Supplementary Material (ESI) for Molecular BioSystems

Alkynyl-farnesol reporters for detection of protein *S*-prenylation in cells

Supporting Information

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Supporting Figures

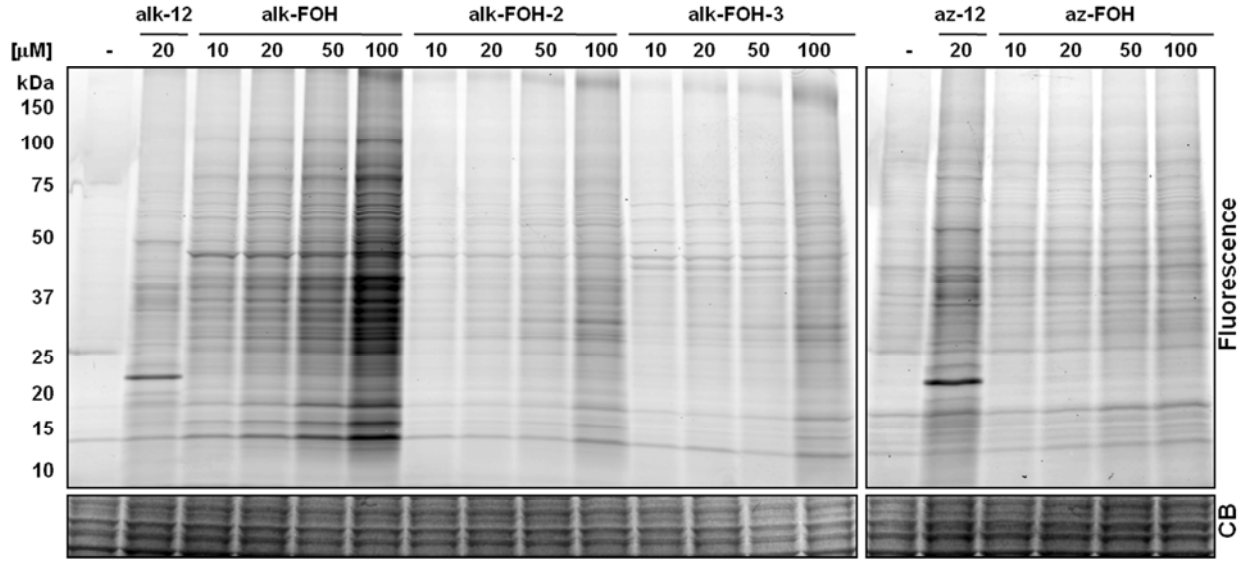


Fig. S1. Dose-dependent metabolic labeling with prenylation reporters. Jurkat cells were treated with different concentrations of prenylation reporters for 4 hours. Cell lysates labeled with alkynyl-isoprenols (alk-FOH, alk-FOH-2 and alk-FOH-3) or azido-isoprenol (az-FOH) were conjugated via CuAAC to azido-rhodamine (az-rho) or alkynyl-rhodamine (alk-rho), respectively. Lysates (20 μg) were separated by SDS-PAGE and scanned for fluorescence (top panel) or stained with Coomassie blue as a loading control (lower panel). Protein *N*-myristoylation reporters alk-12 and az-12 were used as positive controls to compare labeling intensity with protein *S*-prenylation reporters.

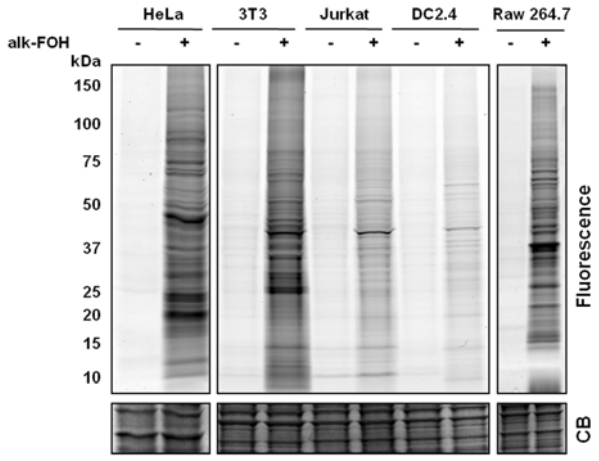
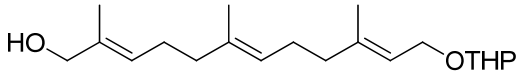


Fig. S2. Profiling of protein *S*-prenylation in different cell types. HeLa, 3T3, Jurkat, DC2.4 or RAW264.7 cells were treated with 10 μM lovastatin for 12 hours before supplementing the media with alk-FOH (20 μM, 1 hour). Cell lysates were conjugated via CuAAC to azido-rhodamine (az-rho). Lysates (20 μg) were separated by SDS-PAGE and scanned for fluorescence (top panel) or stained with Coomassie blue as a loading control (lower panel).

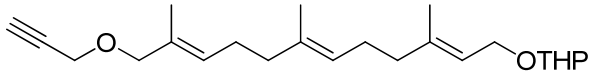
Experimental

All chemicals were obtained either from Sigma-Aldrich, MP Biomedicals, Alfa Aesar, TCI, Fluka or Acros and were used as received unless otherwise noted. The silica gel used in flash column chromatography was Fisher S704 (60-200 Mesh, Chromatographic Grade). Analytical thin layer chromatography (TLC) was conducted on Merck silica gel plates with fluorescent indicator on glass (5-20 μm, 60 Å) with detection by ceric ammonium molybdate, basic $KMnO_4$ or UV light. The ^1H and ^{13}C NMR spectra were obtained on a Bruker DPX-400 spectrometer or a Bruker AVANCE-600 spectrometer equipped with a cryoprobe. Chemical shifts are reported in δ ppm values downfield from tetramethylsilane and *J* values are reported in Hz. Mass spectrometry analysis was conducted on a LTQ MS coupled with Waters UPLC.

Synthesis



(2*E*,6*E*,10*E*)-2,6,10-trimethyl-12-((tetrahydro-2*H*-pyran-2-yl)oxy)dodeca-2,6,10-trien-1-ol (**1**) was produced according to published literature protocols and was identical to previously reported ^1H, ^{13}C NMR and MS analyses.[1] ^1H NMR (600 MHz, $CDCl_3$): δ 1.47-1.88 (m, 6H), 1.59 (s, 3H), 1.65 (s, 3H), 1.67 (s, 3H), 1.98-2.15 (m, 8H), 3.46 (ddd, 1H, *J* = 5.3, 5.3, 10.6), 3.83 (ddd, 1H, *J* = 2.9, 8.2, 11.1), 3.91 (s, 2H), 3.97 (dd, 1H, *J* = 7.6, 11.9), 4.17 (dd, 1H, *J* = 6.4, 11.9), 4.58 (t, 1H, *J* = 3.6), 5.06 (t, 1H, *J* = 6.8), 5.30 (t, 1H, *J* = 6.9), 5.32 (t, 1H, *J* = 6.9). ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.8, 16.1, 16.5, 19.7, 25.6, 26.2, 26.3, 30.8, 39.4, 39.7, 62.4, 63.8, 69.1, 97.9, 120.8, 124.4, 126.0, 134.7, 135.0, 140.2. MS: calculated 322.25, found 345.33 $[M+Na]^+$.

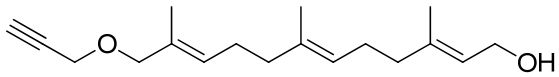


2-(((2*E*,6*E*,10*E*)-3,7,11-trimethyl-12-(prop-2-yn-1-yloxy)dodeca-2,6,10-trien-1-yl)oxy)tetrahydro-2*H*-pyran (**2**) was produced according to published literature protocols and was identical to previously reported ^1H and ^{13}C NMR analyses.[2] ^1H NMR (600 MHz, $CDCl_3$): δ 1.47-1.88 (m, 6H), 1.59 (s, 3H), 1.63 (s, 3H), 1.66 (s, 3H), 1.98-2.15 (m, 8H), 2.39 (t, 1H, *J* = 2.4), 3.50 (ddd, 1H, *J* = 5.2, 5.2, 10.4), 3.88 (ddd, 1H, *J* = 3.0, 8.0, 11.1), 3.92 (s, 2H), 4.01 (dd, 1H, *J* = 7.4, 11.9), 4.06 (d, 2H, *J* = 2.4), 4.22 (dd, 1H, *J* = 6.4, 11.9), 4.61 (t, 1H, *J* = 3.6), 5.10 (t, 1H, *J* = 6.7), 5.35 (t, 1H, *J* = 6.9), 5.41 (t, 1H, *J* = 6.9). ^{13}C NMR (150 MHz,

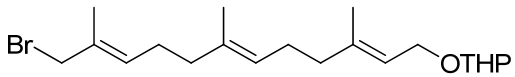
[1] Rose, M. W.; Rose, N. D.; Boggs, J.; Lenevich, S.; Xu, J.; Barany, G.; Distefano, M. D. "Evaluation of geranylazide and farnesylazide diphosphate for incorporation of prenylazides into a CAAX box-containing peptide using protein farnesyltransferase" *Journal of Peptide Research* (2005), 65(6), 529-537.

[2] Hosokawa, Ayako; Wollack, James W.; Zhang, Zhiyuan; Chen, Lin; Barany, George; Distefano, Mark D. "Evaluation of an alkyne-containing analogue of farnesyl diphosphate as a dual substrate for protein-prenyltransferases" *International Journal of Peptide Research and Therapeutics* (2007), 13(1-2), 345-354.

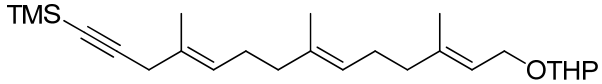
$CDCl_3$): δ 14.0, 16.0, 16.5, 19.7, 25.6, 26.3, 26.4, 30.8, 39.3, 39.7, 56.3, 62.4, 63.7, 74.2, 75.9, 80.1, 97.9, 120.7, 124.4, 129.5, 131.2, 134.9, 140.3. MS: calculated 360.27, found 383.42 $[M+Na]^+$.



(2*E*,6*E*,10*E*)-3,7,11-trimethyl-12-(prop-2-yn-1-yloxy)dodeca-2,6,10-trien-1-ol (**alk-FOH**) was produced according to published literature protocols and was identical to previously reported ^1H, ^{13}C NMR and MS analyses.[2] ^1H NMR (600 MHz, $CDCl_3$): δ 1.60 (s, 3H), 1.65 (s, 3H), 1.68 (s, 3H), 2.00-2.17 (m, 8H), 2.40 (t, 1H, *J* = 2.4), 3.93 (s, 2H), 4.07 (d, 2H, *J* = 2.4), 4.15 (d, 2H, *J* = 6.9), 5.11 (ddd, 1H, *J* = 1.2, 6.8, 6.8), 5.39-5.44 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.1, 16.1, 16.4, 26.4, 26.5, 39.3, 39.6, 56.4, 59.5, 74.2, 76.0, 80.2, 123.5, 124.3, 129.5, 131.3, 135.1, 139.8. MS: calculated 276.21, found 299.33 $[M+Na]^+$.

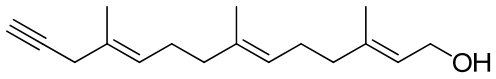


2-(((2*E*,6*E*,10*E*)-12-bromo-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)tetrahydro-2*H*-pyran (**3**): In a previously flame-dried round-bottom flask under argon atmosphere was dissolved *N*-bromosuccinimide (498 mg, 2.80 mmol) in anhydrous dichloromethane (8 mL) cooled to 0 °C. Dimethyl sulfide (247 μL, 3.36 mmol) was added dropwise over 3 minutes, and the resulting reaction mixture was cooled to -20 °C. (**1**) (603 mg, 1.87 mmol) dissolved in anhydrous dichloromethane (4 mL) was then added dropwise over 3 minutes, and the reaction mixture was stirred at 0 °C for 3 hours and then overnight at room temperature. The reaction was quenched with a saturated brine aqueous solution and extracted with dichloromethane (3 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by silica gel flash chromatography (5% EtOAc / 95% hexanes) to yield (**3**) (317 mg, 44%) as a yellow oil. ^1H NMR (600 MHz, $CDCl_3$): δ 1.45-1.87 (m, 6H), 1.58 (s, 3H), 1.66 (s, 3H), 1.73 (s, 3H), 1.97-2.14 (m, 8H), 3.49 (ddd, 1H, *J* = 5.3, 5.3, 10.6), 3.88 (ddd, 1H, *J* = 3.0, 7.9, 11.1), 3.95 (s, 2H), 4.01 (dd, 1H, *J* = 7.4, 11.9), 4.22 (dd, 1H, *J* = 6.4, 11.9), 4.61 (t, 1H, *J* = 3.7), 5.09 (t, 1H, *J* = 6.8), 5.34 (t, 1H, *J* = 6.3), 5.56 (t, 1H, *J* = 6.8). ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.7, 16.0, 16.5, 19.7, 25.6, 26.3, 26.9, 30.8, 38.8, 39.6, 41.9, 62.3, 63.7, 97.9, 120.8, 124.6, 131.3, 132.0, 134.5, 140.1. MS: calculated 384.17, found 407.33 $[M+Na]^+$.

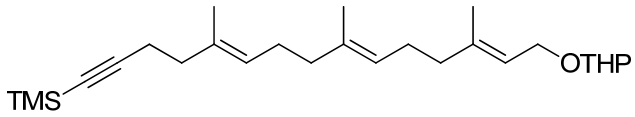


Trimethyl((4*E*,8*E*,12*E*)-4,8,12-trimethyl-14-((tetrahydro-2*H*-pyran-2-yl)oxy)tetradeca-4,8,12-trien-1-yn-1-yl)silane (**4**): In a previously flame-dried round-bottom flask under argon atmosphere was dissolved trimethylsilylacetylene (46 μL, 0.332 mmol) in anhydrous DMF (1 mL) at room temperature. Potassium

carbonate (46 mg, 0.332 mmol), sodium sulfite (21 mg, 0.166 mmol), (**3**) (85 mg, 0.221 mmol) and copper(I) iodide (2 mg, 0.011 mmol) were added sequentially and the reaction mixture was stirred for 4 hours. The reaction was quenched with a saturated ammonium chloride aqueous solution and extracted with dichloromethane (3 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by silica gel flash chromatography (5% EtOAc / 95% hexanes) to yield (**4**) (39 mg, 44%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.15 (s, 9H), 1.48-1.87 (m, 6H), 1.60 (s, 3H), 1.66 (s, 3H), 1.67 (s, 3H), 1.96-2.16 (m, 8H), 2.90 (s, 2H), 3.51 (ddd, 1H, 4.9, 4.9, 10.5), 3.89 (ddd, 1H, *J* = 2.9, 8.0, 11.0), 4.02 (dd, 1H, *J* = 7.4, 11.8), 4.23 (dd, 1H, *J* = 6.4, 11.9), 4.62 (t, 1H, *J* = 3.7), 4.79-4.94 (m, 1H), 5.11 (t, 1H, *J* = 5.8), 5.35 (t, 1H, *J* = 6.4). ^{13}C NMR (150 MHz, $CDCl_3$): δ 0.3, 16.1, 16.6, 19.8, 25.6, 26.5, 26.9, 30.1, 30.9, 32.3, 39.5, 39.8, 62.4, 63.8, 97.9, 104.9, 112.2, 120.8, 124.2, 126.0, 129.7, 135.2, 140.4. MS: calculated 402.30, found 403.33 $[M+H]^+$.

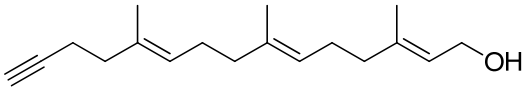


(2*E*,6*E*,10*E*)-3,7,11-trimethyltetradeca-2,6,10-trien-13-yn-1-ol (**alk-FOH-2**): In a round-bottom flask equipped with a condenser, (**4**) (29 mg, 0.072 mmol) was dissolved in EtOH (2 mL). Pyridinium *p*-toluenesulfonate (1.8 mg, 0.007 mmol) was added and the reaction mixture was stirred overnight at 60 °C. The solvent was removed under reduced pressure and the crude material obtained was dissolved in THF (2 mL). Tetra-n-butylammonium fluoride (317 μL of 1 M solution in THF, 0.317 mmol) was added and the reaction mixture stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude material obtained was purified by silica gel flash chromatography (20% EtOAc / 80% hexanes) to yield (**alk-FOH-2**) (13 mg, 73%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.60 (s, 3H), 1.68 (s, 6H), 1.86 (s, 1H), 1.97-2.23 (m, 8H), 2.92 (m, 2H), 4.15 (d, 2H, *J* = 6.9), 5.12 (m, 1H), 5.41 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.6, 16.1, 16.4, 26.4, 27.2, 27.7, 39.4, 39.6, 59.6, 78.0, 98.9, 110.3, 123.5, 124.3, 128.8, 135.1, 139.9. MS: calculated 246.20, found 247.25 $[M+H]^+$.

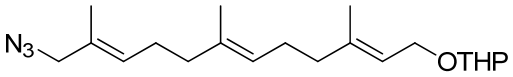


Trimethyl((5*E*,9*E*,13*E*)-5,9,13-trimethyl-15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadeca-5,9,13-trien-1-yn-1-yl)silane (**5**): In a previously flame-dried round-bottom flask under argon atmosphere was dissolved 1-(trimethylsilyl)propyne (88 mg, 0.781 mmol) in anhydrous THF (1 mL) at -20 °C, and n-butyl lithium (488 μL of 1.6 M solution in hexanes, 0.781 mmol) was added dropwise. After 30 minutes, (**3**) (75 mg, 0.195 mmol) dissolved in anhydrous THF (1 mL) was added dropwise, and the reaction mixture was allowed to slowly warm to 0 °C. After stirring 12 hours at 0 °C, the reaction was quenched with ice-cold water (50 mL) and extracted with ether (3 x 50 mL). The combined organic layers were washed with a saturated $NaHCO_3$ aqueous solution (3 x 50 mL) and a saturated brine aqueous solution (150 mL), dried over

Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by silica gel flash chromatography (5% EtOAc / 95% hexanes) to yield (**5**) (77 mg, 94%) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.13 (s, 9H), 1.46-1.76 (m, 6H), 1.56 (s, 3H), 1.60 (s, 3H), 1.68 (s, 3H), 1.98 (t, 2H, *J* = 7.0), 2.00-2.14 (m, 6H), 2.18 (t, 2H, *J* = 7.6), 2.28 (q, 2H, *J* = 6.9), 3.51 (ddd, 1H, *J* = 5.4, 5.4, 10.8), 3.89 (ddd, 1H, *J* = 2.5, 7.9, 10.9), 4.02 (dd, 1H, *J* = 7.5, 11.6), 4.23 (dd, 1H, *J* = 6.4, 12.1), 4.62 (s, 1H), 5.10 (t, 1H, *J* = 5.6), 5.15 (t, 1H, *J* = 6.6), 5.36 (t, 1H, *J* = 6.3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 0.3, 16.0, 16.2, 16.6, 19.4, 19.8, 25.7, 26.5, 26.8, 30.9, 38.8, 39.7, 39.8, 62.4, 63.8, 84.7, 98.0, 107.6, 120.7, 124.1, 125.7, 133.5, 135.3, 140.4. MS: calculated 416.31, found 439.42 $[M+Na]^+$.



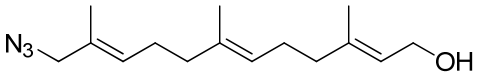
(2*E*,6*E*,10*E*)-3,7,11-trimethylpentadeca-2,6,10-trien-14-yn-1-ol (**alk-FOH-3**): In a round-bottom flask equipped with a condenser, (**5**) (63 mg, 0.151 mmol) was dissolved in EtOH (1.5 mL). Pyridinium *p*-toluenesulfonate (3.8 mg, 0.015 mmol) was added and the reaction mixture was stirred overnight at 60 °C. The solvent was removed under reduced pressure and the crude material obtained was dissolved in THF (1.5 mL). Tetra-n-butylammonium fluoride (332 μL of 1 M solution in THF, 0.332 mmol) was added and the reaction mixture stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude material obtained was purified by silica gel flash chromatography (20% EtOAc / 80% hexanes) to yield (**alk-FOH-3**) (26 mg, 67%) as a colorless oil, and was identical to previously reported ^{1}H, ^{13}C NMR and MS analyses.[3] ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.60 (s, 6H), 1.68 (s, 3H), 1.92-2.15 (m, 9H), 2.17-2.29 (m, 4H), 4.15 (d, 2H, *J* = 6.9), 5.11 (t, 1H, *J* = 6.1), 5.17 (t, 1H, *J* = 6.9), 5.41 (t, 1H, *J* = 6.6). ^{13}C NMR (150 MHz, $CDCl_3$): δ 15.9, 16.1, 16.4, 17.8, 26.4, 26.7, 38.5, 39.6, 39.7, 59.5, 68.5, 84.6, 123.5, 124.1, 125.7, 133.3, 135.3, 139.9. MS: calculated 260.21, found 283.33 $[M+Na]^+$.



2-(((2*E*,6*E*,10*E*)-12-azido-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)tetrahydro-2*H*-pyran (**6**): In a round-bottom flask, (**3**) (75 mg, 0.195 mmol) was dissolved in DMSO (1 mL). Sodium azide (19 mg, 0.293 mmol) was added and the reaction mixture was stirred overnight at room temperature. The reaction was diluted with ice-cold water (50 mL) and extracted with ether (3 x 50 mL). The combined organic layers were washed with a saturated brine aqueous solution (150 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by silica gel flash chromatography (5% EtOAc / 95% hexanes) to yield (**6**) (55 mg, 81%) as a yellow oil isomeric mixture, which was identical to previously reported ^{1}H, ^{13}C NMR and MS analyses.[1] ^{1}H NMR (600 MHz, $CDCl_3$): δ

[3] Cox, Nicholas J. G.; Mills, Stuart D.; Pattenden, Gerald "Macrocyclizations using allylic radical intermediates. A new synthetic approach to natural 14-membered cembranoids" *Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry (1972-1999)* (1992), (11), 1313-21.

1.47-1.87 (m, 6H), 1.58 (s, 3H), 1.67 (s, 3H), 1.72 (s, 3H), 1.91-2.21 (m, 8H), 3.50 (m, 1H), 3.63 (s, 1H), 3.79 (t, 1H, *J* = 7.0), 3.88 (t, 1H, *J* = 9.4), 4.01 (t, 1H, *J* = 8.8), 4.23 (dd, 1H, *J* = 6.6, 11.8), 4.62 (s, 1H), 4.95 (d, 1H, *J* = 23.5), 5.12 (m, 1H), 5.37 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.8, (16.1), 16.6, 17.8, 19.8, 25.7, 26.4, (26.5), (30.8), 30.9, 36.1, 39.4, 39.7, 59.6, 62.4, 63.8, (68.2), 98.0, (114.7), 120.8, 120.9, (124.6), 125.1, 130.4, 134.0, 140.2, (140.3), (142.5). MS: calculated 347.26, found 370.42 $[M+Na]^+$.



(2*E*,6*E*,10*E*)-12-azido-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (**az-FOH**) was produced according to published literature protocols and yielded an isomeric mixture of **az-FOH**, which was identical to previously reported ^{1}H, ^{13}C NMR and MS analyses.[1] ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.60 (s, 3H), 1.68 (s, 3H), 1.73 (s, 3H), 1.92-2.22 (m, 8H), 3.64 (s, 1H), 3.80 (t, 1H, *J* = 7.1), 4.15 (d, 2H, *J* = 6.8), 4.96 (d, 1H, *J* = 15.5), 5.13 (m, 1H), 5.41 (t, 1H, *J* = 6.7). ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.8, 16.1, 16.4, (17.8), 26.3, 26.4, (26.5), (30.7), (36.1), (39.3), 39.5, 39.6, 59.5, 68.1, (114.7), 123.5, 123.6, (124.5), 125.0, 130.4, 134.1, (134.8), 139.6, (139.8), (142.5). MS: calculated 263.20, found 264.25 $[M+H]^+$.