1514, 1464, 1422, 1366, 1344 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.21 (d, 2H, *J* = 8.4 Hz), 6.96 (dd, 1H, *J* = 15.5, 5.7 Hz), 6.84 (m, 1H), 6.83 (d, 2H, *J* = 8.6 Hz), 6.09 (dd, 1H, *J* = 15.8, 1.5 Hz), 6.02 (dd, 1H, *J* = 15.8, 1.5 Hz), 5.93 (m, 1H), 5.81 (m, 1H), 5.32 (d, 1H, *J* = 17.2 Hz), 5.23 (d, 1H, *J* = 17.2 Hz), 5.23 (d, 1H, *J* = 10.4 Hz), 5.14 (d, 1H, *J* = 10.4 Hz), 5.04 (m, 1H), 4.63 (m, 2H), 4.50 (m, 2H), 4.42 (m, 1H), 4.07 (m, 2H), 3.93 (m, 1H), 3.77 (s, 3H), 3.55 (m, 1H), 1.24 (d, 3H, *J* = 6.4 Hz), 1.14 (d, 3H, *J* = 6.4 Hz), 1.04 (m, 21H); LR-MS (FAB+) *m*/*z* 631 (M + H^+); HR-MS (FAB+) calcd for $C_{35}H_{55}O_8Si$ (M + H^+) 631.3666; found 631.3660.

(4*R*,5*S*,*E*)-Allyl 4-(allyloxy)-5-((4*R*,5*S*,*E*)-5-hydroxy-4-(triisopropylsilyloxy)hex-2-enoyloxy)hex-2-enoate (22)

To a solution of PMB ether **21** (234 mg, 0.4 mmol) in CH_2Cl_2 (10 mL) and phosphate buffer solution (1.0 mL, pH 7.0) at ambient temperature was added DDQ (252 mg, 1.1 mmol). The reaction mixture was stirred for 1 h, diluted with CH_2Cl_2, and filtered under reduced pressure. The organic layer was washed with H_2O and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 5) to afford 171 mg (90%) of alcohol **22** as a yellow oil: $[\alpha]_D^{25}$ −18.4 (*c* 1.84, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3515, 3084, 2944, 2892, 2868, 2726, 1724, 1659, 1463, 1423, 1365 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.90 (dd, 1H, *J* = 15.7, 6.2 Hz), 6.83 (dd, 1H, *J* = 15.8, 5.8 Hz), 6.08 (dd, 1H, *J* = 15.8, 1.3 Hz), 5.99 (dd, 1H, *J* = 15.7, 1.1 Hz), 5.92 (m, 1H), 5.83 (m, 1H), 5.31 (d, 1H, *J* = 17.2 Hz), 5.24 (d, 1H, *J* = 17.1 Hz), 5.23 (d, 1H, *J* = 11.4 Hz), 5.14 (d, 1H, *J* = 10.4 Hz), 5.04 (m, 1H), 4.63 (d, 2H, *J* = 5.6 Hz), 4.31 (m, 1H), 4.06 (m, 2H), 3.93 (m, 1H), 3.89 (m, 1H), 1.24 (d, 3H, *J* = 6.6 Hz), 1.10 (d, 3H, *J* = 6.5 Hz), 1.04 (m, 21H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.4, 165.1, 146.5, 144.5, 134.2, 132.0, 123.6, 122.8, 118.4, 117.3, 79.4, 76.0, 71.5, 70.8, 70.7, 65.3, 18.0, 17.5, 14.9, 12.3; LR-MS (FAB+) *m*/*z* 511 (M + H^+); HR-MS (FAB+) calcd for $C_{27}H_{47}O_7Si$ (M + H^+) 511.3091; found 511.3098.

(4*R*,5*S*,*E*)-Allyl 4-(allyloxy)-5-((4*R*,5*S*,*E*)-5-((*S*)-3-(4-methoxybenzyloxy)butanoyloxy)-4-(triisopropylsilyloxy)hex-2-enoyloxy)hex-2-enoate (23)

To a solution of alcohol **22** (40 mg, 0.08 mmol) and acid **8** (53 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) were added EDCI (75.2 mg, 0.4 mmol) and DMAP (48 mg, 0.4 mmol) at ambient temperature. The reaction mixture was stirred for 2 h at the same temperature and quenched with H_2O. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 5) to afford 49 mg (87%) of PMB ether **23** as a yellow oil: $[\alpha]_D^{25}$ −7.27 (*c* 0.307, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3081, 2944, 2893, 2868, 1727, 1660, 1613, 1586, 1514, 1464, 1422, 1378, 1343 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.22 (d, 2H, *J* = 8.5 Hz), 6.88 (dd, 1H, *J* = 15.7, 5.4 Hz), 6.84 (d, 2H, *J* = 8.6 Hz), 6.83 (m, 1H), 6.09 (d, 1H, *J* = 15.8 Hz), 6.04 (d, 1H, *J* = 15.7 Hz), 5.93 (m, 1H), 5.83 (m, 1H), 5.32 (dd, 1H, *J* = 17.3, 1.2 Hz), 5.24 (d, 1H, *J* = 18.5 Hz), 5.23 (d, 1H, *J* = 10.3 Hz), 5.15 (d, 1H, *J* = 10.3 Hz), 5.05 (m, 1H), 4.95 (m, 1H), 4.63 (d, 2H, *J* = 5.2 Hz), 4.52 (m, 1H), 4.43 (m, 2H), 4.06 (m, 2H), 3.94 (m, 2H), 3.77 (s, 3H), 2.64–2.33 (m, 2H), 1.24 (d, 3H, *J* = 6.5 Hz), 1.22 (d, 3H, *J* = 6.2 Hz), 1.15 (d, 3H, *J* = 6.5 Hz), 1.03 (m, 21H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.7, 165.4, 165.2, 159.1, 147.3, 144.5, 134.2, 132.0, 130.6, 129.2, 123.6, 122.4, 118.4, 117.3, 113.8, 79.4, 74.2, 73.1, 71.6, 71.6, 70.8, 70.5, 65.3, 55.3, 42.1, 19.9, 18.0, 14.8, 14.0, 12.4; LR-MS (FAB+) *m*/*z* 739 (M + Na^+); HR-MS (FAB+) calcd for $C_{39}H_{60}O_{10}SiNa$ (M + Na^+) 739.3853; found 739.3875.

(4*R*,5*S*,*E*)-Allyl 4-(allyloxy)-5-((4*R*,5*S*,*E*)-5-((*S*)-3-hydroxybutanoyloxy)-4-(triisopropylsilyloxy)hex-2-enoyloxy)hex-2-enoate (24)

To a solution of PMB ether **23** (123 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) and phosphate buffer solution (0.5 mL, pH 7.0) at ambient temperature was added DDQ (117 mg, 0.5 mmol). The reaction mixture was stirred for 1 h, diluted with CH_2Cl_2, and filtered under reduced pressure. The organic layer was washed with H_2O and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 3) to afford 97 mg (95%) of alcohol **24** as a yellow oil: $[\alpha]_D^{23}$ −8.71 (*c* 0.400, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3516, 3083, 2944, 2893, 2868, 1726, 1660, 1516, 1462, 1367 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.87 (dd, 1H, *J* = 15.9, 5.7 Hz), 6.84 (dd, 1H, *J* = 15.9, 6.0 Hz), 6.09 (dd, 1H, *J* = 15.9, 1.4 Hz), 6.04 (dd, 1H, *J* = 15.7, 1.4 Hz), 5.93 (m, 1H), 5.83 (m, 1H), 5.32 (dd, 1H,

J = 17.2, 1.5 Hz), 5.25 (dd, 1H, J = 17.3, 1.5 Hz), 5.24 (dd, 1H, J = 10.4, 1.3 Hz), 5.16 (dd, 1H, J = 10.5, 1.3 Hz), 5.06 (m, 1H), 5.01 (m, 1H), 4.64 (d, 2H, J = 5.5 Hz), 4.49 (m, 1H), 4.17 (m, 1H), 4.07 (m, 2H), 3.93 (dd, 1H, J = 13.0, 5.9 Hz), 2.47–2.35 (m, 2H), 1.25 (d, 3H, J = 6.5 Hz), 1.20 (d, 3H, J = 6.5 Hz), 1.19 (d, 3H, J = 6.8 Hz), 1.04 (m, 21H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.3, 165.5, 165.2, 146.9, 144.4, 134.2, 132.0, 123.6, 122.7, 118.4, 117.4, 79.4, 74.2, 73.3, 71.7, 70.8, 65.3, 64.2, 42.9, 22.4, 18.0, 14.9, 14.4, 12.4; LR-MS (FAB+) m/z 597 (M + H^+); HR-MS (FAB+) calcd for $C_{31}H_{53}O_9Si$ (M + H^+) 597.3459; found 597.3460.

(4*S*,7*E*,9*R*,10*S*,13*E*,15*R*,16*S*)-9-(Allyloxy)-4,10,16-trimethyl-15-(triisopropylsilyloxy)-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione (25)

To a solution of alcohol **24** (36 mg, 0.1 mmol) in THF (3 mL) were added $Pd(PPh_3)_4$ (14 mg, 0.01 mmol) and morpholine (6 μL, 0.07 mmol) at ambient temperature. The mixture was stirred for 1 h at the same temperature and quenched with 1 N HCl. The mixture was extracted with EtOAc three times. The organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude acid was used for the next step without further purification. To a solution of the above acid in toluene (2 mL) were added Et_3N (50 μL, 0.4 mmol) and 2,4,6-trichlorobenzoyl chloride (47 μL, 0.3 mmol) at 0 °C. The mixture was warmed to ambient temperature and stirred for 1 h. The mixture was diluted with toluene (8 mL) and added slowly to a rapidly stirred solution of DMAP (184 mg, 1.5 mmol) in toluene (10 mL). The reaction mixture was stirred for 2 h at 80 °C and quenched with $NaHCO_3$ at ambient temperature. The mixture was extracted with EtOAc and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 5) to afford 16.3 mg (50%) of macrolactone **25** as a pale yellow oil: $[\alpha]_D^{25}$ −36.7 (c 0.940, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3438, 3081, 2944, 2892, 2868, 2726, 1726, 1660, 1580, 1549, 1462, 1421, 1384, 1367, 1347 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.78 (dd, 1H, J = 15.7, 6.2 Hz), 6.71 (dd, 1H, J = 15.9, 7.4 Hz), 5.91 (dd, 1H, J = 15.6, 0.9 Hz), 5.87 (d, 1H, J = 15.6 Hz), 5.81 (m, 1H), 5.24 (m, 1H), 5.21 (d, 1H, J = 17.3 Hz), 5.15 (d, 1H, J = 10.5 Hz), 4.91 (m, 2H), 4.20 (t, 1H, J = 6.2 Hz), 4.04 (dd, 1H, J = 12.8, 5.2 Hz), 3.81 (dd, 1H, J = 12.7, 6.2 Hz), 3.77 (t, 1H, J = 8.1 Hz), 2.61–2.36 (m, 2H), 1.37 (d, 3H, J = 6.3 Hz), 1.26 (d, 3H, J = 6.5 Hz), 1.25 (d, 3H, J = 6.5 Hz), 0.99 (m, 21H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.5, 164.4, 164.1, 146.8, 145.9, 133.9, 124.3, 122.4, 117.5, 81.3, 74.1, 73.7, 70.6, 70.6, 67.3, 40.5, 19.2, 17.9, 17.4, 12.4; LR-MS (FAB+) m/z 539 (M + H^+); HR-MS (FAB+) calcd for $C_{28}H_{47}O_8Si$ (M + H^+) 539.3040; found 539.3027.

(4*S*,7*E*,9*R*,10*S*,13*E*,15*R*,16*S*)-9-(Allyloxy)-15-hydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione (6)

To a solution of allyl ether **25** (5 mg, 9 μmol) in THF (1 mL) was added TBAF (0.01 mL, 0.01 mmol, 1.0 M in THF) at ambient temperature. The reaction mixture was stirred for 10 min and quenched with H_2O. The mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 2) to afford 3.0 mg (84%) of allyl-MSP **6** as a colorless oil: $[\alpha]_D^{20}$ 4.46 (c 0.100, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3482, 2983, 2936, 1721, 1648, 1453, 1377 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.82 (dd, 1H, J = 15.7, 5.2 Hz), 6.76 (dd, 1H, J = 15.9, 7.3 Hz), 6.02 (d, 1H, J = 15.7 Hz), 5.95 (d, 1H, J = 15.9 Hz), 5.84 (m, 1H), 5.30 (m, 1H), 5.25 (d, 1H, J = 17.3 Hz), 5.19 (d, 1H, J = 10.4 Hz), 4.90 (m, 2H), 4.12 (t, 1H, J = 5.2 Hz), 4.07 (dd, 1H, J = 12.7, 5.1 Hz), 3.85 (m, 2H), 2.55 (m, 2H), 1.40 (d, 3H, J = 6.3 Hz), 1.33 (d, 3H, J = 6.5 Hz), 1.32 (d, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.0, 164.6, 164.4, 145.7, 145.3, 133.9, 124.4, 122.8, 117.8, 81.1, 73.6, 72.9, 71.2, 70.6, 67.7, 40.8, 19.5, 17.9, 17.5; LR-MS (FAB+) m/z 383 (M + H^+); HR-MS (FAB+) calcd for $C_{19}H_{27}O_8$ (M + H^+) 383.1706; found 383.1711.

tert-Butyl dec-9-enylcarbamate (26)

To a solution of dec-9-en-1-ol (1 g, 5.8 mmol, 90%) in CH_2Cl_2 (10 mL) were added i-Pr_2NEt (1.3 mL, 7.5 mmol) and methanesulfonyl chloride (0.5 mL, 6.3 mmol) at 0 °C. The reaction mixture was stirred for 1 h at ambient temperature and quenched with H_2O. The mixture was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude mixture was used for the next step without further purification. To a solution of the above mesylate in DMF (10 mL) were added TBAI (213 mg, 0.6 mmol) and NaN_3 (487 mg, 7.49 mmol) at 0 °C. The reaction mixture was stirred for 14 h at 50 °C and quenched with H_2O. The mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 20) to afford 992 mg (95%) of the corresponding 10-azidodec-1-ene as a colorless oil: FT-IR (thin film, neat) ν_{max} 3328, 3077, 2976, 2928, 2856, 2683, 2511, 2096, 1823, 1640, 1464, 1415, 1349 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.79 (m, 1H), 4.97 (dd, 1H, J = 17.2, 1.8 Hz), 4.91 (d, 1H, J = 10.2 Hz), 3.23 (t, 2H, J = 7.0 Hz), 2.02 (m, 2H), 1.57 (m, 2H), 1.34 (m, 4H), 1.28 (m, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 139.1, 114.1, 51.4, 33.7, 29.3, 29.1, 29.0, 28.8, 28.8, 26.7.

To a solution of the above azide (300 mg, 1.7 mmol) in THF (6 mL) were added H_2O (0.30 mL, 16.5 mmol) and triphenylphosphine (564 mg, 2.1 mmol) at ambient temperature. The reaction mixture was stirred for 18 h at the same temperature and concentrated *in vacuo*. The resulting crude amine was used for the next step without further purification. To a solution of the above amine in THF (4 mL) and H_2O (4 mL) were added Na_2CO_3 (874 mg, 8.2 mmol) and Boc_2O (397 mg, 1.8 mmol) at ambient temperature. The reaction mixture was stirred for 1 h at the same temperature and quenched with H_2O. The mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 1 : 10) to afford 299 mg (71%) of carbamate **26** as a white solid: FT-IR (thin film, neat) ν_{max} 3458, 3353, 3077, 2977, 2927, 2855, 1820, 1694, 1641, 1523, 1455, 1391, 1365 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.77

(m, 1H), 4.96 (dd, 1H, J = 17.2, 1.7 Hz), 4.90 (d, 1H, J = 10.1 Hz), 3.06 (t, 2H, J = 7.2 Hz), 2.00 (m, 2H), 1.41 (m, 12H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.0, 139.1, 114.1, 79.1, 40.7, 33.7, 30.0, 29.3, 29.2, 29.0, 28.8, 28.4, 26.7; LR-MS (FAB+) m/z 278 (M + Na^+); HR-MS (FAB+) calcd for $C_{15}H_{29}NO_2Na$ (M + Na^+) 278.2096; found 278.2090.

***tert*-Butyl (*E*)-11-((4*S*,7*E*,9*R*,10*S*,13*E*,15*R*,16*S*)-15-hydroxy-4,10,16-trimethyl-2,6,12-trioxo-1,5,11-trioxacyclohexadeca-7,13-dien-9-yloxy)undec-9-enylcarbamate (27)**

To a solution of allyl-MSPA **6** (2 mg, 4 μmol) and alkene **26** (5 mg, 0.02 mmol) in CH_2Cl_2 (1 mL) was added Grubbs 2nd catalyst (0.3 mg, 0.4 μmol) at ambient temperature. The reaction mixture was stirred for 30 min at 70 °C and quenched with DMSO. The mixture was again stirred for 12 h and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc–*n*-hexane = 2 : 3) to afford 1.4 mg (52%) of the linker-bound allyl-MSPA **27** as a colorless oil: $[\alpha]_D^{24}$ 26.1 (c 0.120, $CHCl_3$); FT-IR (thin film, neat) ν_{max} 3384, 3082, 2979, 2931, 2856, 1718, 1656, 1524, 1453, 1366 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.94 (dt, 1H, J = 15.6, 7.0 Hz), 6.85 (dd, 1H, J = 15.6, 4.4 Hz), 6.16 (dd, 1H, J = 15.6, 1.8 Hz), 6.00 (m, 1H), 5.76 (m, 2H), 5.35 (m, 1H), 4.98 (m, 2H), 4.88 (m, 1H), 4.65 (m, 2H), 4.45 (m, 1H), 3.07 (t, 2H, J = 7.1 Hz), 2.68–2.51 (m, 2H), 2.16 (m, 2H), 1.42 (m, 14H), 1.31 (d, 3H, J = 6.4 Hz), 1.26 (m, 9H), 1.21 (d, 3H, J = 6.4 Hz), 1.14 (d, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.0, 166.3, 165.5, 156.1, 150.5, 144.6, 128.8, 125.7, 122.8, 121.0, 87.9, 79.2, 76.2, 73.4, 72.6, 72.5, 67.0, 41.4, 40.7, 32.2, 30.0, 29.2, 29.1, 29.0, 28.4, 27.9, 26.7, 20.3, 14.6, 13.9; LR-MS (FAB+) m/z 632 (M + Na^+); HR-MS (FAB+) calcd for $C_{32}H_{51}NO_{10}Na$ (M + Na^+) 632.3411; found 632.3428.

***N*-((*E*)-11-((4*S*,7*E*,9*R*,10*S*,13*E*,15*R*,16*S*)-15-Hydroxy-4,10,16-trimethyl-2,6,12-trioxo-1,5,11-trioxacyclohexadeca-7,13-dien-9-yloxy)undec-9-enyl)-6-(6-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexanamido) hexanamide (3)**

To a solution of allyl alcohol **27** (5 mg, 8 μmol) in CH_2Cl_2 (0.5 mL) was added trifluoroacetic acid (0.5 mL) at ambient temperature. The reaction mixture was stirred for 1 h and concentrated *in vacuo*. The crude mixture was used for the next step without further purification. To a solution of the above amine salt in DMF (1 mL) were added i-Pr_2NEt (3 drops) and *N*-hydroxysuccinimide (NHS)-biotin (**5**, 9 mg, 16.1 mmol) at ambient temperature. The reaction mixture was stirred for 48 h at the same temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (MeOH–CH_2Cl_2 = 1 : 7) to afford 5 mg (66%) of the MSPA probe **3** as a colorless oil: FT-IR (thin film, neat) ν_{max} 3301, 3088, 2926, 2855, 1736, 1703, 1678, 1638, 1554, 1463, 1440, 1372, 1306 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.95 (m, 1H), 6.91 (m, 1H), 6.09 (m, 1H), 6.07 (m, 1H), 5.87 (m, 1H), 5.81–5.77 (m, 1H), 5.35–5.26 (m, 2H), 5.00–4.96 (m, 2H), 4.62 (m, 2H), 4.48 (dd, 1H, J = 7.8, 4.3 Hz), 4.30 (d, 1H, J = 4.5 Hz), 4.28 (d, 1H, J = 4.1 Hz), 3.20 (m, 1H), 3.15 (m, 6H), 2.94–2.60 (m, 4H), 2.19 (m, 8H), 1.71 (m, 2H), 1.61 (m, 6H), 1.47 (m, 14H), 1.32 (m, 8H), 1.29 (d, 3H, J = 6.4 Hz), 1.21 (d, 3H, J = 6.5 Hz), 1.17 (d, 3H, J = 6.4 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.8, 176.8, 172.2, 168.3, 168.0, 166.9, 152.2, 148.8, 130.7, 127.4, 123.0, 122.9, 90.1, 77.9, 75.0, 74.8, 74.6, 74.2, 64.2, 62.4, 57.8, 41.9, 41.2, 41.0, 37.8, 37.6, 34.0, 31.2, 31.1, 31.0, 30.9, 30.6, 30.3, 30.0, 28.8, 28.4, 28.4, 27.7, 27.5, 27.5, 20.9, 16.3, 15.8; LR-MS (FAB+) m/z 962 (M + H^+); HR-MS (FAB+) calcd for $C_{49}H_{80}N_5O_{12}S$ (M + H^+) 962.5524; found 962.5549.

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