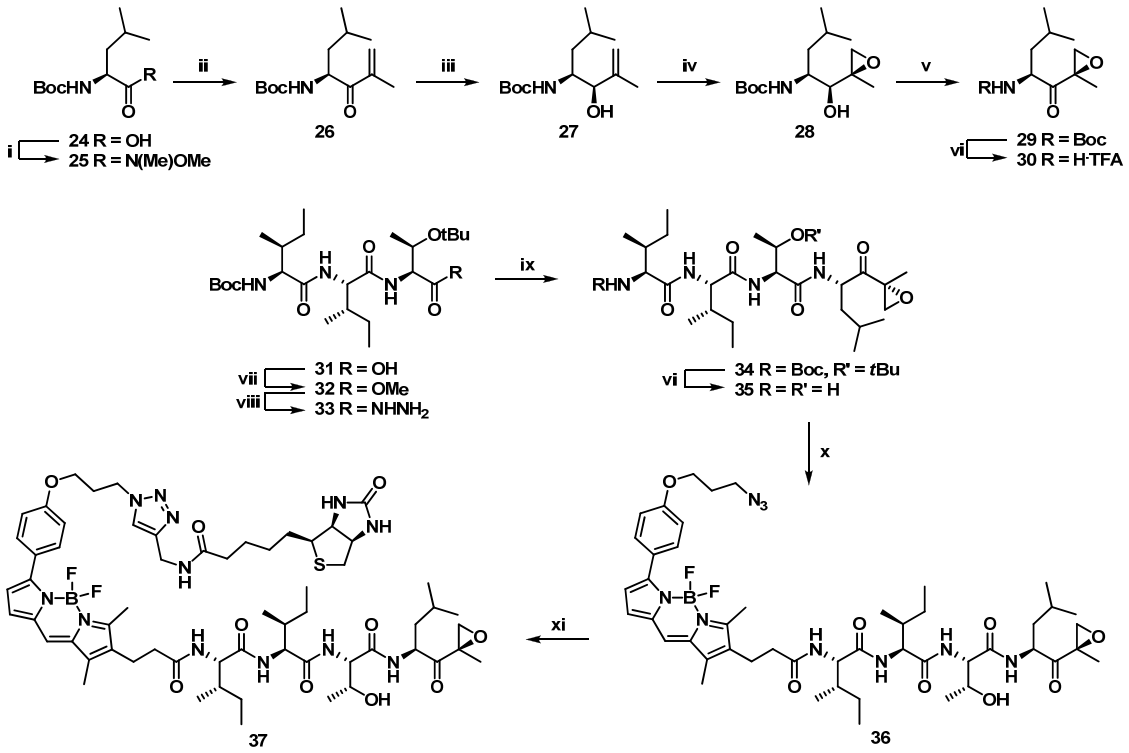
Supplemental document S1: Synthesis of MVB003, MVB070 and MVB072

Proteasome activity imaging and profiling characterizes bacterial effector Syringolin A

BODIPY-epoxomicin (MVB003). 4-methylbenzhydrylamine (MBHA) functionalized polystyrene resin (0.42 g 1.2 mmol/g, 0.5 mmol) was coupled to 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid (HMPB) linker (0.36 g, 1.5 mmol, 3 equiv.) in the presence of BOP (0.67 g, 1.5 mmol, 3 equiv.) and DiPEA (0.53 ml, 3 mmol, 6 equiv.) in NMP. After shaking overnight, the resin was washed with NMP (3x) and DCM (3x). The coupling was monitored for completion by the Kaiser test. The resulting MBHA-HMPB resin was coevaporated with DCE (2x). The resin was then condensed with Fmoc-Thr(*t*Bu)-OH (0.6 g, 1.5 mmol, 3 equiv.) under the influence of DIC (0.26 ml, 1.65 mmol, 3.3 equiv.) and DMAP (9.2 mg, 0.075 mmol, 15 mol%) in DCM for 2 hr. After the resin was filtered and washed with DCM, the condensation cycle was repeated. The resin was then washed with NMP (2x), DCM (2x) and ether (2x) and dried *in vacuo* overnight. The obtained resin was washed with DCM and submitted to two cycles of Fmoc solid phase synthesis with Fmoc-Ile-OH and Boc-Ile-OH, respectively, as follows: after deprotection with 20% piperidine in NMP (20 min.), the resin was washed with NMP (2x) and DCM (2x) and coupled to Fmoc-Ile-OH (0.44 g, 1.25 mmol, 2.5 equiv.) or Boc-Ile-OH (0.29 g, 1.25 mmol, 2.5 equiv.) in the presence of BOP (0.55 g, 1.25 mmol, 2.5 equiv.) and DiPEA (0.26 ml, 1.5 mmol, 3 equiv.) in NMP. The reaction mixture was shaken overnight and for 5 hr. respectively, followed by washing with NMP (3x) and DCM (3x). Couplings were monitored for completion by the Kaiser test. Washing with ether and drying *in vacuo* overnight yielded the fully protected resin tripeptide. MBHA-HMPB-Thr(*t*Bu)-Ile-Ile-Boc resin (~0.5 mmol) was subjected to mild acidic cleavage with 1% TFA in DCM (10 min, 4x). The collected fractions were concentrated in the presence of toluene to yield the crude tripeptide Boc-Ile-Ile-Thr(*t*Bu)-OH, which was used without further purification. (Boc-leucinyl)-(*R*)-2-methyloxirane (synthesized as described by Sin, N.; Kim, K.B.; Elofsson, M.; Meng, L.; Auth, H.; Kwok, B.H.; Crews, C.M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2283 - 2288.) (0.11 g, 0.4 mmol, 1.6 equiv.) was stirred in TFA for 10 min. The reaction mixture was concentrated in the presence of toluene, before the crude leucinyl-2-methyloxirane TFA salt was dissolved in DMF. The crude Boc-Ile-Ile-Thr(*t*Bu)-OH (~0.25 mmol), BOP (0.2 g, 0.45 mmol, 1.1 equiv.) and DiPEA (0.33 ml, 2 mmol, 5 equiv.) were added and the resulting mixture was stirred for 16 hr., before being concentrated *in vacuo*. Purification by column chromatography (PetEt → 30% EtOAc in PetEt) afforded the fully protected epoxyketone tetrapeptide Boc-Ile-Ile-Thr(*t*Bu)-leucinyl-(*R*)-2-methyloxirane as white crystals (144 mg, 0.22 mmol, 88%). Boc-Ile-Ile-Thr(*t*Bu)-leucinyl-(*R*)-2-methyloxirane (77 mg, 0.12 mmol) was dissolved in TFA and stirred for 1 hr. at 0 °C, before being concentrated in the presence of toluene. The resulting TFA salt was dissolved in DCM and DiPEA (79 µl, 0.48 mmol, 4 equiv.) and BODIPY TMR-OSu (60 mg, 0.12 mmol, 1 equiv.) (synthesized as described by Verdoes, M. *et al. Chem. Biol.* **2006**, *13*, 1217-1226) were added. The reaction mixture was stirred 16 hr. to result a mixture of diastereomers. The major product was purified by HPLC purification to give BODIPY-epoxomicin (MVB003) (8.5 mg, 9.7 µmol, 8%). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 7.84 (d, J = 8.8 Hz, 2H), 7.56 (s, 1H), 7.09 (d, J = 3.9 Hz, 1H), 7.01-6.97 (m, 2H), 6.64 (d, J = 3.9 Hz, 1H), 4.39 (dd, J_1 = 10.1, J_2 = 3.6 Hz, 1H), 4.25-4.13 (m, 3H), 3.88 (td, J_1 = 11.0, J_2 = 6.4 Hz, 1H), 3.80 (s, 3H), 3.17 (d, J = 5.2 Hz, 1H), 2.94 (d, J = 5.0 Hz, 1H), 2.72-2.54 (m, 2H), 2.45 (s, 3H), 2.33 (ddd, J_1 = 29.5, J_2 = 13.9, J_3 = 6.4 Hz, 2H), 2.20 (s, 3H), 1.79-1.69 (m, 1H), 1.68-1.57 (m, 2H), 1.48-1.39 (m, 1H), 1.38 (s, 3H), 1.35-1.20 (m, 4H), 1.13-1.02 (m, 1H), 0.99 (d, J = 6.3 Hz, 3H), 0.89-0.76 (m, 12H), 0.74-0.64 (m, 6H).

Scheme 4. Synthesis of the bifunctional epoxomicin derived probes **36** and **37**.



Reagents and conditions: i) *N,O*-dimethyl-hydroxylamine·HCl (1 equiv), BOP (1 equiv.), DiPEA (2 equiv.), DCM, 16 hr., 89%. ii) (a) *t*BuLi (4.5 equiv.), 2-bromopropene (3 equiv.), Et_2O, -78 °C, 15 min. (b) **25**, -78 °C to RT, 2 hr., 79%. iii) $NaBH_4$ (1.4 equiv.), $CeCl_3$ (2.2 equiv.), MeOH, 0 °C, 15 min., 91%. iv) *t*BuOOH (3 equiv.), $VO(acac)_2$ (4 mol%), DCM, 0 °C to RT, 2 hr., 51%. v) Dess-Martin periodinane (3 equiv.), DMSO, 0 °C to RT, 4 hr., 90%. vi) TFA, 30 min. vii) TMS-diazomethane (2 equiv.), MeOH/Tol. (1/1, v/v), 15 min., 64%. viii) Hydrazine monohydrate (60 equiv.), MeOH, reflux, 76%. ix) (a) *t*BuONO (1.1 equiv.), HCl (2.8 equiv.), EtOAc/DMF, 4 hr., -25 °C. (b) DiPEA (4 equiv.), **30** (1.1 equiv.), -25 °C to RT, 16 hr., 89%. x) **13** (1 equiv.), DiPEA (4 equiv.), 12 hr., 47%. xi) **15** (2 equiv.), 10 mol% $CuSO_4$, 20 mol% sodium ascorbate, *t*BuOH/H_2O/Tol. (1/1/1, v/v/v), RT, 12 hr., 85%.

(Boc-leucinyl)-*N,O*-di-methyl-hydroxamide (25). To a solution of Boc-Leu-OH (**24**) (2.5 g, 10 mmol) in DCM were added BOP (5.3 g, 12 mmol, 1.2 equiv.), DiPEA (4.1 ml, 25 mmol, 2.5 equiv.) and *N,O*-dimethylhydroxylamine·HCl (2.9 g, 30 mmol, 3.0 equiv.) and the reaction mixture was stirred overnight, before being concentrated *in vacuo*. The residue was taken up in EtOAc, washed subsequently with 1M aqueous HCl solution (3x), saturated aqueous $NaHCO_3$ solution (3x) and Brine (1x). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (10% EtOAc in Tol. → 20% EtOAc in Tol.), yielding the title compound **25** (2.2 g, 7.9 mmol, 79%). ^{1}H NMR (200 MHz, $CDCl_3$): δ ppm 5.06 (d, J = 9.0 Hz, 1H), 4.66 (td, J_1 = 8.0, J_2 = 7.2 Hz, 1H), 3.74 (s, 3H), 3.15 (s, 3H), 1.79-1.46 (m, 1H), 1.44-1.20 (m, 11H), 0.94-0.83 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ ppm 172.95, 154.85, 78.00, 60.56, 48.13, 40.82, 31.24, 27.51, 23.84, 22.51, 20.72.

(Boc-leucinyl)-isopropene (26). 2-Bromopropene (1.2 ml, 14 mmol, 3.0 equiv.) was dissolved in freshly distilled Et_2O, put under argon atmosphere and cooled to -78 °C. After adding *t*BuLi (13 ml 1.6 M in pentane, 21 mmol, 4.5 equiv.), the reaction mixture was stirred for 15 min. Weinreb amide **25** (1.3 g, 4.6

mmol, 1.0 equiv.) was coevaporated with Tol., dissolved in freshly distilled Et_2O and put under argon atmosphere. The solution was cooled to -78 °C and added to the reaction mixture containing the lithium reagent using an argon-flushed *canula*. The resulting reaction mixture was stirred for 2 hr. and allowed to warm up to room temperature, before being quenched with sat. aq. NH_4Cl. The mixture was extracted with EtOAc (3x) and the combined organics were washed with Brine (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (Tol. → 1% EtOAc in Tol.) afforded compound **26** (0.93 g, 3.6 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 6.11 (s, 1H), 5.88 (s, 1H), 5.44 (d, *J* = 8.8 Hz, 1H), 5.12-5.06 (m, 1H), 1.90 (s, 3H), 1.75-1.68 (m, 1H), 1.49-1.34 (m, 11H), 1.01 (d, *J* = 6.4 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ ppm 201.04, 155.19, 141.96, 125.46, 78.86, 52.19, 42.52, 27.93, 24.55, 22.97, 21.34, 17.42.

(1*S*,2*S*)-2-Boc-amino-4-methyl-1-(isopropenyl)pentan-1-ol (27). A solution of (Boc-leucinyl)-isopropene (**26**, 0.46 g, 1.8 mmol) in methanol was put under argon atmosphere and $CeCl_3{\cdot}7H_2O$ (1.0 g) was added. The solution was cooled to 0 °C, before $NaBH_4$ (95 mg, 2.5 mmol, 1.4 equiv.) was added in 6 portions over 3 min. After stirring for 10 min., the reaction was quenched with glacial acetic acid and Tol. was added after an additional 20 min at 0 °C. The solvents were removed *in vacuo* and the oily residue was dissolved in an EtOAc/H_2O mixture. The organic layer was washed with H_2O and Brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (Tol. → 10% EtOAc in Tol.) yielded **27** (0.40 g, 1.6 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 5.02 (s, 1H), 4.97-4.92 (m, 1H), 4.72 (d, *J* = 8.6 Hz, 1H), 4.14 (s, 1H), 3.93-3.69 (m, 1H), 1.76 (s, 3H), 1.70-1.52 (m, 1H), 1.45 (s, 9H), 1.37-1.08 (m, 2H), 0.97-0.84 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ ppm 156.03, 144.69, 111.03, 79.03, 77.43, 50.65, 36.82, 28.18, 24.39, 23.60, 21.26, 19.17.

1*S*,2*S*)-2-Boc-amino-4-methyl-1-((*R*)-2-methyloxiran-2-yl)pentan-1-ol (28). Compound **27** (0.53 g, 2.1 mmol) was dissolved in anhydrous DCM and cooled to 0 °C. Next, $VO(acac)_2$ (22 mg, 0.080 mmol, 0.04 equiv.) and *t*BuOOH (1.1 ml 5.5 M in decane, 6.2 mmol, 3.0 equiv.) were added and the reaction mixture was stirred for 2 hr., allowing the temperature to rise slowly to RT. The resulting purple solution was concentrated *in vacuo* and the residue was dissolved in EtOAc and washed with a half saturated $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with H_2O and Brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (PetEt → 10% EtOAc in PetEt), yielding epoxide **28** (0.29 g, 1.1 mmol, 51%). ^{1}H NMR (200 MHz, $CDCl_3$): δ ppm 4.88 (d, *J* = 9.5 Hz, 1H), 3.91-3.85 (m, 2H), 2.99 (d, *J* = 5.1 Hz, 1H), 2.64 (d, *J* = 5.1 Hz, 1H), 1.73-1.63 (m, 1H), 1.45 (s, 9H), 1.38 (s, 3H), 1.23-1.00 (m, 2H), 0.96 (d, *J* = 4.0 Hz, 3H), 0.93 (d, *J* = 4.4 Hz, 3H).

(Boc-leucinyl)-(*R*)-2-methyloxirane (29). Dess-Martin periodinane (1.2 g, 2.9 mmol, 3.0 equiv.) was dissolved in DMSO, put under argon atmosphere and cooled to 0 °C. Epoxy alcohol **28** (0.26 g, 0.95 mmol) was coevaporated with Tol., dissolved in DMSO and added to the first solution. The reaction mixture was stirred for 4 hr. and allowed to warm up to room temperature, before sat. aq. $NaHCO_3$ was added. The layers were separated, the aqueous layer was extracted with EtOAc and the combined organic layers were washed with H_2O (3x), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Because DMSO was still present, the residue was dissolved in EtOAc and washed with H_2O (2x) and Brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* and the crude product was purified by column chromatography (PetEt → 5% EtOAc in PetEt) to give the leucine-

derived epoxyketone **29** (0.23 g, 0.85 mmol, 90%). ^{1}H NMR (200 MHz, $CDCl_3$): δ ppm 4.90 (d, *J* = 9.1 Hz, 1H), 4.32 (dt, J_1 = 10.4, J_2 = 3.08 Hz, 1H), 3.30 (d, *J* = 5.0 Hz, 1H), 2.90 (d, *J* = 5.0 Hz, 1H), 1.84-1.59 (m, 1H), 1.52 (s, 3H), 1.41 (s, 9H), 1.27-1.08 (m, 2H), 1.01-0.89 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ ppm 212.13, 155.69, 79.52, 58.74, 52.04, 51.28, 39.82, 27.96, 24.81, 23.02, 20.87, 16.41.

Boc-Ile-Ile-Thr(*t*Bu)-OH (31). 4-methylbenzhydrylamine (MBHA) functionalized polystyrene resin (5.0 g 1.2 mmol/g, 6.0 mmol) was coupled to 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid (HMPB) linker (4.3 g, 18 mmol, 3 equiv.) in the presence of BOP (8.0 g, 18 mmol, 3 equiv.) and DiPEA (6.3 ml, 36 mmol, 6 equiv.) in NMP. After shaking overnight, the resin was washed with NMP (3x) and DCM (3x). The coupling was monitored for completion by the Kaiser test. The resulting MBHA-HMPB resin (~6.0 mmol) was coevaporated with DCE (2x). The resin was then condensed with Fmoc-Thr(*t*Bu)-OH (7.2 g, 18 mmol, 3 equiv.) under the influence of DIC (3.1 ml, 20 mmol, 3.3 equiv.) and DMAP (0.11 g, 0.90 mmol, 15 mol%) in DCM for 2 hr. After the resin was filtered and washed with DCM, the condensation cycle was repeated. The resin was then washed with NMP (2x), DCM (2x) and ether (2x) and dried *in vacuo* overnight. The loading of the resin was determined to be 0.43 mmol/g by spectrophotometric analysis. The obtained resin was washed with DCM and submitted to two cycles of Fmoc solid phase synthesis with Fmoc-Ile-OH and Boc-Ile-OH, respectively, as follows: after deprotection with 20% piperidine in NMP (20 min.), the resin was washed with NMP (2x) and DCM (2x) and coupled to Fmoc-Ile-OH (5.3 g, 15 mmol, 2.5 equiv.) or Boc-Ile-OH (3.5 g, 15 mmol, 2.5 equiv.) in the presence of BOP (6.6 g, 15 mmol, 2.5 equiv.) and DiPEA (3.1 ml, 18 mmol, 3 equiv.) in NMP. The reaction mixture was shaken overnight and for 5 hr. respectively, followed by washing with NMP (3x) and DCM (3x). Couplings were monitored for completion by the Kaiser test. Washing with ether and drying *in vacuo* overnight yielded the fully protected resin tripeptide. MBHA-HMPB-Thr(*t*Bu)-Ile-Ile-Boc resin (~6.0 mmol) was subjected to mild acidic cleavage with 1% TFA in DCM (10 min, 6x). The collected fractions were concentrated in the presence of toluene to yield the crude tripeptide **31**, which was used without further purification. LC/MS analysis: R_t 8.58 min (linear gradient 10 → 90% B in 13.5 min.), *m/z* 502.3 $[M + H]^+$, 524.5 $[M + Na]^+$, 1025.3 $[2M + Na]^+$.

Boc-Ile-Ile-Thr(*t*Bu)-methyl ester (32). The crude Boc-Ile-Ile-Thr(*t*Bu)-OH (**31**) (~3.0 g, ~6.0 mmol) was dissolved in MeOH/Tol (1/1) and treated with TMS-diazomethane (6 ml 2M in hexanes, 12 mmol, 2 equiv.) for 15 min. before being coevaporated with Tol. (3x). Purification by flash column chromatography (DCM → 0.5% MeOH in DCM) yielded the title compound as a white solid (2.0 g, 3.8 mmol, 64%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 6.51 (d, *J* = 8.1 Hz, 1H), 6.43 (d, *J* = 9.3 Hz, 2H), 4.48 (dd, J_1 = 9.1, J_2 = 1.6 Hz, 1H), 4.40 (dd, J_1 = 8.6, J_2 = 6.5 Hz, 1H), 4.24 (dq, J_1 = 6.2, J_2 = 1.6 Hz, 1H), 3.70 (s, 3H), 3.98-3.88 (m, 1H), 2.07-1.97 (m, 2H), 1.92-1.81 (m, 4H), 1.44 (s, 9H), 1.14 (d, *J* = 6.3 Hz, 3H), 1.11 (s, 9H), 0.99-0.86 (m, 12H). ^{13}C NMR (50 MHz, $CDCl_3$): δ ppm 171.052, 170.857, 67.206, 57.828, 57.502, 57.464, 52.138, 37.857, 37.233, 37.215, 37.195, 37.026, 28.320, 28.294, 24.875, 24.790, 24.759, 20.921, 15.532, 15.083, 11.414, 11.313.

Boc-Ile-Ile-Thr(*t*Bu)-hydrazide (33). To the solution of methyl ester **32** (2.0 g, 3.8 mmol) in MeOH was added hydrazine monohydrate (11.1 ml, 228 mmol, 60 equiv.) and the reaction mixture was refluxed overnight, before being concentrated in the presence of toluene. The white precipitate was filtered and washed with MeOH to give the hydrazide **33** (0.88 g, 1.7 mmol, 45%). The filtrate was concentrated in the presence of toluene and recrystallized from toluene/MeOH to yield a second batch of product (**33**)

(0.61 g, 1.2 mmol, 31%). (Total yield: 2.9 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.50 (s, 1H), 7.79-7.71 (m, 1H), 7.54-7.45 (m, 2H), 6.03-5.95 (m, 2H), 4.47-4.35 (m, 1H), 4.28 (d, *J* = 7.3 Hz, 1H), 4.08 (d, *J* = 1.2 Hz, 1H), 3.94 (d, *J* = 6.4 Hz, 1H), 1.97-1.72 (m, 4H), 1.63-1.49 (m, 2H), 1.45 (s, 9H), 1.21 (s, 9H), 1.08 (d, *J* = 6.3 Hz, 3H), 0.92 (dd, J_1 = 14.7, J_2 = 7.6 Hz, 12H). ^{13}C NMR (50 MHz, $CDCl_3$): δ ppm 172.90, 171.27, 169.84, 79.50, 74.62, 66.18, 58.82, 58.74, 57.90, 57.84, 57.74, 56.79, 36.38, 36.14, 27.64, 27.51, 24.32, 24.24, 18.04, 14.96, 14.85, 14.75, 10.36.

Boc-Ile-Ile-Thr(*t*Bu)-leucinyl-(*R*)-2-methyloxirane (34). A solution of Boc-Ile-Ile-Thr(*t*Bu)-hydrazide (**33**) (0.52 g, 1.0 mmol) in DMF/EtOAc (1/1, v/v) was put under argon atmosphere and cooled to -30°C. After adding HCl (0.70 ml 4M in dioxane, 2.8 mmol, 2.8 equiv.) and *t*BuONO (0.13 ml, 1.1 mmol, 1.1 equiv.), the reaction mixture was stirred for 1 hr. to generate the acyl azide. (Boc-leucinyl)-(*R*)-2-methyloxirane (**29**) (0.30 g, 1.1 mmol, 1.1 equiv.) was stirred in TFA for 10 min. The reaction mixture was concentrated in the presence of toluene, the crude leucinyl-2-methyloxirane TFA salt (**30**) was dissolved in DMF and DiPEA (0.19 ml, 1.1 mmol, 1.1 equiv.) was added. The resulting solution was added to the acyl azide reaction mixture at -30°C. DiPEA was added (0.66 ml, 3.8 mmol, 3.8 equiv.) and the reaction mixture was allowed to warm up to room temperature overnight. EtOAc was added and the mixture was washed with H_2O (3x). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (PetEt → 25% EtOAc in PetEt) afforded the fully protected epoxyketone **34** as white crystals (058 g, 0.89 mmol, 89%). ^{1}H NMR (200 MHz, $CDCl_3$): δ ppm 7.64 (d, *J* = 7.1 Hz, 1H), 6.85 (d, *J* = 5.8 Hz, 1H), 6.46 (d, *J* = 8.6 Hz, 1H), 5.07 (d, *J* = 8.3 Hz, 1H), 4.54-4.38 (m, 1H), 4.37-4.25 (m, 2H), 4.20-4.06 (m, 1H), 3.99-3.87 (m, 1H), 3.13 (dd, J_1 = 93.1, J_2 = 5.0 Hz, 2H), 1.97-1.53 (m, 5H), 1.52 (s, 3H), 1.44 (s, 9H), 1.28 (s, 9H), 1.06 (d, *J* = 6.4 Hz, 3H), 1.37-1.02 (m, 4H), 0.99-0.82 (m, 18H). ^{13}C NMR (50 MHz, $CDCl_3$): δ ppm 208.01, 171.74, 171.13, 169.52, 155.91, 79.24, 75.27, 66.48, 59.11, 58.99, 57.29, 56.77, 52.35, 50.62, 39.64, 37.27, 37.06, 28.21, 27.96, 25.30, 24.90, 24.60, 23.26, 21.20, 16.62, 16.38, 15.35, 15.20, 11.23, 11.04.

MVB070: Azido-BODIPY-epoxomicin (36). Boc-Ile-Ile-Thr(*t*Bu)-leucinyl-(*R*)-2-methyloxirane (**34,** 7.9 mg, 12 μmol) was dissolved in TFA (1 ml) and stirred for 30 min., before being coevaporated with Tol. (3x). The crude TFA·H-Ile$_2$-Thr-Leu-epoxyketone (**35**) was dissolved in DMF and azido-BODIPY-OSu (**9,** 6.6 mg, 12 μmol, 1 equiv.) and DiPEA (8 μl, 48 μmol, 4 equiv.) were added and the reaction mixture was stirred for 12 hr. Concentration *in vacuo*, followed by purification by column chromatography (DCM → 2% MeOH in DCM) yielded the title compound as a brown/red solid (5.4 mg, 5.7 μmol, 47%). ^{1}H NMR (600 MHz, MeOD): δ ppm 7.88 (d, *J* = 8.7 Hz, 2H), 7.41 (s, 1H), 7.06 (d, *J* = 3.9 Hz, 1H), 6.99 (d, *J* = 8.7 Hz, 2H), 6.60 (d, *J* = 3.9 Hz, 1H), 4.55 (dd, J_1 = 10.7, J_2 = 2.8 Hz, 1H), 4.30 (d, *J* = 5.0 Hz, 1H), 4.22 (d, *J* = 7.8 Hz, 1H), 4.15-4.12 (m, 3H), 4.02 (p, *J* = 6.1 Hz, 1H), 3.54 (t, *J* = 6.7 Hz, 2H), 3.25 (d, *J* = 5.1 Hz, 1H), 2.92 (d, *J* = 5.1 Hz, 1H), 2.81 (m, 1H), 2.71 (m, 1H), 2.51 (s, 3H), 2.45-2.40 (m, 2H), 2.25 (s, 3H), 2.07 (p, *J* = 6.3 Hz, 2H), 1.89-1.79 (m, 1H), 1.75-1.66 (m, 2H), 1.65-1.52 (m, 2H), 1.53-1.41 (m, 5H), 1.41-1.21 (m, 15H), 1.20-1.06 (m, 5H), 1.05-0.97 (m, 1H), 0.97-0.85 (m, 16H), 0.82 (d, *J* = 6.7 Hz, 3H), 0.76 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, MeOD): δ ppm 209.51, 174.86, 174.06, 173.59, 172.23, 161.03, 160.67, 156.57, 141.79, 136.67, 135.83, 132.45, 131.92, 131.89, 131.86, 131.67, 131.65, 129.91, 129.28, 127.16, 124.70, 119.10, 115.27, 115.19, 69.14, 68.55, 65.98, 60.13, 59.82, 59.42, 59.41, 53.10, 51.84, 40.38, 38.02, 37.71, 36.45, 30.82, 29.90, 26.26, 26.03, 23.81, 21.52, 21.21, 20.02, 17.05, 15.92, 15.86, 11.47, 11.22, 9.67.

MVB0072: Biotin-BODIPY-epoxomicin (37). Azido-BODIPY-epoxomicin (**36**, 4.1 mg, 4.3 μmol) and Biotin-propargylamide (**15**) (2.4 mg, 8.6 μmol, 2 equiv.) were dissolved in *t*BuOH (0.25 ml) and toluene (0.25 ml) before $CuSO_4$ (125 μl 3.4 mM, 10 mol%) and sodium ascorbate (125 μl 6.9 mM, 20 mol%) were added. The reaction mixture was stirred at 80 °C for 12 hr., before being cooled to room temperature and concentrated *in vacuo*. Purification by column chromatography (PE → 50% acetone in PE) yielded the title compound as a brown/red solid (4.5 mg, 3.7 μmol, 85%). ^{1}H NMR (600 MHz, MeOD): δ ppm 7.95-7.78 (m, 3H), 7.42 (s, 1H), 7.07 (d, *J* = 4.1 Hz, 1H), 6.95 (d, *J* = 8.9 Hz, 2H), 6.61 (d, *J* = 4.1 Hz, 1H), 4.70-4.52 (m, 5H), 4.46-4.39 (m, 2H), 4.34-4.26 (m, 1H), 4.25-4.19 (m, 1H), 4.17-4.11 (m, 1H), 4.08-3.99 (m, 3H), 3.95 (t, *J* = 2.2 Hz, 1H), 3.25 (d, *J* = 5.0 Hz, 1H), 3.16-3.10 (m, 1H), 2.92 (d, *J* = 5.1 Hz, 1H), 2.71-2.64 (m, 2H), 2.60-2.56 (m, 1H), 2.51 (s, 3H), 2.46-2.37 (m, 4H), 2.26 (s, 3H), 2.24-2.17 (m, 2H), 1.95-1.21 (m, 32H), 1.21-1.10 (m, 5H), 1.06-0.85 (m, 17H), 0.82 (d, *J* = 6.8 Hz, 3H), 0.76 (t, *J* = 7.3 Hz, 3H).